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ABSTRACTS

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Contents

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CHRONOLOGICAL LIST OF SESSIONS

(See page vii for Thematic Index of Sessions.)

THEMATIC INDEX OF SESSIONS*

Theme A: Development and Plasticity

Session Number	Title	Type	Day	Time
115.	Axonal Growth	Slide	Tu.	1:30 PM
178.	Axonal Growth and Guidance	Poster	W.	2:00 PM
15.	Sprouting and Sprouting Mechanisms I	Slide	M.	10:45 AM
154.	Sprouting and Sprouting Mechanisms II	Poster	W.	9:00 AM
89.	Regeneration I: Central	Slide	Tu.	10:45 AM
153.	Regeneration II: Peripheral	Poster	W.	9:00 AM
221.	Regeneration III:Central	Poster	Th.	9:00 AM
48.	Trophic Factors and Interactions in Development	Slide	M.	2:00 PM
179.	Trophic Agents in Development	Poster	W.	2:00 PM
249.	Trophic Interactions in Development	Poster	Th.	2:00 PM
24.	Competition and Remodeling	Poster	M.	9:00 AM
5.	Synaptogenesis	Slide	M.	9:00 AM
219.	Synapse Formation	Poster	Th.	9:00 AM
60.	Specificity of Synaptic Connections	Poster	M.	2:00 PM
152.	Morphogenesis and Pattern Formation I	Poster	W.	9:00 AM
243.	Morphogenesis and Pattern Formation II	Slide	Th.	3:45 PM
98.	Neuronal Death	Poster	Tu.	9:00 AM
250.	Effects of Activity, Inactivity, and Denervation I	Poster	Th.	2:00 PM
271.	Effects of Activity, Inactivity, and Denervation II	Slide	F.	9:00 AM
23.	Neural Plasticity in Adult Animals I	Poster	M.	9:00 AM
205.	Neural Plasticity in Adult Animals II	Slide	Th.	9:00 AM
61.	Motor Systems II (Motor System I, III, see Theme G)	Poster	M.	2:00 PM
176.	Development of Sensory Systems	Poster	W.	2:00 PM
288.	Limbic System	Poster	F.	9:00 AM
47.	Visual System I: Retino-Geniculo-Cortical Connections	Slide	M.	2:00 PM
132.	Visual System II: Retinotectal Connections	Poster	Tu.	1:30 PM
220.	Visual System III: Retino-Geniculo-Cortical Connections	Poster	Th.	9:00 AM
236.	Visual System IV: Retinotectal Connections	Slide	Th.	2:00 PM
4.	Development in Invertebrates	Slide	M.	9:00 AM
177.	Neurogenetics	Poster	W.	2:00 PM
97.	Endocrine Control of Neural Development	Poster	Tu.	9:00 AM
131.	Biochemical and Pharmacological Correlates of Development	Poster	Tu.	1:30 PM
83.	Development of Excitable Membranes	Slide	Tu.	9:00 AM
273.	Molecular Correlates of Development	Slide	F.	9:00 AM
25.	Nutritional and Prenatal Factors in Development I	Poster	M.	9:00 AM
116.	Nutritional and Prenatal Factors in Development II	Slide	Tu.	1:30 PM
289.	Developmental Disorders	Poster	F.	9:00 AM
62.	Aging I (Aging II, see Theme H)	Poster	M.	2:00 PM
123.	Aging III	Slide	Tu.	3:00 PM
39.	Applications of Recombinant DNA in Neuroscience	Symp.	M.	2:00 PM
73.	CNS Development in Invertebrates	Symp.	Tu.	9:00 AM
145.	Approaches to Studying Gene Expression and Regulation During Neuronal Differentiation and Functioning	Symp.	W.	9:00 AM
265.	Regeneration of Nerve Fibers in Mammalian CNS	Symp.	F.	9:00 AM

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

Theme B: Cell Biology

Session Number	Title	Type	Day	Time
136.	Morphology of Neurons and Glia	Poster	Tu.	1:30 PM
103.	Identified Cells	Poster	Tu.	9:00 AM
158.	Neuronal Tracing by Marker Transport	Poster	W.	9:00 AM
28.	Comparative Neuroanatomy	Poster	M.	9:00 AM
225.	Functions of Glia	Poster	Th.	9:00 AM
50.	Cell and Tissue Culture I	Slide	M.	2:00 PM
224.	Cell and Tissue Culture II	Poster	Th.	9:00 AM
102.	Membrane Structure and Function	Poster	Tu.	9:00 AM
293.	Lipids and Myelin	Poster	F.	9:00 AM
292.	Proteins and Nucleic Acids	Poster	F.	9:00 AM
40.	Specific Immunological Probes	Slide	M.	2:00 PM
101.	Cell Surface Macromolecules	Poster	Tu.	9:00 AM
157.	Axoplasmic Transport I	Poster	W.	9:00 AM
241.	Axoplasmic Transport II	Slide	Th.	2:00 PM
82.	Metabolic Studies I	Slide	Tu.	9:00 AM
254.	Metabolic Studies II	Poster	Th.	2:00 PM
29.	Blood-Brain Barrier	Poster	M.	9:00 AM
52.	Cellular Aspects of Disease I	Slide	M.	3:45 PM
182.	Cellular Aspects of Disease II	Poster	W.	2:00 PM
3.	Lectins: Cytochemical Markers for Nervous System Carbohydrates	Wkshp.	M.	9:00 AM
110.	Quantitative Microscopy: Theory, Methods, and Applications	Wkshp.	Tu.	1:30 PM
168.	Cytoskeletal Determinants of Neuronal Form and Function	Symp.	W.	2:00 PM

Theme C: Excitable Membranes and Synaptic Transmission

Session Number	Title	Type	Day	Time
227.	Synaptic Structure and Function	Poster	Th.	9:00 AM
167.	Electrical Synapses and Gap Junctions	Poster	W.	9:00 AM
140.	Presynaptic Mechanisms	Poster	Tu.	1:30 PM
141.	Postsynaptic Mechanisms I	Poster	Tu.	1:30 PM
233.	Postsynaptic Mechanisms II	Slide	Th.	2:00 PM
270.	Presynaptic and Postsynaptic Mechanisms	Slide	F.	9:00 AM
41.	Pharmacology of Synaptic Transmission I	Slide	M.	2:00 PM
260.	Pharmacology of Synaptic Transmission II	Poster	Th.	2:00 PM
226.	Acetylcholine and Cholinergic Receptors	Poster	Th.	9:00 AM
262.	Receptor Sensitization and Desensitization	Poster	Th.	2:00 PM
34.	Drug Effects on Receptors	Poster	M.	9:00 AM
259.	Metabolism of Receptors and Transmitters	Poster	Th.	2:00 PM
166.	Membrane Biophysics	Poster	W.	9:00 AM
8.	Action Potentials and Ion Channels I	Slide	M.	9:00 AM
165.	Action Potentials and Ion Channels II	Poster	W.	9:00 AM
291.	Action Potentials and Ion Channels III	Poster	F.	9:00 AM
280.	Oscillators and Rhythmic Discharges	Slide	F.	10:45 AM
187.	Epilepsy I: Kindling	Poster	W.	2:00 PM
188.	Epilepsy II	Poster	W.	2:00 PM
206.	Epilepsy III	Slide	Th.	9:00 AM
261.	Epilepsy IV	Poster	Th.	2:00 PM
234.	Sensory Transduction	Slide	Th.	2:00 PM
190.	Studies of Excitable Cells in Culture	Poster	W.	2:00 PM
76.	Electrobiology and Membrane Properties of CNS Neurons I	Slide	Tu.	9:00 AM

189.	Electrobiology and Membrane Properties of CNS Neurons II	Poster	W.	2:00 PM
36.	Electrobiology of Invertebrates	Poster	M.	9:00 AM
191.	Diseases of Synapses and Axons	Poster	W.	2:00 PM
38.	The Neurobiology of Dendrites	Symp.	M.	2:00 PM
109.	Cellular Mechanisms of Epileptogenesis	Symp.	Tu.	1:30 PM
170.	The Growing Relevance of Gap Junctions and Electrotonic Synapses to Neurobiology	Wkshp.	W.	2:00 PM
195.	Synaptic Regulation of Voltage-gated Channels	Symp.	Th.	9:00 AM
231.	Altered Axons: Is Compulsive Conduction Replaced by Spontaneous Spiking?	Wkshp.	Th.	2:00 PM

Theme D: Neurotransmitters, Modulators, and Receptors

Session Number	Title	Type	Day	Time
159.	Acetylcholine	Poster	W.	9:00 AM
35.	Amino Acids I: Pharmacology and Physiology	Poster	M.	9:00 AM
106.	Amino Acids II: Uptake and Binding	Poster	Tu.	9:00 AM
111.	Amino Acids III	Slide	Tu.	1:30 PM
32.	Peptides I: Anatomical Localization in CNS	Poster	M.	9:00 AM
33.	Peptides II: Peripheral Nervous System	Poster	M.	9:00 AM
138.	Peptides III: Receptors	Poster	Tu.	1:30 PM
162.	Peptides IV: Central Physiologic Effects	Poster	W.	9:00 AM
163.	Peptides V: Distribution and Regulation	Poster	W.	9:00 AM
197.	Peptides VI: Distribution and Regulation	Slide	Th.	9:00 AM
30.	Opioid Peptides I: Characterization	Poster	M.	9:00 AM
31.	Opioid Peptides II: Localization	Poster	M.	9:00 AM
43.	Opioid Peptides III: Receptors	Slide	M.	2:00 PM
139.	Opioid Peptides IV: Receptors	Poster	Tu.	1:30 PM
185.	Opioid Peptides V: Electrophysiology	Poster	W.	2:00 PM
257.	Opioids: Neuronal Interactions I	Poster	Th.	2:00 PM
267.	Opioids: Neuronal Interactions II	Slide	F.	9:00 AM
7.	Dopamine I: Receptors	Slide	M.	9:00 AM
42.	Dopamine II: Receptors	Slide	M.	2:00 PM
184.	Dopamine III: Electrophysiology	Poster	W.	2:00 PM
67.	Catecholamines I: Functional Neurochemistry	Poster	M.	2:00 PM
183.	Catecholamines II: Central Physiology	Poster	W.	2:00 PM
258.	Catecholamines III: Central Neuroanatomy	Poster	Th.	2:00 PM
297.	Catecholamines IV: Functional Neurochemistry	Poster	F.	9:00 AM
278.	Biogenic Amines: Histamine and Phenethylamines	Slide	F.	10:45 AM
6.	Serotonin I: Receptors	Slide	M.	9:00 AM
87.	Serotonin II: Electrophysiology	Slide	Tu.	10:45 AM
186.	Serotonin III: Localization, Physiology, and Pharmacology	Poster	W.	2:00 PM
105.	Transmitters in Invertebrates	Poster	Tu.	9:00 AM
86.	Cardioactive Neuropeptides in Arthropods	Slide	Tu.	10:45 AM
255.	Transmitter Cytochemistry and Immunochemistry I: Biogenic Amines	Poster	Th.	2:00 PM
294.	Transmitter Cytochemistry and Immunochemistry II	Poster	F.	9:00 AM
75.	Cellular Localization of Neuroeffector Substances	Slide	Tu.	9:00 AM
51.	Interactions Between Neurotransmitters I	Slide	M.	3:45 PM
256.	Interactions Between Neurotransmitters II	Poster	Th.	2:00 PM
208.	Modulators I: Benzodiazepine Receptors	Slide	Th.	10:45 AM
298.	Modulators II	Poster	F.	9:00 AM
299.	Metabolism of Transmitters and Modulators	Poster	F.	9:00 AM
114.	Cholinergic Receptors I	Slide	Tu.	1:30 PM
160.	Cholinergic Receptors II	Poster	W.	9:00 AM
137.	Adrenergic Receptors	Poster	Tu.	1:30 PM
239.	Benzodiazepine/GABA Receptor Complex	Slide	Th.	2:00 PM
161.	Regional Localization of Receptors I	Poster	W.	9:00 AM
200.	Regional Localization of Receptors II	Slide	Th.	9:00 AM

295.	Cyclic Nucleotides	Poster	F.	9:00 AM
279.	Alcohol	Slide	F.	10:45 AM
104.	Alcohol and Barbiturates	Poster	Tu.	9:00 AM
164.	Transmitters and Receptors in Disease I	Poster	W.	9:00 AM
228.	Transmitters and Receptors in Disease II	Poster	Th.	9:00 AM
66.	Behavioral Pharmacology I	Poster	M.	2:00 PM
238.	Behavioral Pharmacology II	Slide	Th.	2:00 PM
296.	Behavioral Pharmacology III	Poster	F.	9:00 AM
65.	Uptake, Storage, and Secretion I	Poster	M.	2:00 PM
122.	Uptake, Storage, and Secretion II	Slide	Tu.	3:00 PM
74.	Mechanism of Action of Antidepressants	Symp.	Tu.	9:00 AM
266.	In Vivo Electrochemistry: Principles and Applications	Wkshp.	F.	9:00 AM

Theme E: Endocrine and Autonomic Regulation

Session Number	Title	Type	Day	Time
10.	Anterior Pituitary I: Sex Hormones	Slide	M.	9:00 AM
45.	Anterior Pituitary II: Endorphins and ACTH	Slide	M.	2:00 PM
108.	Anterior Pituitary III: Sex Hormones	Poster	Tu.	9:00 AM
107.	Posterior Pituitary	Poster	Tu.	9:00 AM
229.	Pineal Gland: Biological Rhythms	Poster	Th.	9:00 AM
68.	Adrenal Medulla	Poster	M.	2:00 PM
71.	Neural Control of Immune System	Poster	M.	2:00 PM
37.	Cardiovascular Regulation I: Morphological Aspects	Poster	M.	9:00 AM
121.	Cardiovascular Regulation II: Electrophysiology	Slide	Tu.	1:30 PM
207.	Cardiovascular Regulation III: Arterial Pressure and Hypertension	Slide	Th.	9:00 AM
263.	Cardiovascular Regulation IV: Central Transmitters	Poster	Th.	2:00 PM
264.	Cardiovascular Regulation V: Functional Aspects, Stress, and Cerebral Blood Flow	Poster	Th.	2:00 PM
276.	Temperature Regulation	Slide	F.	9:00 AM
300.	Respiratory Regulation	Poster	F.	9:00 AM
69.	Peripheral Autonomic Nervous System	Poster	M.	2:00 PM
70.	Hormones and Behavior I: Sexual Behavior	Poster	M.	2:00 PM
201.	Hormones and Behavior II: Sexual Behavior	Slide	Th.	9:00 AM
232.	Hormones and Behavior III: Receptors in CNS	Slide	Th.	2:00 PM
209.	Neuropeptides in Invertebrates	Slide	Th.	10:45 AM

Theme F: Sensory Systems

Session Number	Title	Type	Day	Time
94.	Skin, Muscle, and Visceral Receptors	Poster	Tu.	9:00 AM
22.	Transduction in Sensory Systems	Poster	M.	9:00 AM
21.	Sensory System Transmitters	Poster	M.	9:00 AM
173.	Spinal Cord I	Poster	W.	2:00 PM
199.	Spinal Cord II	Slide	Th.	9:00 AM
130.	Subcortical Somatosensory Pathways	Poster	Tu.	1:30 PM
247.	Somatosensory Cortex I	Poster	Th.	2:00 PM
269.	Somatosensory Cortex II	Slide	F.	9:00 AM
14.	Evoked Potentials I	Slide	M.	9:00 AM
96.	Evoked Potentials II	Poster	Tu.	9:00 AM
77.	Pain I	Slide	Tu.	9:00 AM
112.	Pain II	Slide	Tu.	1:30 PM
174.	Pain III	Poster	W.	2:00 PM
237.	Pain IV	Slide	Th.	2:00 PM
286.	Pain V	Poster	F.	9:00 AM
203.	Retina: Intrinsic Organization	Slide	Th.	9:00 AM

95.	Retina and Retinofugal Pathways	Poster	Tu.	9:00 AM
11.	Functional Organization of Subcortical Visual Areas	Slide	M.	9:00 AM
151.	Subcortical Visual Pathways	Poster	W.	9:00 AM
59.	Visual Cortex: Functional Organization	Poster	M.	2:00 PM
118.	Vision I: Functional Organization of Striate Cortex	Slide	Tu.	1:30 PM
248.	Vision II: Cortex and Cortico-Subcortical Relationships	Poster	Th.	2:00 PM
268.	Vision III: Extrastriate Visual Areas	Slide	F.	9:00 AM
175.	Auditory and Vestibular Sense Organs	Poster	W.	2:00 PM
49.	Auditory and Vestibular Systems: The Sensory Periphery	Slide	M.	2:00 PM
20.	Subcortical Auditory Pathways	Poster	M.	9:00 AM
129.	Subcortical Auditory Pathways and Auditory Cortex	Poster	Tu.	1:30 PM
78.	Auditory System: Functional Organization	Slide	Tu.	9:00 AM
217.	Chemical Senses: Olfaction and Taste I	Poster	Th.	9:00 AM
235.	Chemical Senses: Olfaction and Taste II	Slide	Th.	2:00 PM
85.	Invertebrate Sensory Systems I	Slide	Tu.	9:00 AM
218.	Invertebrate Sensory Systems II	Poster	Th.	9:00 AM
283.	Modeling and Theoretical Studies	Poster	F.	9:00 AM
2.	The Dorsal Horn: A Profound and a Superficial Analysis	Symp.	M.	9:00 AM
146.	Thalamic Mechanisms of Pain Sensation	Wkshp.	W.	9:00 AM
169.	Parallel and Serial Processing in Vertebrate Visual Pathways	Symp.	W.	2:00 PM
196.	Representation of Space Within Sensory Systems	Symp.	Th.	9:00 AM

Theme G: Motor Systems

Session Number	Title	Type	Day	Time
9.	Motor Systems I: Cortex	Slide	M.	9:00 AM
181.	Motor Systems III: Cortex	Poster	W.	2:00 PM
26.	Cerebellum I	Poster	M.	9:00 AM
211.	Cerebellum II	Slide	Th.	10:45 AM
64.	Basal Ganglia I	Poster	M.	2:00 PM
252.	Basal Ganglia II	Poster	Th.	2:00 PM
274.	Basal Ganglia III	Slide	F.	9:00 AM
27.	Reticular System	Poster	M.	9:00 AM
16.	Vestibular System I	Slide	M.	10:45 AM
156.	Vestibular System II	Poster	W.	9:00 AM
100.	Oculomotor System I	Poster	Tu.	9:00 AM
204.	Oculomotor System II	Slide	Th.	9:00 AM
251.	Oculomotor System III	Poster	Th.	2:00 PM
44.	Visuomotor Integration	Slide	M.	2:00 PM
63.	Sensorimotor Integration	Poster	M.	2:00 PM
120.	Spinal Cord and Brainstem I	Slide	Tu.	1:30 PM
290.	Spinal Cord and Brainstem II	Poster	F.	9:00 AM
84.	Reflex Function I	Slide	Tu.	9:00 AM
180.	Reflex Function II	Poster	W.	2:00 PM
155.	Control of Posture and Movement I	Poster	W.	9:00 AM
223.	Control of Posture and Movement II	Poster	Th.	9:00 AM
240.	Control of Posture and Movement III	Slide	Th.	2:00 PM
133.	Muscle Afferents	Poster	Tu.	1:30 PM
222.	Muscle	Poster	Th.	9:00 AM
253.	Disorders of Motor Systems	Poster	Th.	2:00 PM
134.	Invertebrate Motor Function I	Poster	Tu.	1:30 PM
242.	Invertebrate Motor Function II	Slide	Th.	2:00 PM
99.	Neural Prostheses	Poster	Tu.	9:00 AM

Theme H: Neural Basis of Behavior

Session Number	Title	Type	Day	Time
81.	Higher Cortical Functions I: Evoked Potentials	Slide	Tu.	9:00 AM
171.	Higher Cortical Functions II	Poster	W.	2:00 PM

127.	Interhemispheric Relations	Poster	Tu.	1:30 PM
284.	Motivation	Poster	F.	9:00 AM
287.	Neuropsychology and Memory	Poster	F.	9:00 AM
216.	Attention and Arousal	Poster	Th.	9:00 AM
79.	Sleep I	Slide	Tu.	9:00 AM
285.	Sleep II	Poster	F.	9:00 AM
148.	Evoked Potentials and EEG	Poster	W.	9:00 AM
46.	Circuitry and Pattern Generation	Slide	M.	2:00 PM
18.	Biological Rhythms	Poster	M.	9:00 AM
277.	Circadian Rhythms	Slide	F.	9:00 AM
246.	Subcortical Structures	Poster	Th.	2:00 PM
244.	Toxic Effects on Behavior	Poster	Th.	2:00 PM
55.	Neuroethology I: Invertebrates	Poster	M.	2:00 PM
93.	Neuroethology II: Vertebrates	Poster	Tu.	9:00 AM
272.	Neuroethology III: Vertebrates	Slide	F.	9:00 AM
80.	Learning, Memory, and Habituation I: Lesion and Ablation Studies	Slide	Tu.	9:00 AM
119.	Learning, Memory, and Habituation II: Electrical and Pharmacological Studies	Slide	Tu.	1:30 PM
172.	Learning, Memory, and Habituation III: Pharmacological Studies	Poster	W.	2:00 PM
214.	Learning, Memory, and Habituation IV: Lesion and Ablation Studies	Poster	Th.	9:00 AM
245.	Learning, Memory, and Habituation V: Electrical Recording Studies	Poster	Th.	2:00 PM
212.	Invertebrate Learning and Behavior	Poster	Th.	9:00 AM
117.	Physiology of Invertebrate Behavior and Learning	Slide	Tu.	1:30 PM
91.	Angiotensin and Drinking I	Poster	Tu.	9:00 AM
210.	Angiotensin and Drinking II	Slide	Th.	10:45 AM
12.	Neural Mechanisms in Feeding and Drinking I	Slide	M.	9:00 AM
58.	Neural Mechanisms in Feeding and Drinking II	Poster	M.	2:00 PM
215.	Neural Mechanisms in Feeding and Drinking III	Poster	Th.	9:00 AM
128.	Neuropharmacology of Feeding and Drinking I	Poster	Tu.	1:30 PM
275.	Neuropharmacology of Feeding and Drinking II	Slide	F.	9:00 AM
282.	Stress, Hormones, and the Autonomic Nervous System	Poster	F.	9:00 AM
125.	Brain Metabolism: Effects of Environmental Events and Direct Insult	Poster	Tu.	1:30 PM
150.	Hippocampal Metabolism and Physiology	Poster	W.	9:00 AM
17.	Monoamines and Behavior I	Poster	M.	9:00 AM
53.	Monoamines and Behavior II: Stress and Aggression	Poster	M.	2:00 PM
113.	Monoamines and Behavior III	Slide	Tu.	1:30 PM
149.	Monoamines and Behavior IV	Poster	W.	9:00 AM
13.	Neuropeptides and Behavior I	Slide	M.	9:00 AM
57.	Neuropeptides and Behavior II: Opioid Peptides	Poster	M.	2:00 PM
126.	Neuropeptides and Behavior III	Poster	Tu.	1:30 PM
56.	Effects of Chronic Drug Administration I	Poster	M.	2:00 PM
92.	Effects of Chronic Drug Administration II: Amphetamine	Poster	Tu.	9:00 AM
19.	Opiates I	Poster	M.	9:00 AM
198.	Opiates II	Slide	Th.	9:00 AM
54.	Alcohol: Psychopharmacology	Poster	M.	2:00 PM
147.	Hallucinogens: Behavior and Physiology	Poster	W.	9:00 AM
202.	Neuropharmacology and Biochemistry of Phencyclidine	Slide	Th.	9:00 AM
213.	Psychotherapeutic Drugs I: Affective Disorders	Poster	Th.	9:00 AM
281.	Psychotherapeutic Drugs II	Poster	F.	9:00 AM
90.	Drugs of Abuse: Psychopharmacology	Poster	Tu.	9:00 AM
124.	Aging II	Poster	Tu.	1:30 PM
88.	Neuroanatomical Studies I	Slide	Tu.	10:45 AM
135.	Neuroanatomical Studies II	Poster	Tu.	1:30 PM
144.	Memory and Brain Mechanisms	Symp.	W.	9:00 AM
230.	Neural Mechanisms of Attention	Symp.	Th.	2:00 PM

2 SYMPOSIUM

THE DORSAL HORN: A PROFOUND AND A SUPERFICIAL ANALYSIS.
A.I. Basbaum, University of California, San Francisco (Chmn); A.R. Light, University of North Carolina, G.J. Bennett, NIH-NIDR; E.J. Glazer, University of California, San Francisco; M. Yamamoto-Yoshida, Tsukuba University, Japan.

With the advent of combined morphological and physiological methods for studying the circuitry of the dorsal horn, major advances have been made in our understanding of somatosensory, pain, and pain modulatory systems. This symposium will highlight the results derived from in vivo and from in vitro electrophysiological, morphological, and immunohistochemical studies.

Alan Light will describe the properties and central distribution of primary afferent fibers. His studies involve the intracellular injection of HRP into functionally characterized dorsal root axons, including small, myelinated A delta high threshold mechanoreceptors. An ultrastructural analysis of their central synaptic interactions will also be presented. Gary Bennett will extend these observations to second order neurons of the dorsal horn, including the smallest neurons of the substantia gelatinosa (sg). The application of the technique to the organization of the more ventral, nucleus proprius will also be presented. The pharmacological complexity of dorsal horn circuitry will be next addressed by Ellyn Glazer who has combined EM immunohistochemical and autoradiographic methods to dissect out synaptic interactions between amines, peptides, and other putative neurotransmitters. Finally, Miyuki Yamamoto-Yoshida will discuss the in vitro analysis of the dorsal horn. A variety of pharmacological, morphological, and physiological studies have been effectively combined in the in vivo preparation, providing important new insights into dorsal horn function. Taken together these discussions will provide a comprehensive review of the organization of the superficial and the "deep" dorsal horn.

3 WORKSHOP

LECTINS; CYTOCHEMICAL MARKERS FOR NERVOUS SYSTEM CARBOHYDRATES.
J.D. Coulter (Chairman; Univ. Texas Med. Br.), J.W. Gurd* (Univ. of Toronto), N.K. Gonatas* (Univ. of Penn), M.A. Ruda (NIDR, Nat. Inst. Health), J.H. LaVail (Univ. of Calif., San Francisco), K.H. Pfenninger (Columbia Univ.)

Lectins with selective affinities for mono- and oligo-saccharides provide a powerful tool to identify and localize carbohydrate-containing sites on neuronal membranes. Complex carbohydrates, particularly glycoproteins, are important constituents of the cell surface and have been implicated in the mechanisms of receptor binding, endocytosis, axoplasmic transport and cell-cell recognition. J.W. Gurd describes the application of lectins in the biochemical analysis of membrane and synaptic glycoproteins. Uses of labeled lectins and lectin affinity chromatography to identify and characterize lectin receptors associated with synaptic structures are reviewed. N.K. Gonatas describes studies in cultured neuroblastoma cells comparing the binding, endocytosis and subsequent subcellular localization of lectin-peroxidase conjugates, cholera toxin-peroxidase conjugate and free horseradish peroxidase. Axonal transport of these substances in peripheral nerves is also discussed. M.A. Ruda reviews studies using immunocytochemical methods to localize lectins transported anterogradely or retrogradely in sensory and motor nerves. Evidence that axonally transported lectin may migrate transneuronally into postsynaptic cells is described. J.H. LaVail reports on the use of affinity purified, [^{125}I] labeled wheat germ agglutinin to study axonal transport in chick retinal ganglion cells. Various issues including the specificity of uptake of the lectin, the rate of transport and the ultrastructural localization after transport are discussed. K.H. Pfenninger describes studies using lectins conjugated with the electron dense marker, ferritin, to map quantitatively, plasmalemmal glycoconjugates in developing neurons. How this approach can contribute to understanding cell-cell interactions and regional membrane differentiation in neural development is described.

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4.1 EMBRYONIC ORIGINS OF IDENTIFIED CELLS IN THE LEECH CNS BY TRACER INJECTION AND BY ABLATION OF IDENTIFIED BLASTOMERES. D.A. Weisblat, S.S. Blair* and A.P. Kramer. Dept. Mol. Biol. UCB, Berkeley, CA 94720.

Glossiphoniid leeches are well-suited for neurodevelopmental study because both the early embryo and the segmental ganglia of the adult contain identifiable cells, accessible to observation and experimental manipulation. The early embryo contains one bilateral pair of mesodermal precursors, (M teloblasts) and four bilateral pairs of ectodermal precursors (N,O,P and Q teloblasts). The teloblasts give rise to columns of much smaller stem cells which proliferate to form the segmental tissues of the animal, including the ventral nerve cord.

By injecting horseradish peroxidase (HRP) into identified teloblasts of embryos of the leech Helobdella triserialis, it has been shown that, in normal development, the N,O,P and Q teloblasts each contribute a stereotyped, topographically distinct neuronal subpopulation to the ipsilateral hemiganglia of the nerve cord. Taken with the stereotyped position of identified neurons within the adult ganglia of other leech species, these results suggest that, in normal development, identified neurons arise from specific teloblasts. Further evidence for this hypothesis will be presented from studies involving use of a fluorescent, rhodamine-conjugated synthetic peptide (RDP) as an in vivo cell lineage tracer, coupled with Lucifer Yellow injections of cells in the embryonic nervous system of a giant glossiphoniid leech species, Haementeria ghilianii.

Although the small size of the Helobdella embryo prevents electrophysiological identification of its embryonic neurons, a pair of morphologically identifiable neuropil glia found in each segmental gnalgion have been found to arise from the N teloblasts. Normally, one cell of the pair arises from each N teloblast. The ablation of one N teloblast by intracellular deoxyribonuclease (DNase) injection in early embryogenesis results in a later embryo with ganglia that appear to lack *one neuropil glia, even when progeny of the surviving N teloblast* have crossed the ventral midline and are distributed bilaterally within the ganglion.

4.2 CELL INTERACTIONS AND THE FORMATION OF NORMAL CELL PATTERNING IN THE EARLY EMBRYO OF THE LEECH. S.S. Blair*. Dept. Mol. Biol. UCB, Berkeley, CA 94720.

The embryo of the glossiphoniid leech Helobdella triserialis has been used for the study of cell lineage in the developing nervous system. At the outset of development a series of stereotyped cleavages produce three macromeres (A,B and C), four bilateral pairs of ectoteloblasts (N,O,P and Q) and one bilateral pair of mesoteloblasts (M). The teloblasts give rise to bandlets of stem cells which merge to form the two germinal bands; these two germinal bands eventually coalesce along the ventral midline of the embryo to form the germinal plate, from which arise segmental structures, such as the ventral nerve cord and its segmental ganglia. Through injection of tracer dyes into identified teloblasts or teloblast precursors, it has been shown that the progeny of each teloblast contribute in a characteristic manner to specific structures within the ipsilateral hemiplate. Thus, the progeny of the N teloblast are found mainly in the ventral nerve cord, where they form a spatially stereotyped fraction of the neurons of each ipsilateral hemiganglion. To determine the extent to which formation of this developmental pattern is dependent upon interactions with other cell types, a series of experiments were carried out in which an N teloblast was filled with a fluorescent tracer (rhodamine peptide) and other teloblasts or teloblast precursors were ablated through the intracellular injection of DNase I.

Preliminary results indicate that the ablation of the ipsilateral OPQ or OP teloblast precursors has no visible effect upon the distribution of N teloblast progeny. If instead the ipsilateral M teloblast is ablated, the N teloblast progeny are profoundly disorganized, failing to form ipsilateral hemiganglia or other segmental structures; a portion of the N teloblast progeny may even cross the midline from the mesoderm-deficient side of the germinal plate, apparently becoming incorporated there into the contralateral hemiganglia. As reported earlier, the ablation of the contralateral N teloblast may also cause ipsilateral N teloblast progeny to cross the midline of the germinal plate. *Thus, N teloblast progeny appear to interact with contralateral* N teloblast progeny and with the underlying mesodermal substrate. Interactions with the adjacent O, P or Q ectoteloblasts appear to be less important for normal patterning of N teloblast progeny in the genesis of the segmental ganglion.

4.3 MONOAMINE NEURONS IN EMBRYONIC AND ADULT LEECH. Duncan K. Stuart, Department of Molecular Biology, University of California, Berkeley, CA 94720.

Serotinergic and dopaminergic neurons have been studied in the giant leech, Haementeria ghilianii. In the segmental ganglia apparent homologs of all the previously described serotinergic neurons of Hirudo are present: the giant Retzius pair, the two lateral pairs, the unpaired, posterior medial cell, and the anterior medial pair located only in the first three segmental ganglia. These neurons all stain with neutral red and with the glyoxylic acid fluorescence technique of Torre & Surgeon (Histochem. 49, 1976). However, the unpaired, posterior medial cell is found only in the first seven segmental ganglia of Haementeria whereas it is present in all the segmental ganglia of Hirudo.

In Haementeria two pairs of presumed dopaminergic neurons have been found in each segment, the larger pair in the medial branch of the anterior segmental nerves and the smaller, more peripheral pair in the anterior branch of the anterior nerves. Each dopaminergic neuron projects a major axon centrally which ends in extensive varicose endings in the neuropile of its own and adjacent segmental ganglia. In Hirudo there exists only one corresponding dopaminergic pair.

In Haementeria embryos the serotinergic and dopaminergic neurons begin to contain enough serotonin and dopamine to stain them with the glyoxylic acid method one day after hatching. At that time the larger of the dopamine neurons already sends its major axon into the ganglion, where it branches and sends processes into the adjacent anterior and posterior segments. By this time the isolated nervous system can also be shown to be capable of synthesizing serotonin and dopamine from their radiolabeled precursors. Over the next 7 days the dopaminergic endings in the neuropile increase dramatically, as does the distance between the dopaminergic cell body and its target ganglion. The diameter of the Retzius cell bodies increase relative to other somata.

For the present purpose, the glyoxylic acid procedure was modified for staining of unsectioned material. The preparation, consisting of a dissected leech nerve cord and attached body wall, was stretched and pinned out above the surface of a Sylgard-coated dish filled with ice cold saline and stained for 1-2 min., followed by drying with cold air from a hair dryer for 30 min., and heating in a 95°C oven for 3-5 min. This procedure clears the connective tissue and permits excellent visualization of the serotinergic (yellow) and dopaminergic (blue) cell bodies, axons, and endings.

4.4 SPECIFIC KILLING OF MONOAMINERGIC NEURONS IN THE LEECH EMBRYO BY CYTOTOXIC NEUROTRANSMITTER ANALOGS. J. Glover* and A. Kramer. Graduate Group in Neurobiology, UC Berkeley, Berkeley, CA. 94720

The segmental ganglia of the leech Haementeria ghilianii contain a small number of putative serotonergic neurons. These neurons were found to be susceptible to the cytotoxic effects of the serotonin analogs 5,6 - and 5,7 - dihydroxytryptamine.

Late stage leech embryos, already containing a morphologically intact nerve cord, were injected with 3-4 µl of solutions of the toxic analogs in millimolar concentrations. Within 8 hours, the serotonergic neurons had acquired an intense abnormal brown pigmentation. By the fourth day after injection, the serotonergic neurons showed morphological and physiological signs of cell death, with marked shrinkage of cellular diameter. The cytoplasm had a granular appearance, often with accumulations of large vesicles, and the nucleus was not evident. Often the plasma membrane was clearly absent, and the cell persisted only as a cluster of vesicles or dispersed cellular debris. Cells with intact membranes, when penetrated with a microelectrode, exhibited little or no resting potential and no sign of active membrane properties. Injection of the cell body remnants with lucifer yellow revealed an absence of neurites. These cytotoxic effects were evidently specific since other, non-serotonergic, including putative dopaminergic, neurons appeared morphologically and physiologically normal.

Injection of similar concentrations of toxic analogs into adult leeches produced an apparent disruption of serotonergic terminals. The large serotonergic Retzius cells exhibited significant decreases in resting potential, spike amplitude and electrical coupling. The analogs also appeared to interfere with the production and coordination of swimming movements, a behavior known to be modulated by serotonin in leeches. This effect could be transiently overcome by injection of serotonin.

4.5 DEVELOPMENT OF IDENTIFIED NEURONS IN THE LEECH CNS, J.Y. Kuwada and W.B. Kristan, Jr. Dept. of Biol., UCSD, La Jolla, CA. 92093.

We are investigating the development of a simple, shortening reflex in the leech Haementaria ghilianii. As a first step the central mechanosensitive pressure or P cells, which innervate the skin and provide strong input for the reflex, are being studied.

Eleven days after eggs are laid the chain of ganglia which make up the leech CNS is distinguishable and the P cells have a growth cone directed towards the midline. Later this central process branches sending axons into both anterior and posterior connectives. Simultaneously, many peripherally directed processes appear from the soma. A day later one main peripheral axon, which will set up the cell's major receptive field, is evident. The "extra" smaller processes will subsequently disappear. P cells attain their basic adult morphologies (3 wks) when, in order, peripheral axons grow out roots of adjacent ganglia to set up minor receptive fields in adjacent segments, central neuropilar processes appear, and secondary peripheral axons grow out the ganglion containing their somata.

During the first 2-3 days of process growth, the central process is covered with lateral filopodia which later disappear followed by the growth of neuropilar processes. Thus neuropilar processes do not arise by the selective retention and enlargement of some filopodia. During this time the anterior processes develop faster than the posterior ones and P cells go from displaying passive membrane properties to delayed rectification to noninvading axon spikes. Subsequently, overshooting action potentials can be recorded. Spontaneous synaptic potentials become evident at the time of neuropilar growth.

The initial, primary peripheral axon, like the early central ones, is covered with filopodia as it grows out but the axons which grow out later exhibit much fewer filopodia. This may reflect the fact that the initial axon grows out when roots have not yet formed while the later axons follow roots.

There are 2 P cells per hemiganglion--one mainly innervates the ventral skin and the other the dorsal skin. In the first 3 weeks of development, little if any overlap has been observed between the major and minor fields of a P cell, the major fields of the ventral and dorsal P cells, or the major fields of P cells from adjacent ganglia. These findings plus those of A. Kramer on older leeches (personal comm.) indicate that P cell receptive fields do not arise from an initial overgrowth followed by contraction of peripheral fields.

Presently being investigated are factors influencing the loss of early "extra" peripheral processes and growth of the main peripheral axon, development of an interneuron and motor neuron involved in the reflex, and formation of reflex synapses.

4.6 AN INSECT INTERNEURON DEPRIVED OF SENSORY INNERVATION DURING ITS EMBRYOGENESIS DOES NOT FORM THE NORMAL COMPLEMENT OF DENDRITIC BRANCHES. Marty Shankland, Corey S. Goodman, & David Bentley. Dept. of Zoology, University of California, Berkeley, Ca. 94720 & Dept. of Biol. Sci., Stanford University, Stanford, Ca. 94305.

The Medial Giant Interneuron (MGI) of the grasshopper receives its primary input from the peripheral sensory neurons of the cercus. This synaptic connection is established during embryogenesis, and we have examined the way in which the initial contacts are made between these cells. Cobalt fills of the embryonic cercal nerve have shown that the first sensory axons enter the CNS at 60/65% of embryonic life, and intracellular Lucifer fills of the differentiating MGI demonstrate that its dendrite has very few subsidiary branches at this time. Numerous growth cones begin to spring forth from the main trunk of the dendrite after the sensory axons arrive, and these branches radiate outward until they come into contact with the sensory axon terminals. The dendritic branches do not grow beyond this point. Thus, the coordinate morphogenesis of the cercal sensory axons and the MGI dendrites suggests that developmental interactions between these cells could be responsible for the formation of dendritic growth cones, and the orientation and cessation of their growth.

We addressed this question experimentally by unilaterally ablating the cercus at 60% of embryonic life, i.e. before the first sensory axons reach the CNS. The operated embryos were cultured in a defined medium for 3-9 days and, judging from external criteria, underwent relatively normal development up to 90%. Cobalt fills showed that the cercal sensory axons on the unoperated side of the embryo grow in their normal fashion without forming abnormal crossing projections. This means that the MGI dendrites on the operated side are in fact deprived of contact with cercal sensory axons during the entire course of embryogenesis. Lucifer and cobalt fills of the MGIs on the two sides of the ganglion revealed that dendrites on the deprived side have many fewer branches and smaller branches than dendrites on the undeprived side. Despite this reduction in number, the branches which do form exhibit the appropriate orientation and do not project beyond the point where they would normally have contacted the missing afferents. These findings demonstrate that sensory axon contacts are necessary for the MGI to form and/or retain its normal complement of dendrites. It also appears that these particular sensory axons are not necessary guidance cues for dendritic growth cones to display the usual pattern of growth once they are formed, but it is not known to what extent guidance depends upon other remaining inputs.

4.7 ABNORMAL NEURAL DEVELOPMENT IN DROSOPHILA EMBRYOS CARRYING MUTATIONS AFFECTING SEGMENTAL IDENTITY OR SEGMENT NUMBERS. Lily Yeh Jan* and Yuh Nung Jan. Dept. of Physiology, Univ. of California, San Francisco, CA 94143.

We found that antibodies to horseradish peroxidase (HRP) bind to neuronal membranes and serve as a general neuronal marker in Drosophila. This immunohistochemical staining marks sensory structures, neuromuscular junctions, nerves and fiber tracks in the central nervous system of embryos, larvae, and adult flies. Preadsorption of these antibodies with HRP eliminated all staining. The same pattern of nerve fibers as revealed by fluorescent antibodies to HRP was also marked by brown HRP reaction products when sections were treated sequentially with antibodies to HRP, HRP, diaminobenzidine and hydrogen peroxide. Using an electron microscope, we found that the HRP reaction products were on neuronal membranes.

With this staining, one sees in the embryonic central nervous system two bundles of fibers running in parallel along the entire length of the ventral ganglion and, within each segment, two major commisures connecting the two bundles of longitudinal fibers. Embryos carrying various mutations, deficiencies or duplications have been examined using this staining method. Evidence will be presented to demonstrate that the bithorax gene complex (E.B. Lewis, Nature 276, 565-570, 1978) affects the embryonic nervous system. Also, mutations affecting segment number (C. Nüsslein-Volhard and E. Wieschaus, Nature 287, 795-801, 1980), such as paired, even-skipped, runt, Krüppel and knirps, have been found to alter segment number and organization of the nervous system in embryos.

4.8 DEVELOPMENT OF SYNAPTIC TRANSMISSION IN THE ANTENNAL LOBES OF MANDUCA SEXTA DURING METAMORPHOSIS. Leslie P. Tolbert, Steven G. Matsumoto, and John G. Hildebrand, Dept. of Neurobiology, Harvard Medical School, Boston, MA and Dept. Biology, Columbia Univ., NY, NY.

During the 18 days of metamorphic adult development, sensory neurons in the antenna are born and send their axons to contact developing second-order olfactory neurons in the antennal lobe (AL) of the brain. The axons reach the brain on about day 3 of adult development, when the AL is small and has a light-microscopically homogeneous neuropil. By day 5 glial cells begin to separate peripheral areas of the neuropil into distinct spheroidal glomeruli, and by day 6 the AL looks histologically mature except for its small size.

At day 6, the earliest day at which we have been able to obtain adequate ultrastructural preservation, a few synapses have already begun to form in the newly defined glomeruli. In cross-section, these synapses resemble mature synapses except that they have many fewer synaptic vesicles. In addition to synapses there are numerous desmosome-like contacts, both between glomerular processes and between neurites in extraglomerular (nonsynaptic) regions of the neuropil. In the glomeruli, the desmosome-like contacts often form at the points where three processes meet; these junctions resemble synapses without synaptic vesicles. While the total packing density of ultrastructural junctions in the glomeruli remains fairly constant through the rest of adult development, the percentage of these that are synapses increases until day 12, when it reaches the adult value. Our observations do not reveal whether the early desmosome-like contacts become synapses or whether they simply perform, for instance, an adhesive role during synaptogenesis.

Although synapses can be detected by electron microscopy as early as day 6, intracellular recordings from AL neurons do not detect synaptic transmission from the antennal nerve until day 9, when the number of synapses per unit area in the AL also dramatically increases. The early synapses observed in the electron microscope could be extrinsic (i.e. from antennal axons) or intrinsic (i.e. between AL neurons) in origin. We are currently determining the time course of the maturation of the antennal-axon synapses by labelling this population with horseradish peroxidase. This should reveal whether our ability to detect synaptic transmission between antennal axons and AL neurons correlates with a maturation of the synapses observable before day 9 or with the onset of ultrastructural synapse formation by the antennal axons.
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4.9 DEVELOPMENT OF THE GIANT FIBER SYSTEM IN CRICKETS. G. Jacobs and R.K. Murphey. SUNY Albany, Albany, N.Y. 12222.

Orthopteran insects have a group of giant interneurons located in the abdominal nervous system which have been implicated in escape responses. We are interested in the development of two of these neurons in crickets, the medial giant interneuron and lateral giant interneuron (MGI, LGI). We wanted to determine their segment of origin and whether they were segmentally homologous. We approached this problem by using axonal iontophoresis of cobalt from the mesothoracic ganglion to the terminal ganglion in cricket embryos at different developmental stages (staging is based on Bentley et al., 1980). Neurons in the terminal ganglion whose axons run anteriorly are selectively stained with this method. At 50% of embryonic development the abdominal nervous system consists of 11 primitive segments, the four most posterior ones (7-11) will fuse to form the adult terminal ganglion. The neurons which stain at this stage form bilaterally symmetric clusters whose axons cross in characteristic positions in the anterior and posterior commissures in each of the abdominal segments. (A cluster is defined as a group of neurons whose somata are grouped and whose axons cross in the same position in the commissure.) We have limited the analysis to neurons in the anterior commissure. There are three clusters of neurons with axons in this commissure: cluster 1 is composed of two to four cells with axons in the anterior portion of the commissure, cluster 2 contains four to six neurons whose axons occupy the medial region of the commissure and cluster 3 is limited to a distinctive pair of neurons whose axons are closely apposed just distal to their cell bodies and occupy the most posterior portion of this commissure. At this stage (50%) the neurons are indistinguishable from each other. Between 50% and 70% the abdominal segments undergo fusion to form the terminal ganglion. The clusters are still visible at 70% and certain neurons within each cluster have begun to enlarge with respect to their neighbors. By 90% both MGI and LGI are much larger than any other neurons and by examining successively younger stages we could determine which cluster they were derived from. MGI and LGI come from cluster 2 in the eighth and ninth abdominal segments respectively. If these clusters correspond to the progeny of homologous neuroblasts then it is conceivable that they are serial homologues. In order to confirm this it will be necessary to determine that they come from the same cell division of the same neuroblast in their respective segments. Future experiments will address this question as well as comparisons with other orthopteran insects to discover whether the giant interneurons in those insects have similar lineages to those in crickets.

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4.10 CRICKET CERCAL FILIFORM HAIRS HAVE A SOMATOTOPIC PROJECTION IN THE TERMINAL GANGLION. J.P. Bacon and R.K. Murphey. SUNY Albany, Albany, New York 12222.

The cricket has two kinds of mechanosensitive hairs on its cercus, wind-sensitive filiform hairs which cover the entire cercus and gravity-sensitivity clavate hairs, found only proximally and medially. Cobalt-filling the cercal nerve reveals that most sensory fibres terminate ipsilaterally in the terminal ganglion to form the hollow, club-shaped cercal glomerulus. Identifiable clavate hair afferents project somatotopically into this glomerulus (Murphey et al., 1980). This means that adjacent points on the cercal surface project to adjacent positions in the cercal glomerulus: the cercal surface is represented as a continuum in neuropilar space.

But what of filiform hair afferents, do they also exhibit somatotopy? Their large numbers and the fact that they are not arranged in obvious rows had hitherto hindered their investigation. However, by working with animals from the first to the sixth instar, when there are far fewer hairs, we have been able to demonstrate that each filiform hair is uniquely identifiable, both in terms of its position on the cercus and the position of its afferent arborization within the cercal glomerulus. Filiform hairs on the proximo-medial cercal surface (that region covered by clavate hairs) project to the same regions of neuropile as their clavate neighbors. This supports the notion that the somatotopic maps of the two types of mechanosensitive hairs, concerned with the processing of different sensory modalities, are isomorphic. Clavate hairs occur on only the medial 150° of the cercal surface whereas filiform hairs are equally distributed around the circumference. By cobalt-filling filiform hairs at all circumferential positions, we found that one circumnavigation of the cercus is represented as two trips around the cercal glomerular shell. In other words, opposite sides of the cercus project to approximately the same region of the glomerulus: a clear example of this general principle being provided by medial and lateral hairs both of which project medially in the glomerulus.

This anatomical description provides new insights into the cercal to giant interneurone system. Some of the large interneurons have already been identified. By studying their anatomy we should be able to predict which filiform afferents will or will not connect directly with a given interneuron and thereby explain its response properties. We intend to test this idea by recording simultaneously from identified sensory neurons and the giant neurons. Such an analysis should demonstrate the functional significance of the filiform hair afferent somatotopic organization. Supported by NSF Grant BNS 78 24039 to RKM.

4.11 SYNAPTIC INTERACTIONS BETWEEN THE CLAVATE SENSORY NEURONS OF CRICKETS AND AN IDENTIFIED INTERNEURON. Donald S. Sakaguchi* and R.K. Murphey. Dept. of Biol. Sci., SUNY Albany, Albany, N.Y. 12222.

Club-shaped sensilla, referred to as clavate hairs, have previously been shown to provide the cricket (Acheta domesticus) with equilibrium information regarding its orientation in the gravity field. Each clavate sensillum is innervated by a single sensory neuron which projects to the cercal glomerulus of the last abdominal ganglion (Murphey, Neurosciences Abstract #841, 1979). These sensory neurons form a somatotopic projection in the cercal glomerulus. A major goal of this study was to correlate the dendritic distribution of the identified positional interneuron with the afferent patterning from the clavate sensory receptors. A bilaterally symmetric pair of interneurons in the terminal ganglion was identified and shown to receive their primary afferent input from the clavate sensory cells. We impaled and filled these interneurons with cobalt-chloride. The soma is located ventrally and found at the anterior lateral margin of the terminal ganglion contralateral to its axon and main dendritic processes. The anteriorly projecting axon has been filled to the meso-thoracic ganglion, where it gives off a single collateral within each ganglion. The major dendrites outline the anterior boundaries of the cercal glomerulus and serial reconstructions in transverse section show that the dendritic branches occur in regions of the neuropil corresponding to areas where the clavate afferents terminate.

Physiological characterization of the interneuron was carried out by intracellular recording from the soma. The interneuron response properties were recorded as the cerci were displaced with respect to the animal's body. When the cerci were held in the normal horizontal position there was a low level of baseline spike activity. When the cerci were deflected upwards from this position (analogous to pitch forward) the interneuron was transiently hyperpolarized and no activity was observed invading the soma. With downward deflection of the cerci (analogous to pitch back) a small, tonic depolarization was observed with an increase in frequency of passively invading spikes superimposed on the excitatory postsynaptic potential. During this movement an initial transient dynamic component was observed which decreases to a tonic level of excitation above the baseline level. We are currently examining the connectivity diagram further by selectively deleting various hairs in an attempt to correlate the now well-described afferent projection with the structure and response properties of the postsynaptic neuron. Supported by NIH grant NS 15571 to RKM.

4.12 COMPETITIVE INTERACTIONS AND THE FORMATION OF A SOMATOTOPIC MAP IN AN INSECT. W.W. Walthall and R.K. Murphey. Dept. of Biol. Sci., SUNY Albany, Albany, N.Y. 12222.

Clavate hairs exist in rows which run approximately parallel to the longitudinal axis of the cercus. Each hair is innervated by a single peripheral sensory neuron. All of the axons from cell bodies within a row project to the same general target area in the CNS. Within this target region there exists a gradation in arbor size: the older more distal neurons have arbors which extend into more anterior regions of the target, while younger, more proximal neurons have arbors which are restricted to more posterior areas. Thus, the central projection of a clavate neuron reflects its cell body's position along the proximo-distal axis of the cercus. We have asked whether competitive interactions between neighboring neurons play a role in the construction of this map? To answer this we forced the regeneration of proximal neurons in the absence of their more distal neighbors. Our experimental paradigm was to cut off all of the cercus just distal to the clavate array and then make a notch deletion removing the five most distal clavate hairs in a row. At the same time the cercal nerve was cut at the junction between the cercus and the abdomen, thus forcing axonal regeneration from this point. The result is that proximal neurons regenerate arbors into more anterior regions of the target. They do not normally project to this area, presumably because they are excluded by the presence of their more distal neighbors. Three types of controls were carried out: nerve cut only, to see if regeneration is orderly; notch deletion without nerve cut to see if regeneration is necessary for expansion; and punctate deletions of clavate hairs leaving more distal components intact, to see if smaller deletions provide the necessary impetus for expansion. In these controls no expansion of the arbors was observed. These results demonstrate that expansion of insect sensory neurons into foreign regions of neuropil can occur, but only when the axon is forced to regenerate into a partially deafferented target. We suspect that this expansion is functionally significant, increasing the synaptic efficacy of proximal neurons and intend to test this physiologically.

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5.1 SYNAPSE FORMATION BY REGENERATING PREGANGLIONIC SYMPATHETIC AXONS IN NEONATAL RATS. A. J. Smolen, L. Wright and T. Lindley*
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We have reported that section of the cervical sympathetic trunk (CST) in rats on postnatal day 3 results in some regeneration of the cut axons which form synapses in the superior cervical sympathetic ganglion (SCG). Since there was considerable variability in the numbers of synapses in reinnervated ganglia, we hypothesized that there may be a variable amount of regeneration of cut axons which is responsible for this synaptic variability. The present series of experiments was designed to test this hypothesis.

The left CST was cut approximately 1-2mm caudal to the SCG in neonatal rats on either day 0 (the day of birth) or day 3. The proximal and distal stumps were replaced in a position medial to the common carotid artery, but no special effort was made to approximate the cut ends. After a survival of three months (previously shown to be sufficient for regeneration and synapse formation) the left SCG and its CST were removed and processed for electron microscopical observations. Counts were made of total numbers of axons in the CST and synapses in the SCG.

As reported previously, sectioning the CST on postnatal day 3 resulted in the establishment of approximately one-half the normal number of ganglion synapses (2.5×10^6 in operated animals vs. 4.4×10^6 in controls). Counts of axons revealed that about one-half of the normal number of axons were present in the CST (2600 in operated animals vs. 5900 in controls). Sectioning the CST on the day of birth resulted in the establishment of fewer synapses in the SCG (mean of 1.1×10^6). In this group of animals, the number of axons found in the CST was also greatly reduced (to a mean of 800). Within each age group, the number of ganglionic synapses was correlated with the numbers of axons in the CST.

We have shown that sectioning the CST in neonatal animals results in incomplete regeneration of the preganglionic axons which were cut. The later that the nerve is cut, the less severe is the loss of preganglionic axons. This suggests that the preganglionic neurons may be more susceptible to axotomy-induced direct retrograde cell death, and this susceptibility diminishes during maturation. Thus, the number of ganglionic synapses formed appears to be determined by the number of axons which survive to regenerate into the SCG and not by the number of available target postganglionic neurons.
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5.2 EFFECTS OF NEONATAL TREATMENT WITH TESTOSTERONE ON MATURATION OF PRE- AND POSTGANGLIONIC SYMPATHETIC NEURONS. Linda L. Wright, and Arnold J. Smolen. Department of Anatomy, The Medical College of Pennsylvania, Philadelphia, PA. 19129

Evidence from studies of reinnervation of the superior cervical ganglion (SCG) following section of the preganglionic nerve suggests that there may be a limit to the number of synapses formed by each preganglionic axon (e.g.Ostberg et al. '76). In these studies in the adult rat a loss of preganglionic axons is correlated with a reduction of synapses in the ganglion, and the presence of unoccupied postsynaptic densities. Thus, the reduced numbers of preganglionic axons cannot expand to reinnervate all of the potential postsynaptic sites in the ganglion. Synaptic recovery is also incomplete after neonatal preganglionic section (Smolen, '81). To the extent that reinnervation mimics normal development, these results suggest that the number of preganglionic axons, not the number of ganglion neurons is the limiting factor in the number of synapses formed in the ganglion (see Smolen et al., this volume). We have tested this hypothesis during normal development by determining whether the numbers of synapses are altered when the numbers of ganglion neurons is increased. Testosterone propionate (TP) has been shown to increase neuron numbers in the rat SCG (Dibner and Black,'78), presumably by increasing endogenous levels of Nerve Growth Factor (NGF) and thereby preventing normal cell death. TP (80mg/kg) or vehicle was administered on alternate days from birth to the time of sacrifice on day 15. Neuron and synapse counts revealed that at 15 days, when 85-90% of the adult numbers of synapses have been formed (Smolen and Raisman,'80) the number of synapses in the TP treated ganglia do not differ from controls, even though the number of ganglion neurons is increased by 30%. This result supports the hypothesis that preganglionic axons have a limited synaptic capacity even during the early postnatal period when synaptogenesis normally occurs. The increased numbers of neurons and normal numbers of synapses suggests that either each ganglion cell receives fewer synapses, or that the neurons prevented from dying by the TP treatment are defective in some way and do not receive synaptic input.
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5.3 SELECTIVE CHANGES IN BRAIN SYNAPSE NUMBER ESTIMATED WITH ANTIBODIES TO A POSTSYNAPTIC DENSITY COMPONENT. M.Nieto Sampedro*, C.M. Bussineau* and C.W.Cotman. Dept. Psychobiol., Univ. Calif., Irvine, CA 92717.

Antibodies to molecules present only in the synaptic region of the neuron should be extremely valuable tools in the study of developmental and reactive synaptogenesis. Brain postsynaptic densities (PSDs) contain at least one such antigenic junctional marker, a protein of mol.wt. 95000 (antigen PSD-95). Antibodies raised in rabbits against highly purified PSDs react predominantly (85-90 %) with PSD-95 in SDS-gel electrophoregrams of membrane fractions from rodent brain. By electron microscopy, immunoreactivity is restricted to the PSD, whereas by light microscopy, the three-layer peroxidase-antiperoxidase method (PAP) shows specific staining only in those areas known to contain synapses. Antigen PSD-95 is highly conserved during evolution as it is present in synaptic junctions from the brain of all vertebrate species examined from elasmobranchs to mammals.

Antibodies to PSD-95 can be used in the quantitative estimate of loss and recovery of PSDs during reactive synaptogenesis. This was performed either by microdensitometry of brain slices after immunostaining by the PAP method of by determination of radioactivity in select zones of brain slices after treatment with anti-PSD antibody followed by [^{125}I]protein A. Three days after unilateral ablation of the rat entorhinal cortex, 35-50% of the immunoreactivity was lost in the outer molecular layer of the dentate gyrus ipsilateral to the lesion whereas the inner molecular layer was not affected. Lost immunoreactivity was recovered beginning 10 days post-lesion, in agreement with quantitative electron microscopic studies (Matthews et al., Brain Res.115:121, 1976). Fimbria transection, on the other hand, did not reduce the anti-PSD immunoreactivity in the lateral septal nucleus in agreement with the loss of synapses but conservation of PSDs reported by Raisman and Field (Brain Res. 50:241-264,1973).

These results indicate that antibodies to specific synaptic markers can be used in the rapid quantitative study of changes in synapse number following experimental lesions. Similar determinations should be possible during developmental synaptogenesis, degenerative changes during aging or as a consequence of illness and, perhaps, as a correlate of some types of learning and experience. Supported by grants NS08957 from NIH and AG00538 from NIA.

5.4 Evidence For the Innervation of the Rat Pineal Gland During Late Fetal Development. Anthony Altar*, Tom Motroni*, and Loy D. Lytle (SPON: Harry J. Carlisle). Laboratory of Psychopharmacology, University of California, Santa Barbara, CA 93106.

Previous findings (Altar et al, Fed. Proc. 40: 250, 1981; Yuwiler et al, Am. J. Physiol. 233: 141, 1977) have shown that post-synaptic beta-noradrenergic receptors controlling pineal gland n-acetyltransferase activity (NAT) appear relatively early in development and before the gland has received complete innervation by sympathetic nerve terminals. It has yet to be shown, however, at what age these nascent receptors first become innervated by noradrenergic sympathetic fibers. Catecholamine histofluorescence of the rat pineal gland on the day of birth reveal sympathetic nerves which penetrate the pineal capsule but mainly appear to reside on the surface of the gland (Hakanson et al, Life Sci., 6: 2577, 1967). The present study attempted to determine the age at which pineal gland presynaptic nerve terminals first make functional connections with beta-noradrenergic receptors by incubating pineal glands from rats of various ages with d-amphetamine or l-isoproterenol, sympathomimmetic amines which depend upon pre- or post-synaptic components, respectively, to increase pineal NAT enzyme activity.

Pineal glands from fetuses obtained 19 or 20 days post-conception or from rats 0, 2, 10, or 50 days of age were placed into in vitro organ chambers. Glands were stimulated with either the vehicle or with 10^{-5} M (prenatal pineals) or 10^{-6} M (postnatal pineals) d-amphetamine or l-isoproterenol for 4 hr. Following incubation, pineal glands were assayed for NAT activity according to the procedure of Deguchi and Axelrod (Anal. Biochem. 50: 174, 1972). NAT activity was expressed as nmoles of end product, n-acetyltryptamine, formed per pineal gland per hr.

Daytime NAT activity was relatively low late in gestation, increased after birth to attain a maxima by ten days of age, and declined to a low baseline by 50 days of age. L-isoproterenol markedly increased NAT as soon as 19 days post-conception and, with increasing age, produced successively larger NAT increases. In contrast, relatively high concentrations of d-amphetamine failed to increase pineal NAT 19 or 20 days post-conception. By the day of birth and thereafter, however, NAT was markedly elevated by d-amphetamine.

These results show that the post-synaptic beta-receptor agonist l-isoproterenol stimulates pineal NAT at least 2 days before the presynaptically acting drug, d-amphetamine, is capable of increasing NAT. Thus, functional innervation of the pineal gland by sympathetic, noradrenergic nerve terminals appears to occur during the last 2 days of gestation and after the functional development of post-synaptic beta-noradrenergic receptors. Supported by NIMH grant MH-31134 to LDL and by NIDA predoctoral fellowship award DA-05136 to AA.

5.5 INITIAL SYNAPTIC TRANSMISSION AT THE GROWTH CONE IN XENOPUS NERVE-MUSCLE CULTURES. Y. Kidokoro and E. Yeh.* The Salk Institute, P.O. Box 85800, San Diego, Ca. 92138.

Early events in the formation of the neuromuscular junction have been studied in cell cultures where component cells are clearly visible under a light microscope. Synaptic transmission occurs shortly after nerve-muscle contact, even prior to acetylcholine receptor accumulation to the nerve contact area. We have examined initial synaptic transmission at the growth cone-muscle contact in Xenopus nerve-muscle cultures. The approaching growth cone was observed while the membrane potential of its target muscle cell was continuously monitored. Nerve-evoked synaptic potentials were measured shortly after a growth cone contacted the muscle membrane. The interval between the initial contact of growth cone with muscle membrane and the onset of synaptic transmission was estimated as approximately 8 minutes. Fluctuation of the endplate potential amplitude was analysed by using binomial statistics. It seems that transmitter release at the growth cone occurred in a quantal fashion.

5.6 SYNAPTOGENESIS IN THE DENTATE GYRUS OF PRIMATES: QUANTITATIVE EM STUDY IN RHESUS MONKEY. M.F. Eckenhoff and P. Rakic. Sect. of Neuroanatomy, Yale Univ. Sch. of Med., New Haven, CT. 06510.

The dentate gyrus of 13 rhesus monkeys of embryonic (E) days E71, E73, E75, E78, E91, E110, E130, E149, birth (E165) and postnatal (P) days P34, P64, 10 months and adult were prefused by mixed aldehyde fixative and processed for electron microscopy. Quantitative analyses were conducted using 4-8 EM probes/animal. Each probe consisted of 10-25 consecutive overlapping electron micrographs taken across the full width of the molecular layer of the suprapyramidal (SPL) and infrapyramidal (IPL) limbs. The number of synapses/μ^2 within the inner, middle and outer third of the molecular layer and the density of asymmetrical (ASS) and symmetrical synapses (SS) were determined for each age.

No synapses are present at E71. An occasional synapse can be detected at E73 and E75 but their distribution was quantified only by E78 when their density became measureable. In the IPL, the density of synapses increases rapidly during the second half of gestation and reaches a peak of $0.132/\mu^2$ at birth (66X the number at E78). Although the SPL at E78 contains significantly greater numbers of synapses than the IPL, its peak of synaptic density is also achieved by birth ($0.117/\mu^2$ or 26X the number at E78). The synaptic density levels off after birth; we found no significant density differences between the newborn and adult in either limb although the total number of synapses increases after birth since the hippocampal area continues to grow.

Synaptic densities are similar throughout the inner, middle and outer thirds of the molecular layer of the IPL and SPL although each third receives different inputs. Furthermore, both limbs showed no significant differences in the number of ASS and SS between E78 and E110. However, in the IPL the density of ASS increases 84X from E78 ($0.0015/\mu^2$) to birth and thereafter remains constant, whereas the number of SS increases more gradually (24X) from E78 to E149 ($0.012/\mu^2$) and then decreases to relatively low levels in the adult ($0.002/\mu^2$). In the SPL, the density of ASS increases 40X from E78 ($0.0027/\mu^2$) to birth without significant change thereafter. The number of SS increases more gradually (7X) from E78 to E149 ($0.013/\mu^2$) and then decreases to low levels in the adult ($0.002/\mu^2$).

In summary: (a) Synaptogenesis in the primate hippocampus occurs at considerably earlier embryonic ages than in rodents. (b) There is a biphasic progression of synaptogenesis with a rapid increase from E78 to birth, after which the density levels off. (c) ASS and SS terminals have distinct tempos of development. (d) At early stages synaptogenesis is more robust in the SPL than in the IPL. (e) Time of origin of neurons that form afferents to the molecular layer do not correspond to the sequence of synapse formation. (Supported by NS14841).

6.1 MULTIPLE SEROTONIN RECEPTORS DISCRIMINATED BY ANALOGUES OF 5-METHOXYTRYPTAMINE.[1] M.H. Smit*[a,b], R.L. Glennon*[c], H.I. Yamamura [b,2], and D.L. Nelson.[a] [a]Dept. of Pharmacol. Toxicol., Sch. of Pharm., [b]Dept. of Pharmacol., Sch. of Medicine, Univ. of Arizona, Tucson, AZ 85721; and [c]Department of Pharm. Chem., Sch. of Pharm, Med. Col. of Virginia, Virg. Commonwealth Univ Richmond VA 23298

Evidence has accumulated suggesting the functional presence of multiple serotonin(5-HT) receptors. Ligand-binding studies have revealed a group of high-affinity binding sites (Kd=1-4 nM) for (^{3}H)5-HT on rat brain membranes which this laboratory has previously differentiated into two subpopulations, based on their differential affinities for the neuroleptic spiperone. We are now able to report receptor discrimination using specific analogues of 5-HT. The following compounds were analyzed for their ability to inhibit the binding of [^{3}H]5-HT to its high-affinity sites: 5-methoxy-N,N-dimethyltryptamine [5(MeOH)DMT], 5-methoxy-N,N-diethyltryptamine [5(MeOH)DET], and 5-methoxy-N,N-dipropyltryptamine [5(MeOH)DPT]. Radioligand-binding studies were carried out on a washed membrane preparation from the rat cerebral cortex. Membranes from 10 mg of tissue were incubated in buffer (50 mM Tris-HCl, pH 7.4; 4 mM $CaCl_2$; 10 µM pargyline) containing 2 nM [^{3}H]5-HT and varying concentrations of inhibitor. Bound ligand was isolated by vacuum filtration, and nonspecific binding was defined in the presence of 1 µM metergoline or 0.3 µM 5-HT. All three 5-methoxytryptamines displaced (^{3}H)5-HT from its binding sites in a manner consistent with the presence of more than one type of site. The inhibition curves were shallow, extending over more than a 10,000 fold range of concentrations for the inhibitors and displayed Hill coefficients of less than 1.0. Non-linear regression analysis revealed that each compound fit a two-site model. There was a correlation between their discriminatory behavior and the length of the alkyl substitution. The dissociation constants of the compounds for the high- and low-affinity sites were as follows:

	HIGH	LOW
5(MeOH)DMT	10 nM	500 nM
5(MeOH)DET	15 nM	1000 nM
5(MeOH)DPT	10 nM	1750 nM

The difference in affinity between 5(MeOH)DMT and 5(MeOH)DPT was significant ($p<.05$). These results further support the concept of multiple 5-HT receptors. It is hoped that additional characterization of the structural requirements for fit to these different sites will result in the design of more specific 5-HT agonists and antagonists to aid in the elucidation of the physiological significance of multiple serotonin receptors in the brain. ([1]Supported by grants from the PMA Foundation and the Col. Pharmacy, U. Ariz. [2]Recipient of an RSDA Type II #MS-0095.)

6.2 TISSUE AND SPECIES DIFFERENCES IN ^{3}H-5-HYDROXYTRYPTAMINE BINDING SITES. R.G. Schnellmann* and D.L. Nelson (SPON: B. Schneiderman Fish). Dept. of Pharmacol. & Toxicol., Col of Pharmacy, Univ. of Arizona, Tucson, AZ 85721.

Previous studies have identified two populations of high-affinity (Kd=1-4 nM) ^{3}H-serotonin (^{3}H-5-hydroxytryptamine, ^{3}H-5-HT) binding sites in rat brain which can be distinguished on the basis of their affinities for certain neuroleptics.** Thus, one of these subgroups of sites gave a Kd for spiperone of 2-13 nM and the other a Kd of about 35,000 nM. The present report extends these studies by examining spiperone-sensitive ^{3}H-5-HT binding sites in several different species (rabbit, guinea-pig, and cat) and tissues (frontal cortex, hippocampus, and corpus striatum or caudate nucleus). Saturation studies of ^{3}H-5-HT binding revealed no differences in the affinity of this ligand for its binding sites in any of the species or tissues studied. Kd values for rabbit, guinea-pig, cat and rat ranged from 2.4-2.9 nM. Some small differences were noted in the total number of sites present in tissues from the different species. Examination of spiperone inhibition of ^{3}H-5-HT binding revealed large differences between the species and tissues with IC_{50} values ranging from a low of 87 nM in the guinea-pig hippocampus to 8900 nM in the cat corpus striatum. A heterogeneity of sites was suggested from the finding that logit-log plots of the inhibition curves gave slopes of less than 1.0. Nonlinear regression analysis with computerized curve fitting showed that the inhibition curves for most of the tissues fit a two-site binding model significantly better than a one-site model, as had been shown in the rat brain. However, the affinities for these sites differed from those in the rat. For example, in the guinea-pig the ^{3}H-5-HT binding site having high affinity for spiperone gave Kd values for spiperone in the range of 3-30 nM which were comparable to those described for the rat, but the low-affinity sites gave Kd values which ranged from 400-1300 nM, i.e., at least 25 times lower than the values for the low-affinity site in the rat. Of all the brain areas studied in the different species only 2 gave spiperone inhibition curves which resembeled those found in the rat. These were cat frontal cortex and corpus striatum which gave Kd values of 5-21 nM for the high-affinity spiperone site and 52,000 nM for the low-affinity site. In contrast, cat hippocampus gave Kd values for the high-affinity site of 820-1400 nM which agreed with the values found in the guinea-pig and rabbit. Thus, while the current data agree with the concept of multiple ^{3}H-5-HT binding sites, the classification of these sites is more complex than originally determined in the rat and requires further examination. (Supported by grants from the PMA Fnd. and the Col. of Pharmacy, Univ. AZ.) **Pedigo et al, J. Neurochem. 36:220-226, 1981.

6.3 DETAILED LOCALIZATION OF 5-HYDROXYTRYPTAMINE$_2$ RECEPTORS INVESTIGATED WITH ^{3}H-KETANSERIN, A NEW SELECTIVE LIGAND. J.E. Leysen, M. Verwimp*, R. Geerts*, W. Gommeren* and P.M. Laduron*. Department of Biochemical Pharmacology, Janssen Pharmaceutica, B-2340 Beerse, Belgium.

Ketanserin (R 41 468) is a new 5-hydroxytryptamine (5-HT) antagonist which binds primarily to 5-HT$_2$ receptors and completely lacks interaction with 5-HT$_1$ receptors (Leysen *et al.*, 1981 a). In contrast to previously described ^{3}H-5-HT antagonists, ^{3}H-ketanserin exclusively labels 5-HT$_2$ receptors. Its advantageous binding properties (Leysen *et al.*, 1981 b) renders ^{3}H-ketanserin particularly suitable for investigating the localization of 5-HT$_2$ receptors by *in vitro* and *in vivo* binding. The distribution in the brain and the periphery of specific ^{3}H-ketanserin binding sites studied *in vitro* and *in vivo* in various species shows that the highest receptor density occurs in the prefrontal cortex followed by surrounding cortical areas. Other brain areas and peripheral tissues contain only minor amounts of the receptors. Using neuronal lesions and intracerebral injection techniques the cellular localization and functional role of the frontal cortical 5-HT$_2$ receptors were studied. Hemisection of the frontal cortex by a knife cut just before the tuberculum olfactorium caused 20 to 30 % reduction of the 5-HT$_2$ receptors in the prefrontal cortex anterior to the knife cut, receptors in the cortical areas posterior to the cut were not changed. Hence, part of the receptors seemed to be localized on neurones projecting to the prefrontal cortex. The origin of such neurones was examined by applying electrolesions in various brain areas and by injection of ^{3}H-ketanserin in the prefrontal cortex to verify retrograde axonal transport. Injection of kainic acid in the prefrontal cortex had no noticeable effect on the 5-HT$_2$ receptor population. The possibility exists that a number of the prefrontal cortical 5-HT$_2$ receptors are not located on neurones.

J.E. Leysen, F. Awouters, L. Kennis, P.M. Laduron, J. Vandenberk and P.A.J. Janssen (1981 a). Receptor binding profile of R 41 468, a novel antagonist at 5-HT$_2$ receptors. Life Sci. 28, 1015-1022.

J.E. Leysen, M. Verwimp and P. Laduron (1981 b). Receptor binding properties of ^{3}H-R 41 468, a selective ligand for 5-hydroxytryptamine$_2$ receptors. Abstract - Eight International Congress of Pharmacology - July 1981, Tokyo, Japan.

6.4 SOLUBILIZATION AND PARTIAL PURIFICATION OF ^{3}H-MIANSERIN BINDING SITES. Betty CHAN*, Philip SEEMAN, Anne DUMBRILLE-ROSS and Bertha K. MADRAS (SPON: P. Garfinkel). Psychopharmacology Section, Clarke Institute of Psychiatry and Department of Pharmacology, University of Toronto, Toronto, Ontario, Canada.

The atypical antidepressant mianserin has been shown to bind to S_2 serotonin receptors in cortical tissue (Dumbrille-Ross et al.; Peroutka and Snyder, 1981). ^{3}H-Mianserin has high affinity for the S_2 site although ^{3}H-spiperone is currently used to identify S_2 receptors in non-dopaminergic regions. Purification of ^{3}H-mianserin and ^{3}H-spiperone receptive sites may provide further evidence for their co-existence. ^{3}H-Mianserin receptive sites were solubilized from canine frontal cortex, characterized and partially purified. Binding parameters (drug binding profiles, IC_{50}, K_d) of the membrane and solubilized receptor agreed closely.
R41468 was used to define specific binding.

	IC_{50} (nM) Membrane	Soluble
mianserin	1.3 ± 0.2	4 ± 1
R43448	2.4 ± 0.9	8.4 ± 4
R41468	57 ± 15	64 ± 10
metergoline	125 ± 7	--
d-LSD	206 ± 14	2,150 ± 500
l-LSD	--	35,000 ± 5,000
spiperone	315 ± 70	1,150
haloperidol	867 ± 133	5,700 ± 900
serotonin	7,000	24,000 ± 2,000
tryptamine	45,000	45,000 ± 15,000
histamine	>100,000	>300,000
dopamine	>100,000	>300,000
epinephrine	>100,000	>200,000
norepinephrine	>100,000	>100,000
K_d (nM)	1.1 ± 0.4	2.2 ± 0.5
B_{max} (fmoles/mg)	212 ± 12	95 ± 6

The receptor recovery was 45% whilst protein recovery was 41%. The solubilized receptive site, which yielded a single component on Scatchard analysis was prelabeled with ^{3}H-mianserin and partially purified. Bound ^{3}H-mianserin separated from unbound ^{3}H-mianserin as a single peak on both Sephacryl S-300 columns and on isoelectric focusing columns (pI 5.03 ± 0.16). Both peaks are displaceable by R41468 (1 µM). The same techniques are now being applied to ^{3}H-spiperone binding sites in the frontal cortex and a comparison of both purified preparations is in progress.

6.5 N-ACETYLSEROTONIN BINDING IN HUMAN, CALF AND RAT BRAIN. L.P. Niles*, G.M. Brown and R.K. Mishra*. Neuropharmacology Lab., Depts. Psychiatry & Neurosciences, Faculty of Health Sciences, McMaster Univ., Hamilton, Ontario, Canada, L8N 3Z5.

Prompted by the reported presence of N-acetylserotonin (NAS) and its synthesizing enzyme, N-acetyltransferase, in the rat brain we have studied the binding of NAS in the mammalian CNS in order to determine whether specific central receptors for this N-acetylindolealkylamine exist. Specific binding of H-N-acetylserotonin (^{3}H-NAS) defined as total bound radioactivity minus that not displaced by an excess of non-radioactive NAS was found in synaptosomal membrane fractions from various regions of human, calf and rat brain. Scatchard analysis of equilibrium binding data revealed the presence of saturable high affinity binding sites with dissociation constant (K_D) values ranging from 2-7 nM and binding site concentration (B_{max}) of 0.3-1.25 pmol/mg protein.

Localization studies indicate that cerebellar binding of ^{3}H-NAS exceeds that observed in other brain areas including the hypothalamus, hippocampus, striatum and cortex. Specific binding of ^{3}H-NAS has also been detected in the adrenal, testis, eye and Harderian gland. Initial displacement data indicate that NAS is a more potent inhibitor of ^{3}H-NAS binding than the related tryptamines melatonin, serotonin (5-HT) and N-acetyltryptamine. Furthermore, neither the 5-HT antagonist, methysergide, nor the 5-HT agonist, quipazine are potent displacers of ^{3}H-NAS binding.

These findings are the first evidence that specific high affinity binding sites for NAS are present in the mammalian brain and suggest that specific receptors for NAS may exist in the CNS.

(This work was supported by the Ontario Mental Health Foundation)

6.6 THE CHARACTERISTICS OF ^{3}H SEROTONIN BINDING IN RAT SPINAL CORD TISSUE. P.J. Monroe* and D.J. Smith, Depts. of Pharmacology/Toxicology and Anesthesiology, W.V.U.M.C., Morgantown, WV 26506.

Two distinct serotonin (5-HT) receptor subtypes have been reported in the rat frontal cerebral cortex (Peroutka and Snyder, Mol. Pharmacol 16, 1979). One site (5-HT$_1$ receptor) has a high affinity for ^{3}H-5HT while the other site (5-HT$_2$ receptor) binds ^{3}H-spiroperidol preferentially. In the present study the existence of a similar multiple 5-HT receptor system in rat spinal cord tissue was examined.

A membrane fraction was prepared from either rat frontal cortex or spinal cord according to the method of Peroutka & Snyder (see reference). Binding assays for ^{3}H-5HT were carried out with these membranes at 37°C for 20 min, using 800 µl of tissue suspension (20 mg of tissue/tube), 100 µl of ^{3}H-5HT and 100 µl of drug. Tubes for ^{3}H-spiroperidol binding contained 10 mg tissue and were incubated for 15 min. After the incubation, the tissue was collected on Whatman GF/B filters. The filters were then counted in Hydromix after a 5 hour extraction.

A ^{3}H-5HT binding site could be identified in both spinal cord and frontal cortex tissues. The ratio of specific to total binding was higher in the cortex than in the spinal cord. Values were consistently 60-70% and 35-40%, respectively, when the radioligand was purified, prior to its use, by high performance liquid chromatography. Scatchard analysis of the specific ^{3}H-5HT binding in the spinal cord gave a linear plot yielding a K_D of 9.4 nM and a B max of 5.7 p moles/g. Specifically bound ^{3}H-5HT in the spinal cord could be displaced by unlabeled 5-HT in a similar concentration-dependent manner as in the frontal cortex (50% displacement concentration: 5 nM in spinal cord, 8 nM cortex). Spiroperidol also displaced ^{3}H-5HT but only at concentrations exceeding 10 nM with an apparent 50% displacement concentration of 4 µM. These data agree well with the results obtained by Peroutka and Snyder. However, in contrast to their observations in the rat frontal cortex, there appears to be a lack of saturable ^{3}H-spiroperidol binding in rat spinal cord tissue. Total ^{3}H-spiroperidol binding increased linearly with increasing ligand concentration and was not displaced by either unlabeled spiroperidol or 5-HT, suggesting non-specific interactions. The effectiveness of the two drugs and reletive potencies in displacing ^{3}H-spiroperidol from frontal cortex, where a saturable binding site has been shown to exist, was confirmed.

In conclusion, these results suggest the existence of a 5-HT binding site analogus to the 5HT$_1$ receptor in frontal cortex tissue, but an apparent lack of a putative 5HT$_2$ binding site as distinguished by ^{3}H-spiroperidol.

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6.7 THE ANTIDEPRESSANT RECEPTORS. A. Dumbrille-Ross* and S.W. Tang. Psychopharmacology Section, Clarke Institute of Psychiatry, Toronto, Ontario, Canada M5T 1R8.

In order to examine whether there is a common antidepressant receptor to which antidepressant drugs bind, we examined the binding characteristics of ^{3}H-imipramine (^{3}H-IMI), ^{3}H-desipramine (^{3}H-DMI) and ^{3}H-mianserin (^{3}H-MIAN) to cerebral cortex membranes and platelets of Wistar and Fawn-Hooded (F-H) rats under a number of conditions. [Platelets of F-H rats have a serotonin (5HT) storage pool deficiency and display an abnormally low accumulation of 5HT.]

Binding characteristics are listed below.

1. Binding of ^{3}H-IMI to cortex membranes was potently displaced by serotonin uptake inhibitors like fluoxetine and citalopram (IC_{50} about 20 nM), and displaced by high concentrations (IC_{50} 2-4 µM) of serotonin S_2 antagonists like spiperone and R43 448. Binding of ^{3}H-MIAN was potently displaced by serotonin S_2 antagonists (IC_{50} about 40 nM) and by high concentrations of the uptake inhibitors (IC_{50} 1-2 µM), showing a profile similar to that of ^{3}H-spiperone binding.
2. Antidepressant drugs compete with very different potencies with both ^{3}H-IMI and ^{3}H-MIAN binding.
3. Binding of ^{3}H-IMI but not ^{3}H-MIAN is sodium dependent.
4. Raphé lesions abolished binding of ^{3}H-IMI but did not alter binding of ^{3}H-MIAN to cortex membranes.
5. ^{3}H-IMI but not ^{3}H-MIAN binds to platelet membranes of Wistar rats. But ^{3}H-IMI binds to neither platelet nor cortical tissue of F-H rats. Binding of ^{3}H-MIAN is similar in the cortex of Wistar and F-H rats. (The binding of the two antidepressants to platelets and cortex membranes will be compared with that of other serotonergic ligands.)
6. There is no high affinity binding site demonstrable for ^{3}H-DMI in cortex membrane preparations examined under similar conditions.

The results show that ^{3}H-IMI and ^{3}H-MIAN label different sites while ^{3}H-DMI failed to bind to membrane surface receptors with high affinity. It is unlikely that there is a "common antidepressant receptor" to which all antidepressants bind in the CNS or platelets.

6.8 DENERVATION SUPERSENSITIVITY OF THE BULBOSPINAL SEROTONIN SYSTEM: 5-HT$_1$ RECEPTOR BINDING AND FUNCTIONAL DATA. Fredrick Sautter*, Lee-Ming Kow, Donald W. Pfaff and Frank P. Zemlan. The Rockefeller University, N.Y., N.Y. 10021 and Dept. of Psychiatry, University of Cincinnati College of Medicine, Cincinnati, OH 45267.

The present data demonstrate: 1) a functional denervation supersensitivity of the spinal cord serotonin (5-HT) system, indicated by shifts to the left of the 5-HT agonist dose-response curves for facilitation of spinal reflexes and 2) a "supersensitivity" of spinal 5-HT$_1$ receptors, indicated by increased specific high affinity binding of ^{3}H-5-HT after denervation.

The effects of 5-HT and alpha-noradrenaline (NA) receptor agonists on the receptive field (RF) area and response magnitude of spinal reflexes elicted from the lumbar dorsum were examined from 6 hrs to 3 wks after spinal transection (T_{10}) in the rat. A dose-response related increase in reflex RF area and response magnitude was observed after administration of the 5-HT agonists quipazine (0,5,10 & 20 mg/kg) and 5-MeODMT (0,0.1,0.5 & 2.0 mg/kg) for all spinal reflexes examined across the entire postoperative period, while NA agonists were without effect. An observed shift to the left in the 5-HT agonist dose-response curves with time after transection indicated a functional denervation supersensitivity of the spinal 5-HT system which could be demonstrated as soon as 1 to 2 days postoperatively.

The effect of spinal cord transection (T_{10}) or sham operation on specific ^{3}H-5-HT (2nM) binding to membranes prepared from the lumbar enlargement was examined 0, 1, 2 and 7 days postoperatively. Seven days after transection ^{3}H-5-HT binding (64.3 ± 3.7 fmoles/mg protein) was elevated when compared to the sham control group (47.1 ± 2.6 fmoles/mg protein). Scatchard analysis (0.5 to 16 nM ^{3}H-5-HT) revealed that the maximum number of ^{3}H-5-HT binding sites (B_{max}) was significantly greater ($p<0.01$) 7 days after transection while no change in the apparent 5-HT receptor affinity constant (K_D) was found (transected: B_{max} = 199 ± 19 fmoles/mg protein, K_D = 2.2 ± 0.2 nM, sham: B_{max} = 107 ± 15 fmoles/mg protein, K_D = 1.2 ± 0.2 nM for 4 experiments performed in triplicate).

The present data suggest that the functional supersensitivity observed 7 or more days after denervation may have been due to an increase in the number of spinal 5-HT receptors, while the functional supersensitivity found beforehand was probably due to presynaptic mechanisms altered by axotomy.

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6.9 EFFECTS OF CHRONIC AMPHETAMINE TREATMENT ON SEROTONIN RECEPTORS. G. Battaglia* and M. Titeler (SPON: J. Khanna). Dept. of Pharmacol. University of Toronto, Toronto, Ontario M5S 1A8.

Recent evidence has shown potent effects of chronic amphetamine treatment on the serotonergic systems of the brain (1). Meanwhile, ^{3}H-serotonin (S_1 receptors) and ^{3}H-spiperone (S_2 receptors) have been found to label distinct serotonergic receptor sites in rat brain (2). We decided to investigate the effects of chronic amphetamine treatment on serotonin receptors in rat brain to see if chronic treatment with this drug results in changes in the number or the pharmacological properties of these receptors.

Treated and untreated (control) rats were housed separately and fed ad libitum. Treated rats were given 5 mg/kg/day amphetamine sulphate in their drinking water. After six weeks the rats were decapitated and their cortices and caudates dissected. The caudates were prepared and assayed for dopamine receptors as previously described (3). The cortices were prepared and assayed for serotonin receptors as previously described (4).

Table 1

	Control		Treated	
	K_D (nM)	B_{max} (fmol/mg)	K_D	B_{max} (fmol/mg)
^{3}H-ser (cortex)	0.91 ± .09	129 ± 10 (n=8)	1.3 ± .18	137 ± 7 (n=11)
^{3}H-spip (cortex)	1.2 ± .12	347 ± 38 (n=8)	1.1 ± .13	238 ± 25 (n=11)
^{3}H-spip (caudate)	0.1 ± .01	411 ± 26 (n=15)	.07 ± .007	327 ± 14 (n=15)

There were no significant differences between ^{3}H-serotonin binding in treated and untreated rat cortices, whereas there was a 31% decrease in ^{3}H-spiperone binding. In the caudate ^{3}H-spiperone binding was reduced by 20%.

These preliminary results are being extended to varying dosages of amphetamine and to examining the reversibility of the neurochemical effects of chronic amphetamine treatment. This work was supported by a grant from the Hospital for Sick Children Foundation.

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6.10 SEROTONIN RECEPTOR LOCALIZATION BY AUTORADIOGRAPHY: QUANTITATIVE ANALYSIS AND THE EFFECT OF STEROID HORMONES. A. Biegon*, T.C. Rainbow and B.S. McEwen, Rockefeller University, New York NY 10021

A modification of the method of Young and Kuhar (Eur. J. Pharmacol. 62:237, 1980) was used to obtain a quantitative analysis of the density of 5HT receptors in rat brain nuclei prior to a study of the effects of steroid hormones on these receptors. Coronal cryostat sections were incubated with 2nM ^{3}H-5HT and placed against tritium sensitive LKB film in the dark for 2 months. The resulting images were analyzed by a densitometer (Rainbow et al., J. Neurosci. Methods, in press) and also subjected to color-enhanced computer analysis. The highest concentrations of 5HT receptor (densitometer readings > 10 units) were found in the anterior hippocampus, subiculum and dentate gyrus, dorsal raphe and the substantia nigra. High values (6-9 density units) were found in the globus pallidus, lateral preoptic area, hippocampal field CA1 and the interpeduncular nucleus. Moderate levels (3-5 units) were found in the nucleus accumbens, medial amygdala, cortical amygdala, arcuate nucleus, bed nucleus of the stria terminalis, olfactory tubercle, superior colliculus, central grey, layer 4 of the cortex, ventromedial and dorsomedial hypothalamus, dorsal tegmental nucleus, n. locus ceruleus and thalamic n. parafascicularis. Low levels (1-3 units) were observed in the cingulate cortex, caudate nucleus, medial habenula, medial preoptic n., median raphe and medial septum. Very low levels were found in hippocampal field CA3, cerebellum, most of the thalamus and all white matter. These data mostly confirm and extend previous reports (Meibach et al., Soc. Neurosci. Abst. 6:357, 1980; Young and Kuhar, cited above). It is noteworthy that most of the brain regions known to concentrate steroids have moderate to high levels of 5HT receptors, and this raises the possibility of an interrelationship between these systems. Thus we are studying the effect of corticosterone on hippocampal 5HT receptors in adrenalectomized rats and the effect of estrogen on 5HT receptors in ovariectomized (OVX) rats. Estradiol benzoate (10µg/day) was injected twice and rats were killed 48h after the second injection; OVX controls received sesame oil vehicle. Estrogen treatment produced an increase in 5HT binding in several estrogen concentrating nuclei: e.g., arcuate nucleus (Control: 3.8±0.2 density units; Estrogen:4.9±0.3; lateral preoptic area near bed nucleus of stria terminalis: 6.2 ± 0.4 vs 7.5±0.3; and ventrolateral septum: 6.4±0.4 vs 9.8±0.4). No change was observed in cortex, caudate, or dorsal raphe nucleus. These results are similar to results of in vitro ^{3}H 5HT binding measurements on homogenates (Fischette et al., This Meeting). Grant support: NSO7080 (BMc), Rockefeller Foundation (RF70095); and by NIMH, MH08055 (TCR) and C. Weizmann (AB) postdoctoral fellowships.

6.11 SEROTONIN RECEPTORS IN ESTROGEN CONCENTRATING BRAIN NUCLEI AND THEIR MODULATION BY ESTRADIOL. C.T.Fischette, A.Biegon* and B.S.McEwen. Rockefeller University, New York, New York 10021

Serotonin (5-HT) receptors have been shown to fluctuate over the estrous cycle in the basal forebrain in intact cycling female rats(Biegon et.al., Brain Res.,187:221,1980). In order to localize the effect of estradiol on these receptors, we have developed a microassay for 5-HT receptor binding in individual brain nuclei. For each of 5 experiments 3 ovariectomized rats were given sesame oil and 3 were given 10µg estradiol benzoate (EB)sc. for 2 days; they were sacrificed 48h after the last injection. Nuclei were removed from frozen 300µm thick coronal sections according to the method of Palkovits et. al.(Brain Res., 77;137,1974) and pooled. Previously described assay procedures (Biegon et.al.,1980) were modified for small samples. Following incubation of the homogenates in 50mM Tris buffer at 37°C, 300µl Tris buffer containing pargyline (10^{-4}M) and ascorbate (0.02%) were added to the homogenates and incubated again for 10min; then, 100µl aliquots were assayed in a final volume of 0.3ml containing 2nM ^{3}H-5HT, with 1µM cold 5-HT used to estimate nonspecific binding. Saturation experiments under these conditions exhibited Kd(1.6nM) and Bmax(170fm/mg pro.) values similar to those previously reported. Binding was linearly dependent on protein concentration from 10-100µg protein.

Moderate to high levels of 5-HT receptor binding were found in all of the estrogen concentrating areas sampled. EB treatment produced a significant (35-50%) increase in 5-HT receptor binding in the preoptic area, lateral septum and arcuate nucleus-median eminence. Non-significant increases were observed in the bed nucleus of the stria terminalis and anterior hypothalamic nucleus. No change was observed in the ventromedial nucleus or corticomedial amygdala. Overall levels of 5-HT receptor binding that were insensitive to EB treatment were very high in the dorsal raphe and substantia nigra, and very low in the ventral thalamus and cerebellum.

The distribution of 5-HT receptors is in agreement with the results obtained by autoradiography (Biegon et.al., this meeting; Meibach et.al., Soc. for Neuroscience, 6:357, 1980). We conclude that EB causes an increase in 5-HT receptor binding in some but not all brain regions which contain estrogen receptors. The specific anatomical localization of the effect may be related to the specific roles of these nuclei in the control of ovulation and/or estrogen-mediated behaviors in the female rat. (Supported by PHS NIH AM06122(CF), Weizmann Postdoctoral Fellowship(AB) and NIH NS 07080(BMc). An institutional grant was also obtained from the Rockefeller Foundation, RF 70095.

7.1 BRAIN D_2 DOPAMINE RECEPTORS: PARTIAL PURIFICATION. Leslie LILLY*, Alan DAVIS, Bertha K. MADRAS, Claire M. FRASER, J. Craig VENTER* and Philip SEEMAN (SPON: P. Brawley). Department of Pharmacology, University of Toronto, Toronto, Canada; Psychopharmacology Section, Clarke Institute of Psychiatry, Toronto; and State University New York at Buffalo, New York, U.S.A.

We used gel chromatography and isoelectric focusing to purify the D_2 dopamine receptors from canine brain striatum. The membrane fraction of the tissue was solubilized in 1% digitonin in phosphate buffer, and the receptors labelled with 4 nM ^{3}H-spiperone. The specific binding was defined as that displaced by 1 μM (+)-butaclamol. The purified preparation was characterized (Madras et al., Soc. Neurosci. Abstr., 1981) and used to raise monoclonal antibodies (Davis et al., Soc. Neurosci. Abstr., 1981).

a. Sephacryl S-300 was used to partly purify the D_2 dopamine receptors. Soluble material, incubated in 4 nM ^{3}H-spiperone and added to a column pre-equilibrated in a phosphate-Triton buffer system, eluted at a volume corresponding to a MW of 220,000 daltons and a Stokes radius of 5.8 nm (N=3). Of the specifically bound fmoles spiperone added to the column, 17 ± 3% were recovered in the peak fraction of the effluent. Concentration of this peak, followed by assay, showed that ^{3}H-spiperone binding was 100% specific in that peak.

b. Using preparative column isoelectric focusing, we focused unlabeled material and assayed each fraction for the presence of D_2 receptors using Sephadex G-50 mini-columns or a polyethylene glycol precipitation method (Chan et al., submitted).

c. We also focused material prelabeled with 4 nM ^{3}H-spiperone alone and in the presence of 1 μM (+)-butaclamol. D_2 receptors were detected by radioactivity in the effluent.

Two peaks of bound ^{3}H-spiperone were obtained in the effluent, with isoelectric points (pI) of 4.93 ± 0.11 (N=13) and 7.76 ± 0.11 (N=9). These peaks were stereoselectively displaced by 1 μM (+)-butaclamol. The peak at 4.9 was always associated with the presence of a visible protein precipitate within the column. (Free ^{3}H-spiperone had a pI value of 11.1). The pH 7.8 fraction represented a 40-fold purification of the D_2 receptors. [Supported by The Medical Research Council of Canada (to PS) grants AHA 79688 and HL 21239 (Nat. Inst. of Health; to CMF & JCV), and NATO/SRC U.K. Fellowship (to AD).]

7.2 PARTIALLY PURIFIED DOPAMINE/NEUROLEPTIC RECEPTORS (D_2-TYPE). Bertha K. MADRAS, A. DAVIS, Leslie LILLY, Claire FRASER, Craig VENTER, Philip SEEMAN. Psychopharmacology Section, Clarke Institute of Psychiatry, Department of Pharmacology, University of Toronto, Toronto, Ontario, Canada; and State University New York at Buffalo, New York, U.S.A.

Solubilized dopamine/neuroleptic receptors (D_2) of canine caudate are stable at 0°C and retain the binding properties of the membrane receptor. Prelabelled (^{3}H-spiperone) soluble receptors were separated by isoelectric focusing into a bound ^{3}H-spiperone fraction (displaceable with (+)-butaclamol) and an unbound ^{3}H-spiperone peak (Lilly et al., Soc. Neurosci. Abstr., 1981). This purified preparation was used to raise monoclonal antibodies (Davis et al., Soc. Neurosci. Abstr., 1981).

Pharmacological characterization of the bound ^{3}H-spiperone peak confirmed that this fraction was the neuroleptic receptor.

Prelabeled soluble receptors were eluted from isoelectric focusing columns as a single peak with a pI of 6.4 ± 0.2. The fractions containing radioactivity were dialyzed overnight and re-labeled with ^{3}H-spiperone (4 nM) in the presence or absence of neuroleptic drugs and dopamine agonists. The recovery of specific - but not total - binding was greater than 82%.

	IC_{50} (nM) Soluble	Purified
Spiperone	3.5 ± 2	2.3 ± 1.3
(+)-Butaclamol	4.6 ± 1	8.0 ± 1.7
Fluphenazine	4.2 ± 1.2	10 ± 0.5
Haloperidol	40 ± 8	58 ± 6
Chlorpromazine	145 ± 15	197 ± 36
(-)-Butaclamol	~10,000	~10,000
Dopamine	5,000 ± 1800	100,000*
Apomorphine	1,650 ± 570	3,800 ± 450
ADTN	950 ± 280	--
Norepinephrine	>100,000	>100,000
Serotonin	>100,000	>100,000

*20% inhibition

The affinity, rank order of potencies of neuroleptics, and stereoselectivity of the site were retained during the focusing procedure.

The reduced affinity of the purified receptor for dopamine could be a consequence of (1) dialysis or buffer conditions; (2) conformational change in the binding site; (3) removal of a dopamine binding cofactor; (4) separation of neuroleptic and dopamine binding sites. These possibilities are being explored.

7.3 MONOCLONAL ANTIBODIES TO PARTIALLY PURIFIED D_2 DOPAMINE RECEPTORS. Alan DAVIS, Claire M. FRASER*, Leslie LILLY*, Bertha K. MADRAS, J. Craig VENTER* and Philip SEEMAN. Department of Pharmacology, University of Toronto, Canada M5S 1A8; Psychopharmacology Section, Clarke Institute of Psychiatry, Toronto; and State University New York at Buffalo, New York, U.S.A.

D_2 dopamine receptors were partially purified and characterized from canine brain striatum by isoelectric focusing (Lilly et al., Madras et al., Neurosci. Abstr., 1981) and used to raise antibodies in mice. Balb/C mice were immunized with a solubilized fraction which was 40-fold enriched in D_2 receptors and which had an isoelectric point of 7.76. After a booster injection, splenic cells from the immunized mice were fused with mouse myeloma SP/O-Ag14 cells, using the method of Fraser and Venter (Proc. Nat. Acad. Sci. 77, 7034-7038, 1980). Spleen and myeloma cells were fused using warm polyethylene glycol 1000. Unfused cells were eliminated by means of selective media, and the fused cells (hybridomas) were grown in 96-well plates. The culture media of active hybridomas were then screened for the presence of antibody to the D_2 dopamine receptor. Membrane preparations of the D_2 receptor were incubated with the culture medium for 18 hr, followed by the addition of 4 nM ^{3}H-spiperone. Specific binding of ^{3}H-spiperone was defined as that displaced by excess (+)-butaclamol. The medium of one hybridoma (D2D3) markedly inhibited specific binding in a concentration-dependent manner.

The hybridoma medium was also screened for its ability to precipitate soluble receptors, using an indirect immunoprecipitation assay. Samples of culture media were incubated with aliquots of digitonin-solubilized receptors for 18 hr, and the receptor-antibody complexes were then precipitated with an excess of rabbit anti-mouse IgG. The supernatant was then assayed for remaining soluble D_2 dopamine receptors, using 4 nM ^{3}H-spiperone and Sephadex G-50 mini-columns. The culture medium from one hybridoma (designated D1D3) effectively precipitated soluble D_2 receptors in a concentration-dependent fashion. At a 1:4 dilution of the D1D3 culture medium, 18.0 ± 3.2 fmoles (N=3) of D_2 dopamine receptors were specifically precipitated. [Supported by The Medical Research Council of Canada (to PS), grant AHA 79688 and HL 21239 (Nat. Inst. of Health; to CMF & JCV), and NATO/SRC U.K. Fellowship (to AD).]

7.4 D_3 AND D_4 DOPAMINERGIC BINDING SITES DETECTED BY ^{3}H-ADTN. Stephen J. LIST and Philip SEEMAN (SPON: Y. Israel). Dept. of Pharmacology, Univ. of Toronto, Toronto, Canada M5S 1A8.

The D_3 binding site is characterized by its high-affinity for ^{3}H-dopamine (K_D = 2 nM) and its low-affinity (200-50,000 nM) for neuroleptics. The D_3 site is also detected by ^{3}H-apomorphine, but less selectively. We now report that ^{3}H-ADTN detects both the D_3 site and another site which we call D_4.

The specific binding to rat striatum of 0.8 nM ^{3}H-ADTN (defined as that inhibited by 1 μM apomorphine) was approximately 60% of total binding. ADTN, apomorphine and dopamine showed monophasic competition curves with IC_{50} values of 3.5 nM, 4.0 nM and 10 nM, respectively. Neuroleptics competed for ^{3}H-ADTN binding with both high- and low-affinity components: spiperone competed for 50% of the specific binding between 0.01 nM and 5.0 nM, and competed for the remaining specific binding between 100 nM and 10,000 nM. Thus, ^{3}H-ADTN detected two sites, one with high-affinity and the other with low-affinity for neuroleptics. The high-affinity neuroleptic site was defined as that displaceable by 30 nM spiperone: the low-affinity site (measured in the presence of 30 nM spiperone) was defined as that displaceable by 1 μM apomorphine.

The density (B_{max}) of the D_3 site (low-affinity for neuroleptics) was 70 fmoles/mg protein, and that for the D_4 sites (high-affinity for neuroleptics) was 90 fmoles/mg protein; the K_D values were both 2 nM.

	IC_{50} (nM) D_3 Site ^{3}H-dopamine	D_3 Site ^{3}H-ADTN	D_4 Site ^{3}H-ADTN
ADTN	1.5	-	4.0
Apomorphine	3.5	3.0	5.0
Dopamine	5.0	3.0	17.0
Haloperidol	200	250	15
Bromocryptine	400	200	10
Chlorpromazine	700	1,600	40
Spiperone	1,500	4,000	0.15
Sulpiride	50,000	-	5.0

The table indicates that the D_3 ^{3}H-ADTN binding site is the same as that detected by ^{3}H-dopamine. The D_4 site detected by ^{3}H-ADTN has a distinct pharmacology characterized by its high affinity for both dopamine agonists and neuroleptics. (Supported by the Medical Research Council of Canada and the Ontario Mental Health Foundation.)

7.5 ^{3}H-NPA LABELS A SUBSET OF D_2-DOPAMINE RECEPTORS IN CALF AND RAT BRAIN. M. Titeler and G. Battaglia*, Department of Pharmacology, University of Toronto, Toronto, Ontario M5S 1A8.

In using ^{3}H-spiperone to label D_2-dopamine receptors evidence has accumulated for the existence of the labelling of multiple dopaminergic sites (1,2). Most notably, catecholamines compete for specific ^{3}H-spiperone binding in caudate homogenates with very shallow slopes, while ergots and neuroleptics compete for this binding with steep slopes (1,2). N-propyl-apomorphine shows a very high affinity and a low-affinity component of competition for specific ^{3}H-spiperone binding. When ^{3}H-NPA is used to label sites in caudate tissue at very low concentrations (10^{-10}M) it clearly labels a site for which neuroleptics and catecholamines can compete with high affinity and steep slopes (see table 1). Scatchard analysis in calf tissue indicates that the ^{3}H-NPA binding to high affinity neuroleptic sites has a B_{max} of 120 fmol/mg, while the B_{max} of ^{3}H-spiperone binding in the same tissue was 380 fmol/mg. Thus ^{3}H-NPA labels a sub-set of high affinity neuroleptic-dopamine receptors, while ^{3}H-spiperone labels two sites, both of which have high affinity for neuroleptics and one of which has a high affinity for catecholamines and one of which has a low affinity for catecholamines. We are currently pursuing investigations designed to determine whether these are two distinct dopamine receptors or two states of a single receptor. Sibley and Creese have recently reached similar conclusions about D_2 dopamine receptors in the pituitary (3).

IC_{50} vs. 10^{-10} ^{3}H-NPA

Drug	Calf (M)	Rat (M)
(+)-Butaclamol	4.1×10^{-9}	
Spiperone	1.6×10^{-10}	4×10^{-11}
Haloperidol	1.8×10^{-9}	
Sulpiride	9.5×10^{-8}	
Dopamine	3×10^{-10}	1.6×10^{-9}
NPA	3×10^{-10}	4.8×10^{-10}
ADTN	1.9×10^{-9}	2.5×10^{-10}
Apomorphine	1.4×10^{-9}	

1. Burt, D.R., Creese, I., Snyder, S.H., 1976, Mol. Pharmacol. 12, 800-812.
2. Titeler, M., Weinreich, P., Sinclair, P., Seeman, P., 1978, P.N.A.S. 75, 1153-1156.
3. Sibley, D.R. and Creese, I., 1980, Eur. J. Pharmacol. 65, 131-133.

7.6 CHARACTERIZATION OF DOPAMINERGIC BINDING SITES IN THE CYTOSOL FRACTION OF THE STEER ANTERIOR PITUITARY. Andrea S. Weisman*, Robert W. Kuhn* and Richard I. Weiner. Reproductive Endocrinology Center, Univ. of CA School of Medicine, San Francisco, CA 94143.

We have recently shown the presence of high affinity, stereoselective dopamine (DA) binding sites in the cytosol fraction of steer anterior pituitary (AP) homogenates (Kerdelhue et al, Endocrinology, 109, in press). We have further characterized these sites by sucrose density sedimentation, gel filtration, salt precipitation, reaction with N-ethylmaleimide (NEM) and enzymatic digestion. Cytosol was prepared for binding studies by homogenizing steer AP in buffer (15 mM Tris, 1 mM EDTA, 0.01 mM Bacitracin, pH 7.4) containing 304 mM sucrose and centrifuging for 1 hr at 145,000 x g and 4°C. Specific ^{3}H-Spiperone (^{3}H-SPIP) binding was defined as the counts displaced by 10^{-6} M d-butaclamol. Initial experiments showed that the separation of bound from free ligand by gel filtration on Sephadex G-25 or by charcoal adsorption, gave essentially identical values for the K_d (0.26 vs 0.29 nM) and Bmax (9.1 and 11.9 fmole/mg protein). Because of its rapidity and accuracy, the charcoal assay was used in subsequent studies. The ^{3}H-SPIP binding site was found to be precipitable by 40% saturation with ammonium sulfate. Following this partial purification and concentration, the binding sites were analyzed by sucrose density sedimentation and gel filtration. The binding site sedimented as a 6 S molecule and was slightly retained by a Sephacryl S-200 column. These results are consistent with a soluble molecule having a molecular weight in excess of 200,000 daltons. The requirement for a free sulfhydryl group for ligand binding was shown by sensitivity to treatment with NEM: 20 mM NEM eliminated 74% of specific binding while 100 mM completely eliminated specific binding. Incubation for 1 hr at 37°C with Viokase (0.5 mg/ml), Pronase (0.5 mg/ml), or trypsin (0.3 mg/ml) decreased specific binding by 94%, 58%, and 27% respectively, while incubation with DNAse (1 mg/ml) and RNAse (1 mg/ml) were without effect. It thus appears that the DA binding site is a large protein which requires a free sulfhydryl group for binding. The presence of the cytosol receptor does not appear to be due to mechanical disruption of membrane receptors since it is not present in the cytosol fraction from caudate nucleus homogenates, and rehomogenization of the microsomal fractions of the AP does not result in the extraction of additional soluble sites. These sites could result from internalization of membrane receptors, de novo synthesis prior to incorporation into the membrane, or an additional class of sites involved in an intracellular site of action of DA.

This work is supported by NIH HD 08924 and the Mellon Foundation.

7.7 ^{3}H-SPIROPERIDOL BINDING TO HUMAN CAUDATE: SPECIES VARIATION IN SITE NUMBER, AFFINITY, DENSITY AND SELECTIVITY. L.H. Matt*, J.D. Reimnitz*, M.E. Maguire, A.C. Andorn*. Depts. of Psychiat. and Pharmacol. Case Western Reserve Univ. Schl. of Med., Cleve. OH 44106.

^{3}H-Spiroperidol binds specifically to homogenates of human caudate, obtained from normal brains less than 16 hours postmortem, and prepared according to published methods (Andorn and Maguire, J. Neurochem., 35, 1105-1113, 1980). This high affinity binding is saturable, reversible, stereoselective with regard to (+) and (-) butaclamol, linear with regard to defined protein concentrations, and apparently occurs at multiple sites of limited density. Association is biphasic and rapid, with rates consistent with sites of $K_D \leq$ nM. Equilibrium is achieved by 20 min. Dissociation initiated at 30 min. by excess haloperidol is biphasic; approximate $t_{1/2}$ are 2.0 and 17 min. These rates are again consistent with sites of $K_D \leq$ nM. Equilibrium saturation analyses show at least two sets of sites. The K_{Dapp} are 20 +/- 10 pM and 260 +/- 80 pM, while the respective B_{max} are 90 +/- 10 and 100 +/- 10 fmol/mg protein. The K_D 20 pM sites are selective for monoaminergic agonists with serotonin (5HT) $\geq$ dopamine (DA) > (-)-norepinephrine (NE). The K_D 260 pM sites are also selective for these agents but with DA>>5HT$\geq$(-)NE. This selectivity is consistent with a dopamine receptor. In fact, the selectivity of these K_D 260 pM sites is the same as we have observed at rat striatal sites having K_D 20 pM. These findings contrast with previous reports of ^{3}H-Spiroperidol binding in caudate of other mammals. Whether site homogeneity or multiplicity has been shown, the highest affinity site demonstrated has had a selectivity consistent with a dopaminergic receptor, unlike these present observation sin human caudate. These findings suggest that antipsychotic ligand binding site properties may vary between species of the same phylogenetic class.

7.8 EVIDENCE FOR DIRECT DOPAMINE RECEPTOR EFFECTS BY AMANTADINE. R.M. Allen. Psychiatry Research Unit, Department of Psychiatry, Louisiana State University Medical Center, Shreveport, LA 71130.

Amantadine has been shown to prevent haloperidol-induced dopamine (DA) receptor hypersensitivity in the rat striatum using a stereotyped behavior model (Allen, et al, Biol. Psychiatry 14:541), direct receptor binding techniques (Allen, et al, Eur. J. Pharmacol. 65:313), without apparent effect in the mesolimbic DA system (Allen, et al, Soc. for Neurosci. Abstracts 6). Previous behavioral work has demonstrated that amantadine blocks amphetamine-induced excitability (Clark, et al, Proc. Soc. for Exp. Biol. and Medicine 151:434), blocks amphetamine or apomorphine-induced stereotypy (Schneiden, et al, Eur. J. Pharmacol. 39:133), and inhibits neuroleptic-induced catalepsy (Maj, et al, Psychopharmacologia 24:296). It has been shown to depress cortical DA neuron firing when applied microiontophoretically (Stone, Br. J. Pharmacology 56:101). These results indicate a direct receptor effect. In this study, direct displacing effects on [^{3}H]-spiroperidol and ADTN were examined. Pooled rat striatum crude membrane preparation was used as the study substrate. The radioreceptor assays were performed in a Tris-salt-EDTA buffer system as described by Leysen, et al (J. Neurochem. 36:201). Scatchard analysis of [^{3}H]-spiroperidol was performed using 10^{-5} (+) butaclamol (But) or 10^{-5} amantadine (AM) as displacing agents. Amantadine displaced 32% of specifically bound spiroperidol (Bmax = 90 fmol/mg protein) compared to (+) But (Bmax = 281 fmol/mg protein) with only slighly differing affinity (K_D with AM 111 pM, (+) But 188 pM). Hill plot analysis revealed a Hill coefficient of 0.95 for AM and 1.01 for (+) But. The IC_{50} for (+) But using 300 pM [^{3}H]-spiroperidol was 5 nM, for AM, 10 nM. Using similar conditions except for incubation temperature and time (25°C, 20 min), Scatchard analysis of [^{3}H]-ADTN was performed with apomorphine and amantadine. 10^{-5} amantadine displaced 1.4 times as much [^{3}H]-ADTN (Bmax = 1276 fmol/mg) as 10^{-5} apomorphine (Bmax = 743 fmol/mg) but with almost half the affinity (K_D AM = 4.8 nM, K_D APO = 2.6). Both AM and APO had Hill coefficient of 0.9. These preliminary results indicate that amantadine has direct receptor effects on both antagonist and agonist receptor sites which may explain its effects on neuroleptic-induced behavioral effects and its greater usefulness in neuroleptic-induced extrapyramidal side effects than idiopathic Parkison's disease. Further studies on agonist binding effects and speculation about its possible mechanism of action will be presented.

7.9 CHARACTERIZATION OF ^{3}H-ADTN BINDING SITES IN DOG RENAL CORTEX AND THEIR SENSITIVITY TO REDUCING AGENTS. C.K. Scott*, and C. VanderWende (SPON: A. Feldstein). College of Pharmacy, Rutgers, The State University, Box 789, Piscataway, New Jersey 08854

There has been much evidence to support the existence of a peripheral dopamine receptor located in the kidney which is responsible for renal vasodilation and subsequent increase in renal blood flow. We have identified this receptor utilizing the dopamine agonist ^{3}H-ADTN, in membrane preparations of canine renal cortex. ^{3}H-ADTN binding displays all of the necessary criteria for specific receptor interaction in that it is linear over a given protein concentration, is saturable, and displays appropriate kinetics, (Kd ∿5nM). The receptor displays the appropriate pharmacological specificity for the dopamine agonists: dopamine, 6,7-ADTN, apomorphine and epinine. In addition, the receptor shows a marked sensitivity to a number of reducing agents such as ascorbic acid, sodium metabisulfite and dithiothreitol. Evaluation of saturation kinetics and subsequent scatchard analysis in the presence of 0.1% ascorbic acid, a concentration routinely used in dopamine binding assays, demonstrates a marked reduction in the number of binding sites with no effect on the dissociation constant.

These data indicate that the ^{3}H-ADTN binding site in canine cortex membranes may represent the proposed peripheral dopamine receptor and that this receptor may be similar to the receptor in the brain in regard to the kinetics of ^{3}H-ADTN binding and sensitivity to reducing agents.

7.10 DOPAMINE RECEPTORS, AMPHETAMINE RESPONSE, AND HYPERKINETIC SYNDROME. Daiga Helmeste and Philip Seeman. Dept. of Pharmacology, University of Toronto, Toronto, Canada M5S 1A8.

In order to examine the potential relation between the density of dopamine receptors (D_2 type) and the behavioural response to amphetamine, the densities (B_{max}) of brain region dopamine receptors in eight strains of mice were measured using ^{3}H-spiperone (0.05 to 2 nM).

The mice with high densities of D_2 receptors in the striatum and olfactory tubercle (C3H/HeJ, CD-1, BALB/cJ and A/J) had B_{max} values 15 to 25% higher than the low-density mice (CBA/J, C57BL/6J, SEC/IReJ and DBA/2J). The K_D values were not significantly different between the two groups.

Locomotor activity after 0.5, 1.0 or 5.0 mg/kg i.p. d-amphetamine - sulphate was qualitatively different depending on whether the strain had high or low D_2 receptor density. The inbred strains (C3H/HeJ, BALB/cJ, A/J) with high D_2 density decreased their activity after 0.5 to 1.0 mg/kg amphetamine. The low-density mice either increased or showed no change in locomotor activity after 0.5 to 1.0 mg/kg amphetamine. All strains showed increased locomotion after 5.0 mg/kg amphetamine (compared to vehicle-injected controls).

The high density of D_2 receptors may reflect a functionally overactive dopamine system in those mice who responded to amphetamine with hypolocomotion. This qualitative difference in behavioural response to amphetamine is similar to that found in hyperkinetic children, with some children being good responders and others being non-responders to equivalent doses of amphetamine.

(This work was supported by the Hospital for Sick Children Foundation, the Ontario Mental Health Foundation and the Medical Research Council of Canada.)

7.11 INCREASED STEREOSPECIFIC DOPAMINE RECEPTOR BINDING AFTER REMOVAL OF ENDOGENOUS DOPAMINE FROM RAT BRAIN REGIONS. N.G. Bacopoulos, T.L. Miller * and P.L. Ware *. Departments of Pharmacology and Psychiatry, Dartmouth Medical School, Hanover, NH, 03755.

Endogenous dopamine contaminating radioreceptor binding assays can seriously interfere with the measurement of stereospecific 3H-dopamine (3H-DA) and 3H-apomorphine (3H-APO) binding sites in particulate fractions of rat caudate nucleus (CN) or mesolimbic brain regions. Binding was measured with 0.2-15.0 nM 3H-DA or 3H-APO (40-41 Ci/mmole, New England Nuclear) and 0.2 mg tissue protein in a final volume of 1.0 ml containing 20 mM Tris-HCl buffer, pH 7.4, 1.1 mM ascorbic acid 5.0 mM EDTA-4Na, 1.1 mM ascorbic acid and 15 uM pargyline. After a 30 min incubation at 23° C, samples were passed through GF/B filters under suction. Radioactivity on the filters was measured with 10 ml 3% Protosol-Econofluor (New England Nuclear) 24 hr later. Nonspecific binding was measured with 10 uM d-butaclamol. Endogenous dopamine was measured radioenzymatically in perchloric acid extracts of tissue suspensions used in the binding assay. The method of tissue preparation had significant effects on residual dopamine content and on the stereospecific binding sites of 3H-DA or 3H-APO. Successive "washes" (centrifugations and resuspensions of the 20,000 X g total particulate pellet in 20 mM Tris-HCl buffer, pH 7.4) of the crude homogenate reduced dopamine content and increased the number of binding sites of both radioligands proportionately. Preincubation of the tissue suspension at 37° C for 15 min resulted in an additional reduction in endogenous dopamine content and a 2-3 fold increase in stereospecific binding. This was due to an increase in the total binding rather than a reduction in the nonspecific binding. In preincubated tissue suspensions, the stereospecific binding sites of 3H-DA or 3H-APO were saturable, with a Bmax of 250-300 fmoles/mg protein in the CN and 2/3 that value in the mesolimbic region. The Kd of 3H-APO in the CN was 1.1-1.6 nM and of 3H-DA 0.8-1.3 nM. Values shown are the range of 4 experiments. Subfractionation studies revealed that the crude synaptosomal pellet contained most of the saturable stereospecific sites of the two radioligands, but in this tissue preparation preincubation was again necessary for the removal of the majority of endogenous dopamine. It appears that the portion of endogenous dopamine that is removed by preincubation at 37° C may be bound to dopamine receptors, therefore its removal is essential for the accurate measurement of dopamine receptors with 3H-agonists. Supported by PHS research grant MH33958-01.

7.12 THE DOPAMINE RECEPTOR IN THE INTERMEDIATE LOBE OF THE RAT PITUITARY GLAND IS NEGATIVELY COUPLED TO ADENYLATE CYCLASE. H. Meunier* and F. Labrie* (SPON: C. Radouco-Thomas), MRC Group in Molecular Endocrinology, CHUL, Québec, Canada G1V 4G2.

The predominant role of dopamine (DA) in normal brain function, as well as in diseases such as schizophrenia and Parkinson's disease, has been strong stimulus for research on the DA receptor and its mechanism of action. Functionally, the best known action of the DA receptor is its stimulatory effect on adenylate cyclase activity. However, the heterogeneity of brain tissue complicates the analysis of DA action in central nervous system. The intermediate lobe of the rat pituitary gland is a pure population of cells specialized in the secretion of peptides derived from proopiocortin. We have taken advantage of this system to study the interactions of the DA receptor with adenylate cyclase using a series of agonists and antagonists of known pharmacological activity in pars intermedia cells in primary culture. DA agents inhibit cyclic AMP (cAMP) accumulation with the following order of potency (K_d): 2-bromo-α-ergocryptine (0.20 nM) ≥ pergolide (0.27 nM) ≥ dihydroergocryptine (0.5 nM) > apomorphine (1.5 nM) > DA (5.0 nM) > (-)norepinephrine (30 nM) ≥ (-) epinephrine (50 nM). Propranolol (100 nM) was present during incubations with (-)epinephrine and (-)norepinephrine in order to block their interaction with the β-adrenergic receptor which is stimulatory on cAMP accumulation in pars intermedia cells. That changes in cAMP levels in pars intermedia are involved in the control of α-MSH secretion is strongly suggested by the finding that 25 to 35% inhibition by the DA agonist 2-bromo-α-ergocryptine of basal and 100 nM (-)isoproterenol-stimulated cAMP levels is accompanied by 50 to 70% inhibition of α-MSH release respectively. DA antagonists reverse the inhibition of cyclic nucleotide levels induced by 10 nM DA with the following order of potency: spiperone (0.02 nM) ≥ thioproperazine (0.04 nM) > haloperidol (0.5 nM) ≥ fluphenazine (0.6 nM) ≥ pimozide (0.8 nM). The potent neuroleptic (+)butaclamol reverses the inhibitory action of DA (K_d = 1.5 nM) while its pharmacologically weak enantiomer, (-)butaclamol, is 80 times less potent. The serotonergic antagonists cyproheptadine and methysergide can also act as weak DA antagonists while serotonin has no effect on cAMP accumulation or α-MSH secretion. The intermediate lobe of the rat pituitary gland offers a unique model of a dopaminergic receptor which inhibits adenylate cyclase activity in parallel with α-MSH secretion, thus indicating the presence of DA_- receptor. It is expected that the knowledge gained with this relatively simple model will yield useful information for the less accessible dopaminergic systems of the brain.

7.13 THE REGULATION OF OLFACTORY BULB ADENYLATE CYCLASE: INTERACTIONS BETWEEN DOPAMINE AND INSULIN. M. L. Barbaccia*, D. M. Chuang* and E. Costa. Lab. Preclin. Pharmacol., NIMH, St. Elizabeths Hosp., Washington, D.C. 20032.

Havrankova and her collegues (Nature 272:827, 1978) reported that different rat brain areas contain various densities of saturable, specific, high affinity, recognition sites for insulin. Moreover, the brain areas that contain the highest density of recognition sites also contain the highest density of insulin-like immunoreactivity (PNAS 75:5737, 1978). Olfactory bulb which has one of the highest contents of insulin and insulin binding sites was used to elucidate the location and role of brain insulin. Since rat olfactory bulbs contain dopaminergic cell bodies we investigated whether they also contain a dopamine dependent adenylate cyclase. Using 300μ slices of olfactory bulb we measured basal cAMP content at 10 minutes after a 30 minute period of preincubation. Dopamine in 3×10^{-5}M or greater increased cAMP content in olfactory bulb slices. Monolateral bulbectomy increased the responsiveness of the contralateral olfactory bulb adenylate cyclase to dopamine stimulation. The increase of cAMP caused by dopamine was partially inhibited by haloperidol (5×10^{-6} M) and facilitated bysulpiride (5×10^{-6}M) and IBMX (5×10^{-6}M). Insulin failed to cause an accumulation of cAMP in striatum and frontal cortex slices. We then tested the action of insulin (3 to 9×10^{-7}M) on the cAMP content of olfactory bulb. We found that also insulin increased the cAMP content in a concentration dependent manner. This action of insulin was unaffected by (5×10^{-6} M) haloperidol and increased by (5×10^{-6}M) sulpiride and IBMX (5×10^{-6}M). The data obtained are compatible with the view that activation of receptors for insulin and dopamine can increase the adenylate cyclase activity. Moreover, monolateral bulbectomy facilitated the accumulation of cAMP in the contralateral bulb elicited by insulin and dopamine. Hence the two neuromodulators appear to interact as if insulin receptors regulating cAMP content were modulated by D-2 receptors. Moreover, our data suggest that the contralateral bulb can modulate the dopamine activation of the adenylate cyclase of an olfactory bulb.

8.1 RUTHENIUM RED INDUCES A NONINACTIVATING NA CURRENT IN PERFUSED SNAIL NEURONS. W. L. Byerly & J. R. Stimers. Dept. Biological Sciences, University of Southern California, Los Angeles, CA 90007.

Ruthenium Red (RuR) blocks Ca binding and/or uptake in a variety of systems. Also, evolked and spontaneous release from synaptosomes and the frog neuromuscular junction is blocked by RuR. Since this later effect is dependent upon Ca current in the membrane, we chose to study its effects on the currents of a voltage clamped membrane. Using isolated, perfused, Limnea neurons, RuR was found to block the Ca current and induce a noninactivating Na current.

The methods are those of Byerly and Hagiwara (in press). Limnea neurons are perfused both intracellularly and extracellularly. All experiments are done using a suction electrode voltage clamp. All external solutions contain Ca^{2+} and Mg^{2+}.

When a cell is intracellularly perfused with CsAsp and bathed in a solution with Ca^{2+} as the only permeant cation, a prominent Ca current is seen. Within 5 minutes of adding RuR to the bath, 95% or more of the Ca current disappears. This indicates that RuR does block the Ca current in this preparation. As with other Ca blockers, the effect is not completely specific. RuR also blocks nearly 50% of the outward currents (with either K^+ or Cs^+ inside the cell). The presence of 5mM EGTA inside precludes this being a secondary loss of the Ca-dependent K current.

RuR has another effect, in that it induces a noninactivating Na current. The normal Na current seen in this preparation inactivates very fast, decaying to near zero within 10ms. However, in the presence of RuR a Na conductance turns on when the membrane potential is stepped above -30mV, fully activating above +10mV. The conductance decays less than 40% during a 60ms pulse. This is very similar to the kinetics and voltage dependence seen for the Ca current. However, when Na^+ is replaced by Tris in the bathing solution, no inward current is seen. The reversal potential of this current agrees with the Nernst potential for Na^+. It is selective for Na^+, in that neither K^+, Cs^+, Ca^{2+} nor Mg^{2+} contribute significantly to this current. Experiments are in progress to determine if this Na current passes through the normal Na channels, Ca channels or by some other mechanism.

8.2 CALCIUM-DEPENDENT SODIUM CONDUCTANCE IN MAMMALIAN DORSAL ROOT GANGLION NEURONS IN PRIMARY DISSOCIATED CELL CULTURE. E.J. Heyer, R.L. Macdonald. Dept. Neurology, Univ. of Michigan, Ann Arbor, MI 48109. Present address (EJH): Dept. Neurology, Mt. Sinai School of Medicine, New York, N.Y. 10029.

Action potentials (APs) dependent on sodium, calcium and potassium conductances can be elicited from mouse dorsal root ganglion (DRG) neurons in primary dissociated cell (PDC) culture. The rising phase of the action potential depends on activation of sodium conductance; its maximum rate of rise ($\dot{V}_{max}$) is directly proportional to peak sodium conductance. In addition, at resting membrane potential (RMP) neuronal input resistance (R_{in}) is due in part to sodium conductance. We have demonstrated that intracellular calcium reduced $\dot{V}_{max}$ and increased R_{in} suggesting that at least a portion of membrane sodium conductance was blocked by intracellular calcium. Tetrodotoxin (TTX), a sodium conductance blocker, also only partially blocked $\dot{V}_{max}$. Its action was probably on that part of the sodium conductance that was calcium sensitive.

PDC cultures of spinal cord dissected from 12-14 day old fetal mice were grown by conventional techniques. After 4-6 weeks in culture, intracellular recordings with 4M KAc-filled microelectrodes were made in neurons bathed in Tris buffered saline containing 5 mM calcium (TBS).

In calcium-free TBS, $\dot{V}_{max}$, AP duration and R_{in} were 231.6 V/sec, 1.0 msec and 26.0 MΩ. In bathing solutions containing calcium (2.5 or 5.0 mM) $\dot{V}_{max}$ was decreased, and AP duration and R_{in} were increased. Since DRG neurons contain a large voltage-dependent calcium conductance, it was possible that this effect was due to calcium entry into the neuron. Therefore, we performed two experiments. First, we blocked extracellular calcium entry by adding manganese (5.0 mM), a calcium conductance blocker, to TBS. $\dot{V}_{max}$, AP duration and R_{in} became 242.0 V/sec, 0.8 msec and 34.8 MΩ which were similar to values obtained in calcium-free TBS. Second, in manganese-containing TBS we restored the effect of calcium on $\dot{V}_{max}$ by injecting calcium intracellularly with a second micropipette containing 0.5 M $CaCl_2$ and 0.5 M KCl. No restoration was possible by injecting manganese intracellularly. Other divalent cations were studied to see if they would substitute for calcium. Barium and strontium substituted for calcium to permit long duration APs to be evoked in DRG neurons. However, only barium substituted for calcium to block $\dot{V}_{max}$ and therefore reduced sodium conductance. Addition of TTX to calcium-, manganese-, barium- and strontium-containing TBS reduced $\dot{V}_{max}$ to the same values, implying that TTX blocked that portion of sodium conductance blocked by some divalent cations. Supported by the Grass Foundation (Morison Fellowship) (E.J.H.),and NINCDS (NS 15225) and RCDA (NS 00408) (R.L.M.).

8.3 Ca^{2+} ACTIVATED K^+CURRENT NOISE AND RELAXATION MEASUREMENTS IN HELIX NEURONES. A. Hermann+ and K. Hartung+ (SPON: A.L.F.Gorman) Fakultät für Biologie, Universität Konstanz, D-7750 Konstanz, FRG.

Ionophoretic injection of Ca^{2+} ions into voltage clamped Helix neurones causes an increase of K^+ current which is associated with an increase of membrane current fluctuations. Membrane current noise generated by the Ca^{2+} injections disappears at the K^+ equilibrium potential and current relaxation reverses at a similar potential. For small outward currents the relationship between the variance of current fluctuations and the mean current is linear. The single channel conductance calculated from the current variance measurements is 3.4+0.4 pS (S.E.M., n=15). Relaxation measurements revealed that with small doses of Ca^{2+} injections the relaxation current can be fitted by a single exponential. At higher doses of Ca^{2+} injections two or more time constants were needed for a fit. The relaxation measurements further showed that the instantaneous current-voltage relation of the Ca^{2+} activated K^+ current is almost linear whereas in the steady state there is strong outward rectification. Relaxation time constants were in the range of 20-60 msec at potentials between -100 mV and +10 mV and increased in a voltage dependent manner with membrane depolarization. The Ca^{2+} activated current relaxation is also voltage dependent and changes e-fold per 28 mV change in membrane potential.

Prolonged injections of high amounts of Ca^{2+} depresses the outward current and the current fluctuations. Subsequent injection of Ca^{2+} into the cell could not activate the outward current again.

Extracellular TEA (tetraethylammonium) effectively blocks the Ca^{2+} activated K^+ current within seconds after application with a half-maximum blockade achieved at 0.7 mM. 4-AP (4-aminopyridine) had no blocking effect at these cells.

It is concluded that ionophoretic injection of Ca^{2+} ions into Helix neurones activates a K^+ conductance specific to K^+ ions and that opening and closing of K^+ channels is voltage- and time dependent. High amounts of intracellular Ca^{2+} or extracellular TEA block the Ca^{2+} activated K^+ conductance.

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8.4 CALCIUM ACTIVATED OUTWARD CURRENTS (I_c) IN VOLTAGE-CLAMPED BULLFROG SYMPATHETIC NEURONES. A. Constanti*, P.R. Adams and D.A. Brown* (SPON: D. Kunze). Department of Physiology and Biophysics, University of Texas Medical Branch, Galveston, TX 77550.

I_c's were studied in 2 ways: (1) from clamp-currents generated by depolarizing voltage-commands before and after suppressing inward Ca-currents; and (2) as outward currents induced by intracellular Ca-iontophoresis.

(1) Steady-state I/V curves following 40-100 ms depolarizing commands from a holding potential (V_H) of -30 mV frequently showed an "N"-shape, peaking at between +20 and +50 mV. The "N"-peak was reduced or abolished by omitting external Ca or by adding Cd (0.1 mM), Co (1 mM) or Mn (1 mM). Outward current trajectories at command potentials equal to or more positive than -10 mV showed an early phase, peaking in 5-10 ms, suppressible in Ca-free solution and/or 0.1 -0.5 mM Cd. Additionally a Ca-sensitive transient outward current was sometimes detected on depolarizing to $\geq$ -35 mV from -100 mV. This peaked in $<$ 5 ms and decayed within 20 ms. Both early outward and transient currents were inhibited by 1 mM TEA but not by 4-AP.

(2) Outward I_c- currents following iontophoretic injection of $CaCl_2$ were recorded using a single electrode voltage-clamp. During steady injection I_c peaked within 2-5 sec. Currents declined slowly thereafter but recovered rapidly on stopping injection. Brief (0.2-1 s) injections generated non-desensitizing responses offsetting in 0.5-5 s. Such responses increased linearly with iontophoretic dose up to 6 nC. For constant calcium doses, I_c increased steeply as V_H was made more positive from -60 mV, corresponding to a 12.5 mV increase in conductance (G_c) per e-fold depolarization up to -10 mV. In 25 mM [K], currents reversed in direction at -35 $\pm$ 5 mV (mean $\pm$ S.D., n = 8). V_{rev} shifted by 41 $\pm$ 15 mV (mean $\pm$ S.D., n = 6) on raising [K] from 2.5 to 25 mM. I_c evoked by intracellular Ca-injection was insensitive to external Ca, Cd or 4-AP but was inhibited 50% by 1 mM TEA.

These experiments show that many (though not all) bullfrog sympathetic neurones possess Ca-sensitive K-channels which are also voltage-sensitive, are blocked by low concentrations of TEA, and are activated sufficiently rapidly to aid spike repolarization.

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8.5 SINGLE CHANNEL AND NOISE ANALYSIS OF OUTWARD CURRENTS IN BULLFROG GANGLION CELLS. P.R. Adams, A. Constanti*, R.B. Clark*, C.E.Y. Adams* and D.A. Brown*. (SPON: O.S. Steinsland). Department of Physiology and Biophysics, University of Texas Medical Branch, Galveston, TX 77550.

Fragments of adult bullfrog ganglia maintained in culture for several weeks developed very smooth and round protruding neurons upon which it was routinely possible to establish gigohm seals. Both fresh and cultured neurons were used to perform noise analysis of maintained outward currents. After about 30 sec depolarization to between -10 and +20 mV, a small residual and noisy outward current was observed. The power spectrum of the current noise was approximately Lorentzian with a 1/f admixture. In 8 tests near 0 mV, the mean corner frequency was 8 Hz. The mean single channel conductance in 17 tests was 101 pS (80 mV driving force assumed). Both the residual outward current and the associated extra variance were abolished by perfusing cadmium (100 μM), TEA (10 mM) or calcium-free Ringer. Thus this highly conducting channel is assumed to be a calcium activated K-channel. In one test intracellular calcium injection yielded a sustained outward current with an elementary conductance of 97 pS, and a similar spectral shape.

Depolarized patches exhibited 2 main types of outward event. The large type exhibited a single channel conductance close to 100 pS, and was thus identified as a Ca-activated K-channel. The current through this channel at first ohmically increased with potential, and then decreased with further depolarization. At around +80 with respect to rest the open channel showed glitchy, rapid closings; at more positive potentials these became shorter and more frequent, so that the open channel was very noisy.

At very positive potentials ($\sim$ +200 mV) relative to rest, upward and downward glitches, though very short, seemed to be equally present. The rapid vibration of the channel in a partially open state may expain the apparent decrease in conductance at very positive potentials. The open times of these putative Ca-activated K-channnels was very variable from patch to patch, and could spontaneously increase at a given patch. The open time increased strongly with depolarization (e-fold for 22 mV). Since these channels could be recorded using Ca-free pipettes, they are probably activated by spontaneous internal calcium release.

Patches also usually showed a smaller (2-3x) shorter lived channel whose opening frequency increased strongly with depolarization, and may be a delayed rectifier.

Supported by NS 14986 and NS 14920.

8.6 INTRACELLULAR CALCIUM TRANSIENTS ELICITED BY SYNAPTIC AND ELECTRICAL MEMBRANE ACTIVATION AND BY THEOPHYLLINE MEASURED IN BULLFROG NEURONS USING ARSENAZO III. S.J Smith*, A.B. MacDermott F.F. Weight. Lab. Preclin. Studies, NIAAA, Rockville, MD 20852

We have used the indicator dye arsenazo III to measure three different kinds of intracellular calcium transients in neuronal cell bodies of isolated bullfrog sympathetic ganglia. B-cells were loaded with the dye by microiontophoresis, and their optical absorbance was measured simultaneously at four wavelength bands centered at 570, 610, 660, and 700 nm. Absorbance changes were identified as calcium signals on the basis of spectral characteristics. We found the following. (1) Voltage clamp depolarizations to potentials between -30 and +50 mV and trains of antidromic action potentials produced calcium signals which increased progressively during stimuli up to several seconds in duration. These signals are presumed to result from calcium entry through voltage-dependent channels. Upon return to resting membrane potential, the calcium signals returned to baseline over a monotonic time course lasting approximately one minute. The time course of calcium signal recovery resembles that of the post-tetanic hyperpolarization (PTH) observed in these neurons. It has been suggested that the slow potassium conductance underlying the PTH is activated by intracellular calcium ions (Minota, S., Jap. J. Physiol., 24:501, 1974). Our observations suggest that the accumulation and removal of cytoplasmic calcium ions may directly determine the time-dependence of that slow potassium conductance. (2) Trains of orthodromic synaptic stimuli produce a calcium signal even when the postsynaptic membrane potential is held constant by a voltage clamp. This calcium transient is presumed to result from the opening of transmitter-controlled channels. The amplitudes and kinetics of calcium signals elicited synaptically are roughly similar to those of the signals elicited by comparable trains of antidromic spikes. (3) In the presence of the methylxanthines caffeine or theophylline, rhythmic hyperpolarizations lasting 10-30 seconds occur spontaneously at intervals of a few minutes (Kuba, K., J. Physiol. 298:251, 1980; McCort, S.M. and Weight, F.F., Neurosci. Abstr. 5:47, 1979). In dye-loaded neurons treated with theophylline (5 mM), we observed increases in cytoplasmic calcium coincident with such spontaneous hyperpolarizations. This observation supports earlier suggestions that these hyperpolarizations result from cytoplasmic calcium increases acting by way of the calcium-activated potassium conductance mechanism. There are indications that methylxanthine-induced calcium oscillations may involve calcium movement between cytosol and another intracellular store. (This work partially supported by PHS NS 16671-01).

8.7 DIFFUSION OF Ca^{2+}, Ba^{2+}, H^+ AND ARSENAZO III IN NEURAL CYTOPLASM. J. A. Connor, Z. Ahmed and G. Ebert*. Univ. of IL, Dept. of Physiol. & Biophys. Urbana, IL 61801.

Experiments have been run to compare the movement rates of several small cations in nerve cell cytoplasm to their movement rates in saline solution. The relative diffusional rates of the indicator dye, arsenazo III, have also been examined. The purpose of the study has been to assess the effect of molluscan nerve cytoplasm on the diffusion coefficient of Ca^{2+} and H^+. Neural somata (300-600 µm diameter) from Archidoris monterreyensis were injected with indicator dyes, either arsenazo III (for divalent ions) or phenol red (for H^+) to concentrations of approximately 0.3 mM and 0.5 mM respectively. Following injection the electrode was removed from the cell and a second electrode containing either $CaCl_2$, $BaCl_2$ or HCl inserted for iontophoretic injection. Current pulses of 1 to 2 sec were used and the quantity of injected cations was in the neighborhood of 1 to 5 pmoles. The transient, local concentration changes of indicator-cation complex resulting from injections were monitored by measuring transcellular absorbance changes with two independent light collecting fibers (50 µm diameter). One optic fiber was centered directly over the injection site while the other was positioned at different locations about the cell surface. The injected quantities of ions were too small to cause appreciable steady baseline changes. Identical experiments were carried out using saline droplets of comparable size to the neurons. The droplets were formed under mineral oil and saline contained (in mM) 50 NaCl, 350 KCl, 3 $MgCl_2$, 10 mM Mops buffer at pH 7.4, plus the indicator dye.

The most striking differences between cell and saline droplet experiments occurred for injections of Ba^{2+}, an ion which is apparently poorly buffered by cytoplasm, and H^+. The time course of both signals was slowed by around an order of magnitude. For Ba^{2+} it required between 10 and 20 sec for the absorbance change to decay e-fold at the injection site in the neurons while in saline the corresponding period was about 1 second. The decay of the cellular signal for Ca^{2+} injection was much faster, probably due to high affinity buffering of this ion by cytoplasm. Also in the neurons the second optic fiber detected appreciable absorbance changes for Ba^{2+} injections even when separated from the injection site by 50 to 100 µm. For similar Ca^{2+} injections there was almost no signal. The time course of arsenazo III spread was also slower in the cytoplasm and a fraction of the injected dye appears to be bound and relatively immobile. Simulation studies are being run in an attempt to assess the relative importance of binding to other factors which might also slow diffusion. Supported by NS 15186 from NINCDS.

8.8 CHARACTERIZATION AND PARTIAL PURIFICATION OF FACTORS CONTROLLING THE GENERATION OF ACTION POTENTIALS IN PARAMECIUM. N. Haga,* M. A. Forte, Y. Saimi* and C. Kung,* Laboratory of Molecular Biology, University of Wisconsin, Madison, WI 53706.

The ciliated protozoan, Paramecium tetraurelia, offers a unique opportunity to utilize the combined techniques of genetics, biochemistry and electrophysiology in the analysis of the molecular interaction necessary for the generation of action potentials. Mutants unable to generate action potentials have been isolated as cells which show no backward swimming in response to ionic stimulation. Standard genetic crosses of these recessive mutants called pawns separate them into at least three complementation groups designated pwA, pwB and pwC. We have found that microinjection of cytoplasm from a donor wild-type cell into a recipient pawn of any of the three complementation groups restores the ability of the pawn cell to swim backward when stimulated with 20 mM K+ and hence restores the ability to generate action potentials in these cells. The restoration is not due to a simple addition of ions in the cytoplasm but represents a profound change in the excitable membrane of the recipient pawn cells as demonstrated by electrophysiological analysis under a voltage clamp. Restoration is maximal by eight hours and lasts up to 3 days. The factors from wild-type cytoplasm which restores backward swimming in the pawn mutants have been further characterized and purified. A cell homogenate is centrifuged at 27,000 xg and the supernatant (S_1) further centrifuged at 100,000 xg. The pellet from the 100,000 xg spin (P_2), the original supernatant (S_1) and the cell homogenate from the wild type cells were all active in curing pwA, pwB or pwC cells when injected. The restoration of backward swimming by these fractions can occur in the absence of protein synthesis. The activity for pwC is apparently not sensitive to endogenous or exogenous RNase's, is destroyed by trypsin and is inhibited slightly by divalent cations. P_2 has been further fractionated on sucrose gradients. The activity for pwC is associated predominantly with a membrane vessicle band at 45% sucrose. This final fraction represents about a 30 fold purification from the original cell homogenate. Further fractionation is in progress. Supported by P.H.S. grant GM22714 and N.S.F. grant BNS79-18554 to C.K. and N.I.H. postdoctoral fellowship GM06491 to M.F.

8.9 A DROSOPHILA MUTANT AFFECTS SODIUM CHANNEL LEVELS. L.M. Hall, S.D. Wilson*, J. Gitschier and G.R. Strichartz. Dept. of Genetics, Albert Einstein Coll. of Med., Bronx, NY 10461 and Department of Physiology and Biophysics, SUNY at Stony Brook, NY 11794.

We have used ^{3}H-saxitoxin as a probe in binding studies of the voltage-sensitive sodium channel in mutant and wild-type strains of Drosophila melanogaster. Of particular interest is the mutant nap^{ts} (no action potential, temperature-sensitive) in which nerve conduction is blocked at elevated temperatures (34°C) but recovers rapidly when the temperature is decreased. Both larvae and adults show a corresponding temperature-induced paralysis which is rapidly reversed at lower temperatures (Wu, C. F., B. Ganetzky, L.Y. Jan, Y.-N. Jan, and S. Benzer, Proc. Natl. Acad. Sci. U.S.A., 75: 4047, 1978). Our ^{3}H-saxitoxin binding studies have revealed that this mutant has a reduced number of saturable saxitoxin-binding sites compared to wild-type controls. When binding assays are conducted on Drosophila head extracts at 4°C, the nap^{ts} mutant has 69.7 ± 3.2 fmoles of toxin-binding sites per mg protein while the wild-type has 114.8 ± 24.3 fmoles/mg protein. This same difference persists if the assays are conducted at 30°C. There are no differences between nap^{ts} and the wild-type with respect to K_D, pH sensitivity, thermal inactivation, ion sensitivity, and tetrodotoxin inhibition of the saxitoxin-binding receptor. From these results we suggest that there are no structural differences between sodium channels in the mutant and wild-type. The nap^{ts} gene appears to affect regulation of sodium channel levels.

As one test of this suggestion, we have pharmacologically reduced the number of functional sodium channels in the wild-type strain by feeding flies sublethal doses of tetrodotoxin. After this treatment wild-type flies resemble the nap^{ts} mutant in that they can be paralyzed at temperatures which normally have no effect on wild-type locomotion. We will discuss how all of the nap^{ts} characteristics (temperature-induced paralysis, temperature-induced blockade of action potentials, altered refractory period, and enhanced sensitivity to tetrodotoxin) can be explained on the basis of reduced numbers of sodium channels. (Supported by N.I.H. grant NS 16204 to L.M.H. and NS 12828 to G.R.S.)

8.10 GENETIC MANIPULATION OF POTASSIUM CHANNEL KINETICS AND MATURATION TIME IN DROSOPHILA: A VOLTAGE CLAMP STUDY. Lawrence Salkoff* (SPON: J.L. Rosenbaum). Dept. of Biology, Yale Univ., Box 6666, New Haven, CT. 06511.

Mutations mapping at the X-linked Shaker locus of Drosophila melanogaster alter different properties of the potassium channels that carry the fast, transient, outward current (the A-current) in the dorsal longitudinal flight muscles (DLM). One mutation Sh^5, alters the biophysical properties of the A-current channels. Two other mutations, Sh^{rK0120} and Sh^{KS133}, suppress the normal developmental appearance of the channels during the pupal period. The Sh^5 mutation affects only inactivation: it causes the channels to inactivate (close) twice as fast as wild-type channels, and it accelerates recovery from inactivation. The channels of heterozygote (Sh^5/wild-type) animals close at a rate intermediate to mutant and wild-type channels. Heterozygotes also recover from inactivation at a rate intermediate between wild-type and mutant. Other active and passive membrane properties are not apparently altered by this mutation. Thus, the Sh^5 A-current activation curve and steady-state inactivation curve are indistinguishable from wild-type. Membrane input resistance is also unaffected.

The Sh^{rK0120} and Sh^{KS133} mutations suppress the normal maturation of the A-current channels during pupal development. The pupal period in D. melanogaster lasts for approximately 96 hrs. During this time the DLM fibers are formed. The DLM contains two distinct sets of potassium channels, the A-current channels, and the delayed rectification channels that carry a slower activating, noninactivating current. These channels appear in the membrane at different times. The A-current channels appear in the normal membrane after 55 hrs of pupal development and reach their final density by 72 hrs. Delayed rectification channels are sparse or absent at 72 hrs (Salkoff & Wyman, 1981, Science: 212:461-463). Delayed rectification then develops and is mature at 96 hrs, the time of adult eclosion. Sh^{rK0120}/Sh^{rK0120} or Sh^{KS133}/Sh^{KS133} muscle cells have no A-current response even at 96 hrs. At this stage these mutant cells have only delayed rectification. Heterozygous (Sh^{rK0120}/+ or Sh^{KS133}/+) animals have half the normal A-current response at all pupal stages. In the homozygous mutants the A-current finally appears 24 hrs after eclosion in the majority of adult muscle cells. The expression of these mutant genes may be "leaky" or a different gene may be active in the adults.

8.11 BLOCKADE OF INWARD RECTIFICATION IN IMMATURE STARFISH OOCYTES BY INTRACELLULAR H^+. W.J. Moody and S. Hagiwara. Jerry Lewis Research Center & Dept. of Physiology, UCLA, Los Angeles, CA 90024 and Dept. of Biological Sciences, USC, Los Angeles, CA 90007.

The sensitivity of inwardly rectifying K currents to decreases in intracellular pH (pH_i) was studied in immature oocytes of the starfish, M. aequalis. K currents were measured under voltage-clamp and pH_i was recorded with recessed-tip pH microelectrodes. Average values for pH_i and V_m in normal Ringer were 7.09 ± 0.08 and -71.2 ± 1.6 mV, respectively (n=14). When the external pH was decreased from 7.8 to 5.0 using the membrane-impermeant buffer biphthalate (20mM), pH_i decreased only slightly or not at all (0-0.12 unit), and the inward rectifier currents were affected only slightly or were unaffected (0-12% block). In pH 5 biphthalate solutions, the greatest blocking effects did not correlate with the largest pH_i changes. When the external pH was decreased from 7.8 to 5.0 using the membrane-permeant buffer acetate (10mM), pH_i decreased rapidly to 5.84-6.0, and the inward rectifier currents were virtually eliminated (90-98% block). Since the buffer strength of 20mM biphthalate is 60% greater than 10mM acetate at pH 5.0, it is unlikely that these results are explained by differing abilities of the two solutions to change the pH in a highly buffered extracellular space. Titration curves of inward rectifier currents vs. pH_i were obtained by exposing single cells to a series of acetate-buffered solutions with pH values between 7.8 and 5.0. pH_i was recorded continuously and voltage-clamp pulses delivered after pH_i had attained a steady-state in each solution. The titration curves were very consistent among cells. The pooled data could be described adequately by assuming that three H^+ ions bind to a single site to block the channel. The apparent pK of the site is 6.27. We examined the possibility that the internal H^+ block could be explained by a decrease in internal Na^+ activity ($[Na^+]_i$), since the channel is known to be blocked by low $[Na^+]_i$ (Hagiwara and Yoshii, J. Physiol. 292:251). $[Na^+]_i$ was recorded with Na-sensitive microelectrodes during exposure to low pH acetate solutions. Rather than a decrease, and increase in $[Na^+]_i$ was recorded when pH_i was decreased. This suggests that the inward rectification channel may be even more sensitive to changes in intracellular pH than our titration curves indicate.

8.12 PARTIAL DEMYELINATION AS A TEST FOR LATERAL DIFFUSION OF SURFACE CHARGES IN THE FROG NODE OF RANVIER. P.A. Pappone* and M.D. Cahalan. Dept. of Physiology and Biophysics, Univ. of Calif., Irvine, CA 92717.

We have examined the effects of the amino group reagent trinitrobenzene sulfonic acid (TNBS) on the sodium currents of voltage clamped frog myelinated nerve and skeletal muscle fibers. TNBS is membrane impermeant and converts normally positively charged amino groups into neutral trinitrophenylated derivatives. In both fiber types externally applied TNBS caused a rapid shift in the voltage dependence of steady state sodium current inactivation and in the time constants for development of and recovery from inactivation to more hyperpolarized potentials. Sodium current activation was shifted in the same direction, but to a lesser extent. These effects suggest that TNBS treatment results in an increase in the negative surface potential of the membrane. The effects of TNBS are largely irreversible in muscle fibers, but reverse rapidly (within 1-3 min) in nerve fibers following short exposures (<1-2 min) to TNBS. Nerve fibers recover more slowly from the TNBS-induced shift following longer exposures. Since the reaction of TNBS with amines results in a stable product, we suggest that the reversibility of the TNBS effect in the nerve may be due to diffusion of the modified membrane components laterally into the internodal membrane. In the nerve fibers only the 0.5-1μ node of Ranvier is exposed to the reagent, and diffusion of membrane components with mobility like that of lipids in bilayers would be quite rapid over this distance. To test this hypothesis we examined the effects of TNBS on partially demyelinated nerves. Normal nerve fibers were exposed to 0.5M urea in Ringers for 2-5 min. Following washout of the urea there was a 2-16x increase in the membrane capacity and a 1.5-6x increase in voltage-dependent potassium current, indicating that extranodal nerve membrane was exposed by the treatment. In the demyelinated nerve fibers recovery from the TNBS-induced shift was slow or absent following short (~1 min) exposure to the reagent, supporting the hypothesis that the TNBS-modified membrane components are free to diffuse laterally in the nerve membrane. This result suggests that TNBS may be modifying membrane lipids. If so these results indicate that the charge on membrane lipids can influence the gating of sodium channels. (Supported by a postdoctoral fellowship to P.A.P. from the Muscular Dystrophy Association of America and NIH Grant NS14609).

8.13 ACTION POTENTIAL MECHANISM OF RAT CORTICAL NEURONS IN CELL CULTURE, Marc A. Dichter and James Lisak*, Dept. of Neurology, Harvard Medical School, Children's Hospital, Boston, MA 02115.

Rat cortical neurons maintained in dissociated cell culture have thin, rapidly rising, overshooting action potentials (APs) (Dichter, Brain Res. 149:279-293, 1978). Removal of Ca or application of the Ca channel blocking agent, Co, has minimal effects on AP morphology, but these APs disappear in the presence of tetrodotoxin (TTX) at 10^{-7} g/ml. Thus, these thin APs appear to be generated by a Na-dependent mechanism. In the presence of TTX, many neurons have small to moderate sized, somewhat wider, action potentials which are eliminated by Co. If the voltage sensitive K conductance in these cells is blocked by TEA (25 mM), or if Ba is substituted for Ca, wide and less rapidly rising, overshooting APs appear in all the neurons. These APs appear to be generated by voltage sensitive Ca channels in that they are unaffected by tetrodotoxin, are blocked by Co and verapamil, and their overshoots are a function of the extracellular Ca or Ba concentrations (with slopes of overshoot vs log Ca or log Ba being close to 30 mV/decade change). We conclude that the cortical neurons in culture have voltage sensitive Ca channels which under normal conditions contribute relatively little to the soma action potential. When K channels are blocked or when Ca currents are enhanced, Ca dependent APs can be expressed by essentially all the neurons.

In studies using intermediate concentrations of TEA (10 mM) where Ca APs are more variably present, low threshold APs and APs elicited with short current pulses appear to be Na-dependent APs, whereas spontaneous, synaptically driven APs (possibly arising from EPSPs in dendrites) and APs triggered by larger, longer current pulses are Ca-dependent APs. These data suggest that the Ca-dependent APs are generated in dendrites and the Na-dependent APs are generated in the soma or axon hillock.

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9.1 ORGANIZATION OF CORTICOSPINAL PROJECTIONS AS DEMONSTRATED BY THE COMBINED TECHNIQUES OF MICROSTIMULATION AND METABOLIC LABELING. E. Kosar*, and H. Asanuma (SPON: V.J. Wilson). The Rockefeller University, New York, N.Y. 10021.

Activation of restricted portions of the ventrolateral horn of the spinal cord by microstimulation of the motor cortex in both the rat and the monkey could be demonstrated using the [^{14}C] 2-deoxyglucose technique. Under halothane anesthesia, the motor cortex was exposed and a closed chamber installed. Anesthesia was then discontinued. Microstimulation (6-20μamp x 10-13 pulses of 0.2msec duration, 300Hz) was applied to a cortical focus via a tungsten-in-glass microelectrode to elicit movement of individual muscles of the distal forelimb or digits. [^{14}C] 2-DG was injected intravenously (100μCi/1kg of body weight) while microstimulation was continued for 45 minutes. The elicited movement remained stable throughout the full experimental period. The animal was subsequently overdosed with barbiturate and perfused intracardially with 5% glutaraldehyde-0.5% paraformaldehyde. The brain and spinal cord were removed immediately, frozen in Freon XXII, sectioned on a cryostat (20μm) and processed according to the procedure of Sokoloff et al. (J. Neurochem. 28: 1977). Following the appropriate exposure period, alternate sections of the brain and spinal cord were examined by projecting the autoradiograms onto a television screen. Sections were traced from the screen and labeling was reconstructed 3-dimensionally. Metabolic labeling at the stimulation site extended approximately 1.5mm rostro-caudally in motor cortex. Unilateral and bilateral labeling was observed in the spinal cord. Unilateral labeling was located contralaterally in only a restricted portion of the ventrolateral cervical cord in all animals and was localized to the lateral portion of the motoneuron pools. This labeling extended rostro-caudally in the cervical cord approximately 4mm in the rat and 7mm in the monkey, maintaining the same medio-lateral position throughout its full extent. The bilateral labeling was located in the position of the ventromedial motoneurons related to axial musculature. Metabolic labeling was also observed at other levels of the spinal cord but the density of labeling appeared equal on both sides. This is in contrast to the restricted unilateral labeling in the cervical cord described above. The combination of these two techniques has thus far proven useful in analyzing the pattern of activity within the CNS originating from a small restricted area of the cortex.

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9.2 INPUT TO CAT'S PERICRUCIATE MOTOR CORTEX (AREA 4γ) FROM PARIETAL CORTEX (AREA 5b AND AREA 7). R. S. Waters, A. Mori*, E. Kosar, R. S. Babb and H. Asanuma. The Rockefeller University, New York, N.Y. 10021.

We have reported (Babb et al. Neurosci. Abstr. 6: 159, 1980) that horseradish peroxidase (HRP) injected into the forelimb region of the motor cortex (area 4γ) labelled cell bodies within the suprasylvian gyrus (areas 5b and 7). The present study was undertaken to examine the physiological properties of these neurons and to determine the caudal extent of the neurons that project to area 4γ. Under halothane anesthesia a double-barreled chamber was installed over the motor and parietal cortices. An array of 8 stimulating microelectrodes was inserted into area 4γ. The receptive field of neurons around each electrode was examined first and then threshold current for muscle contraction was determined. A single recording microelectrode was driven systematically into areas 5b, 7 or 19 while ICMS was delivered simultaneously through the 8 stimulating electrodes. Whenever an antidromically activated neuron was encountered, the effective stimulating electrode was determined and an examination of the receptive field of the neuron was made using natural stimulation by air puffs, brush stroke, pressure to deep structures, and passive movement of joints. Receptive fields of neurons were also examined in penetrations where antidromically activated neurons were not encountered. At the conclusion of an experiment HRP was injected through the recording electrode, at the site where an area 7 neuron was activated antidromically from area 4γ. After a survival time between 44-48 hrs., the subject was sacrificed, the brain perfused, and the tissue processed using the tetramethyl benzidine method.

The following results were obtained: (1) Neurons in area 5b and area 7 could be activated antidromically from electrodes in 4γ. (2) Neurons in caudal 5b and area 7 did not respond to peripheral stimulation nor to auditory or visual stimuli. (3) The majority of the 5b and area 7 neurons projected to the precruciate region of area 4γ. The highly organized projections to motor cortex we reported for neurons in the anterior and posterior ansate area (Waters et al., Neurosci. Abstr. 6: 124, 1980) were not found for areas 5b and 7. (4) Following injection of HRP into the region of antidromically activated neurons in area 7, labelled neurons were found in area 19. Since area 19 is known to receive higher order visual information it is likely that some complex visual information may reach the motor cortex through areas 5b and 7.

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9.3 THE MONKEY'S PREMOTOR CORTEX. S. P. Wise and M. Weinrich, Laboratory of Neurophysiology, NIMH, Bethesda, MD.

The definition of a premotor cortex (PM), as distinguished from the precentral motor cortex (MI) or any other motor representation, remains uncertain. Investigations employing microelectrode methods should improve the understanding of PM, but previously neither the location of isolated neurons nor the cytoarchitectural characteristics of the region have been clearly described. Therefore, we have initiated a re-investigation of PM.

A rhesus monkey was operantly conditioned to depress one of four keys located in a perimeter at arms length. While the monkey pressed one key, another of the four keys, selected randomly, was illuminated after a randomly varied delay. This key thereby became the next target. A barely discernible visual cue near the target key, appearing after another variable delay, signaled the monkey to move and depress the target. The monkey was required to make the movement within a short period of time, near the limit of reaction time. Reaction time was typically 350 msec; movement time, an additional 250 msec. EMG of selected muscles and EOG were monitored periodically during the recording sessions.

(1) Many (149/221) neurons in PM show changes in activity markedly (90-190 msec) before the onset of a voluntary movement (20-120 msec before the onset of EMG change) and usually continue their altered discharge rate throughout the movement and for 50-350 msec after the termination of movement. Their activity is often (57/149) specific for the direction of arm movement. (2) These neurons are located within the frontal agranular cortex, corresponding to a part of area 6 as defined by the absence of a large population of giant, layer V pyramidal cells in addition to the lack of an internal granular layer. (3) PM can be distinguished from the MI representation by its markedly increased threshold for intracortical microstimulation effects (constant current, 30 msec trains, 0.2 msec pulses at 333 Hz). The threshold exceeds 90 μA in 37/40 penetrations. Conversely, MI can be defined by the consistently low microstimulation thresholds (28/30 penetrations at <50 μA). And (4) a substantial population of neurons (102/221) change their activity in relation to motor set and/or signals which indicate the location of motor targets.

These features are consistent with a role of this part of area 6 in the control of movement, especially the dynamic phase of a voluntary movement, and with the concept of a distinct premotor cortical field which constitutes part of a belt of somatic sensorimotor cortex surrounding the "primary" motor (MI) and somatic sensory (SI) cortex.

9.4 THE MOTOR CORTEX OF THE RAT: DEFINITION BY MICROSTIMULATION AND CYTOARCHITECTONICS. J. P. Donoghue and S. P. Wise, Laboratory of Neurophysiology, NIMH, Bethesda, MD. 20205

The somatic sensorimotor cortex of the rat contains several distinct cytoarchitectonic fields. Welker (Brain Res., 26:259, 1971) showed that the first somatic sensory (SI) cortex, as defined by cells sensitive to cutaneous inputs, corresponds to the most densely granular part of the cortex. But there is not, as yet, a clear cytoarchitectural definition of the rat's motor cortex. We have used intracortical microstimulation and standard neuroanatomical methods to define the first motor (MI) cortex in the albino rat.

Retrograde axonal transport experiments demonstrate that the corticospinal projection originates, in part, from two cytoarchitecturally distinct fields of the frontal agranular cortex (FAC). Anterograde transport experiments show that fibers from FAC project to the ventral horn of the spinal cord, in addition to a denser projection to the intermediate spinal laminae. These findings suggest that both of these FAC fields are part of the somatic sensorimotor cortex. Under ketamine anesthesia, relatively low threshold microstimulation (10-65 uA, 30 msec trains, 0.2 msec pulses, 333 Hz) elicits movements of the contralateral head, trunk, or forelimb from only one of these FAC fields. In the other field, stimulation thresholds generally exceed 100 uA. The low threshold field can be characterized by the relative homogeneity of its superficial laminae This appearance contrasts, in thionin stained sections, with the less densely staining layer III of the adjacent higher threshold FAC field. We therefore conclude that the low threshold FAC field corresponds to MI, while the higher threshold, spinally projecting part of the FAC is a distinct cortical field within the somatic sensorimotor cortex.

Caudolateral to the low threshold FAC, we observed a continuation of the low threshold (hindlimb and forelimb) microstimulation responses into the granular cortex. Such an overlap between the SI and MI representations was originally described by Hall and Lindholm (Brain Res., 66:23, 1974). Microstimulation of other parts of the granular cortex at <65 uA does not evoke movement.

These experiments demonstrate that a cytoarchitecturally distinct cortical field can be identified as a major part of MI in the rat. The rat's MI representation apparently differs from that of certain primates in that it includes granular cortex which is also part of the SI cortex.

9.5 CLASSIFICATION OF HINDLIMB AREA 4 UNITS AS RELATED TO FORCE DURATION AND DIRECTION IN THE CONDITIONED MONKEY. S. A. Sahrmann, M.H. Clare, E. B. Montgomery Jr.*, and W. M. Landau. Dept. Neurol., School. Med., Washington U., St. Louis, MO 63110.

Rhesus monkeys were conditioned with light signals to perform four randomly selected tasks with the hindfeet. Force was exerted bilaterally on fixed bars with attached strain gauges. The tasks were: (1) to exert a strong plantarflexion force, hold it a random time, on cue exert a strong, brief, rapid dorsiflexion force and return to rest (rapid-relax); (2) to do the converse; (3) to plantarflex into a small force window, holding until signalled to return to rest; and (4) to perform the converse of (3).

A preliminary report (Sahrmann, S.A., et al. Soc. Neurosci. Abstr., Vol. 5, p. 384, 1979) designated units as phasic if their firing rate did not remain increased for the 200 msec. after achievement of force criterion for hold tasks. The tonic units showed sustained activity during hold. Present analysis of 228 units recorded from 3 rhesus monkeys indicated that 65% were phasic and 17% were tonic (sustained increased activity for at least 500 msec. after force criterion).

Units were further classified as Directional if increased activity was consistently related to development of either plantarflexion or dorsiflexion force (103/228, 45%). Bi-directional units were those with increased activity during both plantarflexion and dorsiflexion force (99/228, 43%). Inhibitory units were those with decreased activity during plantar and/or dorsiflexion (26/228, 11%).

Directional units were further subgrouped according to the presence or absence of additional phases of increased activity other than that with primary agonist contraction. Some units were similar to spinal motoneurons since increased activity exclusively paralleled initiation of agonist contraction (26/103, 25%). Others, those with high resting frequencies, were inhibited during antagonistic contraction (37/103, 36%). A third group showed increased activity during antagonistic relaxation as well as during agonist contraction (40/103, 39%).

Bi-directional units were designated as symmetric if activity during both plantar and dorsiflexion was similar (17/99, 83%). Asymmetric units were more strongly associated with force development in one direction than in the other (82/99, 83%).

9.6 RELATIONSHIP OF CLASSIFIED HINDLIMB AREA 4 UNITS TO FORCE MAGNITUDE IN THE CONDITIONED MONKEY . M.H. Clare, S.A. Sahrmann, E.B. Mongtomery Jr.* and W. M. Landau. Dept. Neurol., School. Med., Washington U., St. Louis, MO 63110.

Rhesus monkeys were conditioned with light signals to perform four randomly selected tasks with the hind feet. Force was exerted bilaterally on fixed bars with attached strain gauges. The tasks required either a large (1 kg) plantar or a large dorsiflexion force maintained for a random period, and at a light cue, a large rapid force in the opposite direction (rapid reversal) followed by relaxation. A small plantar or dorsiflexion force (200 gm) held for a random period, with relaxation on light cue was also required. Thus the activity of each cortical unit was recorded during large maintained, large rapid-reversal and small maintained forces in both plantar and dorsiflexion.

The large rapid-reversal force was associated with the peak discharge frequency of 56% of the phasic units (as classified in Sahrmann, S.A., et al., Soc. Neurosci. Abstr., vol. 7, 1981) but with only 24% of the tonic units.

Few units were found with frequency changes proportional to small and large forces (Directional 12% and Bi-directional 8%). Peak frequency was associated with either or both large forces for 27% of the Directional and 46% of the Bi-directional. The asymmetric units usually had greater frequency changes for both large forces than for the small forces.

The Directional units had the highest incidence of the peak frequency during the small force equalling or exceeding that of the large force, (35% Directional; 20% Bi-directional). Twenty-five percent of the Directional and 8% of the Bi-directional units increased their firing rate with large forces exclusively.

Most Inhibitory units showed decreased discharge during force in both directions. The magnitude of the decrease in firing frequency was not related to the magnitude of the force but did vary for plantar and dorsiflexion.

10.1 EVIDENCE THAT ENDOPEPTIDASE-CATALYZED LHRH CLEAVAGE CONTRIBUTES TO THE REGULATION OF MEDIAN EMINENCE LHRH LEVELS DURING POSITIVE FEEDBACK. J.P. Advis*, J.E. Krause* and J.F. McKelvy, Department of Neurobiology and Behavior, SUNY Stony Brook, New York 11796.

In order to examine the possible physiological relevance of peptidases acting on LHRH, we have studied the degradation of LHRH by homogenates of median eminence from ovariectomized, estradiol-progesterone treated rats, decapitated at three hour intervals after progesterone administration (10.00 h). It is known that under these conditions an increase in median eminence LHRH content (13.00 h) precedes the release of LHRH into the portal vessels, which subsequently gives rise to a surge-like release of pituitary LH (16.00 h). Furthermore, both the increase in LHRH content and of serum LH can be prevented by prior administration of the noradrenergic synthesis inhibitor diethyldithiocarbamate (DDC).

Total LHRH peptidase activity was assessed by HPLC estimation of the loss of synthetic LHRH incurred when aliquots of supernatant (5'/10,000g) were incubated (37°/30 min.) with LHRH under optimal conditions. LHRH degradation was measured as initial velocities using saturating substrate concentrations. Characterization of degradation products by HPLC and amino acid analysis revealed a rate limiting step to TYR^5-GLY^6 bond scission. Other studies showed that the responsible activity is a metalloendopeptidase. LHRH content and serum levels of gonadotropins were estimated by RIA.

LHRH degradation was decreased ($p<0.01$) three hours after progesterone administration, at a time when LHRH content is increased ($p<0.01$), and serum LH remained at basal levels (13.00 h). At the time of the LH surge (16.00 h) LHRH degradation was still low and LHRH content had returned to basal levels. LHRH degradation in animals which received a single dose of DDC, one hour before progesterone administration was higher ($p<0.01$) than in saline treated controls at 13.00 h and 16.00 h.

We conclude that the degradation of LHRH by a metalloendopeptidase may contribute to the regulation of LHRH levels appropriate for gonadotropin release.

Supported by NSF BNS 7684506 and NIH RCDA AM 00751 (JFM).

10.2 IN VIVO LHRH RELEASE ESTIMATED WITH PUSH-PULL CANNULAE AND SIMULTANEOUS SERUM LH MEASUREMENT IN OVARIECTOMIZED EWES. J.E. Levine, F. Pau*, V.D. Ramirez, and G.L. Jackson*. Dept. of Physiology and Biophysics, and Dept. of Veterinary Biosciences, University of Illinois, Urbana, IL 61801

A neural oscillator may regulate pulsatile release of LH. This regulation likely is achieved through rhythmic discharge of LHRH from axon terminals in the median eminence (ME). Recently we showed that release of LHRH can be estimated in unanesthetized animals using the push-pull cannula (PPC) (Endo 107:1782). Combining this technique with measurement of serum LH, we attempted to determine the: 1) pattern of spontaneous LHRH output from the ME of the ovariectomized (OVX) sheep, and 2) temporal relationship between LHRH and LH release. Thirty days after ovariectomy, ewes received a permanently implanted PPC (20g). The tip of the PCC was directed into the lateral ME. Post-implantation recovery was at least 6d. Before perfusion, a stylette was removed from the outer PPC and an inner cannula assembly (28g) inserted. Artificial CSF was pumped through the inner cannula and pulled between the cannulae at 18ul/min. Continuous 10min fractions were collected, acidified, and stored for LHRH RIA. Blood samples were obtained every 10min via jugular catheter, each being drawn 5min after the start of a perfusate collection interval. Duration of sampling was 5-7h. Plasma LH levels were determined by RIA.

LHRH output was distinctly pulsatile, occurring at a frequency of about 1 pulse/40min. Peak values were consistently within 1.0-1.6pg/10min, while trough values were ≤0.6pg/10min. The pattern of LHRH output remained unchanged throughout all perfusions (5-7h). Plasma LH values also were pulsatile, and LH peaks occurred either during the same interval or interval after an LHRH pulse. LH pulses always were preceded by LHRH pulses, but LHRH pulses were not always followed by LH pulses. Data from one sheep were subjected to spectral analysis. Peak frequencies for LHRH and LH were identical (1.5cycles/h) and the squared coherence was .77. Histological examination revealed that each cannula tip was placed in the lateral ME. These experiments demonstrate for the first time that hypothalamic LHRH release in the OVX ewe occurs in discrete pulses, and that LH pulses appear to be preceded by LHRH pulses. Furthermore, this study establishes the push-pull perfusion technique as a direct method by which the output of releasing factors can be measured and correlated with circulating pituitary hormone levels. Supported by NSF Grants SER 76-18255 and PCM 77-04656 and NIH Grant HD 13037.

10.3 IDENTIFICATION OF LUTEINIZING HORMONE-RELEASING HORMONE (LHRH) NEURONS IN THE ARCUATE NUCLEUS OF HYPOTHALAMIC SLICES. M.J. Kelly, O.K. Ronnekleiv Dept. of Physiology (MJK), and Dept. of Psychiatry (OKR), Univeristy of Pittsburgh School of Medicine, Pittsburgh, PA 15208.

Arcuate (ARC) neurons have been found to be acutely sensitive to 10^{-10} M 17β-estradiol when applied to the medium bathing hypothalamic slices (Kelly et al., Exp. Brain Res. 40:440 (1980). We have labeled these neurons with procion yellow and have found that these estrogen-responsive neurons are small fusiform cells with little branching of the dendrites and little or no spine-like appendages. In order to identify these neurons, it was necessary to develope an immunocytochemical technique which was compatible with the procion yellow fluorescence. Cycling female rats were killed and sagittal hypothalamic slices were prepared as previously described (Kelly et al., 1980). The 400 μm thick slices were then immediately immersed in cold 4% Paraformaldehyde in .1 M phosphate buffer (pH 7.2) for 1-1/4 hrs. Then the slices were transfered to phosphate buffered saline (10% sucrose, pH 7.2) and refrigerated until cutting. The slices then were taken, frozen rapidly in isopentane cooled to -75°C in a dry ice/ethanol mixture, and 16 μm sections were cut at -17°C on a cryostat. The sections were kept frozen until the PAP immunochemical procedure (Sternberger, 1974) was done using the WP-1 antiserum (courtesy of Dr. R.L. Eskay) for LHRH. LHRH-immunoreactive neurons were identified scattered throughout the ARC region. This staining was abolished by pre-incubation of the antiserum with synthetic LHRH. The LHRH-immunoreactive neurons have a similar morphology as the identified estrogen-sensitive neurons previously described. We cannot say at this point that these are the same cells, but the compatibility of this staining procedure with the procion yellow fluorescence will allow us to correlate the electrophysiological response to estrogen with the peptide content of the cell.

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10.4 IMMUNOCYTOCHEMICAL LOCALIZATION OF LHRH IN MALE AND FEMALE RAT BRAIN: A QUANTITATIVE COMPARISON. B.D. Shivers, R.E. Harlan*, J.I. Morrell and D.W. Pfaff. The Rockefeller University, New York, NY 10021.

Luteinizing hormone releasing hormone (LHRH) regulates functions which are sexually dimorphic and steroid dependent. In this study LHRH was immunocytochemically localized in the brains of adult rats of both sexes, using Sternberger's peroxidase-antiperoxidase method and Silverman Rb III primary antiserum. Four groups of rats were used: intact males, gonadectomized (gdx) males, gdx females and gdx females with estradiol (E2) capsules. Each brain was examined from midseptal through midbrain intercollicular levels inclusive.

	intact ♂	gdx ♂	gdx ♀ + E2	gdx ♀
Total number LHRH cells ($\bar{x}$ + SE, N=4)	507±52*	130±58	146±41	82±30
% LHRH cells dark	81.9*	54.5	51.2	47.1
% LHRH cells in DBB+POA	75.1#	55.4	63.7	65.8

* $p < .01$ vs. three other groups
\# $p < .005$ vs. gdx male and gdx female with E2.

Intact male rats had a significantly greater number of visualized LHRH cell bodies than did the three other groups. Cells were classified as dark or light based on the amount of immunoreactive cytoplasmic material. Intact males showed a significantly greater percentage of dark cells than did the three other groups. No differences in either cell number or percentage of dark cells were found among the three other groups. The majority of LHRH cells was seen in the diagonal bands of Broca (DBB) and preoptic area (POA). Intact males had a significantly higher percentage of LHRH cells in the DBB+POA than did gdx males or gdx females with E2.

The results suggest that LHRH content in cell bodies is greater in intact males than in the three other groups. One interpretation of the decrease in the percentage of LHRH cells in the DBB+POA following gonadectomy in the male is that some LHRH cells are more responsive to testicular steroids than are others.

(Research was supported by a grant from Rockefeller University.)

10.5 EFFECTS OF ELEVATED LEVELS OF PROLACTIN ON LORDOSIS BEHAVIOR AND LHRH-STAINED CELLS IN THE FEMALE RAT. C.A. Dudley, S. Jamison and R.L. Moss. Dept. of Physiology, University of Texas Health Science Center, Dallas, Texas 75235.

Elevated plasma levels of prolactin (PRL) induced either by pituitary transplants under the renal capsule or by direct peripheral administration have been shown to suppress reproductive behavior in the male rat and to interfere with vaginal cyclicity in the female rat. The purpose of the present study was to investigate the effects of persistent, elevated levels of PRL on lordosis behavior and on brain LHRH-staining in the intact, normal cyclic female rat. Hyperprolactinemia was induced in 11 female rats by grafting whole pituitaries under the kidney capsule, while sham surgery was completed on 12 control female rats. Serum PRL levels were measured via radioimmunoassay and levels of sexual receptivity were determined in the grafted and control animals at one and three months after transplantation. At the fourth month, the grafted and control animals were sacrificed and the extent of brain LHRH staining determined immunocytochemically. Serum PRL levels were found to be 2 to 3 times higher in the grafted than the control animals at both times of assay. On the other hand, high levels of mating behavior were successfully induced in both groups of animals at one month by exogenous administration of estradiol benzoate (E) and progesterone (P). The lordosis to mount ratio (L/M) for the grafted animals was 1.00± .00 whereas that for the sham group was .95±.01. However, at three months, E-P induced mating behavior could not be obtained in the grafted animals (L/M=0.0) whereas the control group still exhibited good lordotic responsiveness (L/M=.62). The animals were then sacrificed (at four months) via perfusion with a mixture of paraformaldehyde and glutaraldehyde. The brains were removed and 50 μ sections were cut on a Vibratome from the septal area through the median eminence. The sections were then processed immunocytochemically according to standard peroxidase anti-peroxidase techniques using a specific antibody to LHRH provided by W. Vale. The number of LHRH stained cell bodies in each section was counted and a mean per animal was then obtained without knowledge as to the condition of the animal. Subsequently, a mean for the grafted and control animals was calculated. The number of LHRH stained cell bodies was dramatically lower in hyperprolactinemic animals than in the control animals. The mean number of LHRH stained cell bodies per tissue section was 1.8± .58 for the grafted animals and 3.1±.77 for the sham controls.

The absence of mating behavior in the hyperprolactinemic animals may be due to decreased LHRH synthesis as reflected by a reduction in the number of stained cell bodies in these animals.

This research was supported by NIH grant HD 09988.

10.6 ACTIONS OF OVARIAN HORMONES AND MICROELECTROPHORETICALLY APPLIED PROLACTIN ON LHRH-SENSITIVE NEURONS IN THE NUCLEUS VENTROMEDIALIS HYPOTHALAMI. A. Chan*, C.A. Dudley* and R.L. Moss (SPON: J. Schadt). Dept. Physiol., Univ. Tx. Hlth. Sci. Ctr., Dallas, TX.

Previous reports have demonstrated that luteinizing hormone-releasing hormone (LHRH) exerts a facilitatory effect on mating behavior in the female rat. Prolactin (PRL), administered intraventricularly, has been shown to suppress mating behavior in the estrogen-progesterone (E-P) primed ovariectomized rat. The inhibitory effect of PRL on mating has been suggested to be mediated through LHRH. Since the ventromedial nucleus (VMH) has been implicated in the control of mating behavior, the present experiment was designed to study the actions of ovarian hormones and microelectrophoretically applied PRL on the LHRH-sensitive units in the VMH. Extracellular potentials were recorded via 4 M NaCl-filled central barrel (4-8 MΩ) of a seven-barreled micropipette (composite tip diameter of 1.5-3 μm). Each of the outer barrels contained one of the following solutions: 0.1 M norepinephrine (NE); 0.5 M dopamine (DA); 0.5 M sodium glutamate (GLUT); 0.1 mM PRL; 1 mM LHRH; 1 mM D-Pro, D-Phe^2, D-Trp^{3,6}-LHRH (LHRH$^-$, LHRH antagonist which inhibits the release of LH from the pituitary gland in a concentration ratio of 0.5:1).

Summary of pharmacological responses of VMH neurons

Agents	Untreated OVX rats				E-P treated OVX rats			
	n	↑	↓	→	n	↑	↓	→
LHRH	39	13%	18%	69%	80	16%	31%	53%
LHRH$^-$	26	8%	27%	65%	59	17%	41%	42%
DA	47	11%	72%	17%	37	8%	73%	19%
GLUT	21	62%	5%	33%	52	69%	8%	23%
PRL	77	68%	13%	19%				

The results tabled above indicate that more nerve cells are inhibited by LHRH and LHRH$^-$ in E-P treated than untreated OVX rats. No such hormonally dependent differences were observed with DA or GLUT. The stimulatory effect of PRL appeared to be dose-dependent (0-30 nA). Of all the LHRH-inhibited units, PRL excited 65%, inhibited 13% and had no effect on 22% of neurons tested (n=23). Of all PRL-activated units, LHRH excited 23%, inhibited 32%, and had no effect on 45% of these units tested (n=47). This result indicates that the LHRH-inhibited units are predominantly excited by PRL, but the stimulatory action of PRL is not specific for the LHRH-inhibited units. The above findings can be interpreted to suggest that the inhibitory response of LHRH in the VMH, which is dependent on ovarian hormones, may be related to mating behavior. The mechanism by which PRL suppresses mating behavior could be mediated by stimulating the LHRH-inhibited units and thus exerting an opposite effect to LHRH in the VMH. Research supported by NIH grant NS 10434 to RLM.

10.7 EFFECTS OF LHRH ON MULTIUNIT ELECTRICAL ACTIVITY OF THE PREOPTIC-ANTERIOR HYPOTHALAMUS IN THE OVARIECTOMIZED RAT. David I. Whitmoyer, Peter C.K. Leung and Charles H. Sawyer. Department of Anatomy and Laboratory of Neuroendocrinology, Brain Research Institute, UCLA, Los Angeles, CA 90024.

Luteinizing hormone-releasing hormone (LHRH) has been implicated as a possible mediator of "ultrashort-loop feedback" in the control of LH release. In the rat, perikarya of LHRH neurons have been located primarily rostral to and within the preoptic-anterior hypothalamic region. In the present study, effects of intraventricular infusions of LHRH on the multiunit activity (MUA) of this rostral brain region were investigated in long-term ovariectomized rats.

Adult female Sprague-Dawley rats were chronically implanted with third-ventricle cannulas and electrode arrays for recording MUA and cortical EEG. All animals were ovariectomized 2 weeks prior to implantation and recordings were taken from the unanesthetized freely-moving animals 2 weeks post-implantation. MUA records were obtained from 25 electrode sites in the preoptic-anterior hypothalamus. Intraventricular infusion of saline (2 μl, pH 5.5) had no effect on MUA. On the other hand, infusion of LHRH (0.5 μg in 2 μl saline, pH 5.5) led to changes in MUA in 11 of 25 trials. Of these 11 responses, 5 were increases; i.e., MUA rose by 40-50% by 40 min post-infusion. The inhibitory effects were striking, with 5 sites showing 50% decreases by 50 min post-infusion and lasting through the end of the 90-min recording period.

In ovariectomized rats, norepinephrine (NE) has been shown to inhibit LH release. The effects of NE (10 μg in 2 μl saline, pH 5.5) were assessed for 17 of the 25 sites. NE infusions were separated from the LHRH tests, either 2 days before or 2 days after. In 15 of the 17 cases, NE led to dramatic decreases in MUA within 10 min. Thus, in rostral brain sites some populations of neurons were responsive to both NE and LHRH. However, although the time course of MUA changes following LHRH infusion may be a direct result of LHRH exposure, the possible involvement of secondary effects such as a change in plasma LH cannot be excluded.

Previous MUA studies from our laboratory have employed several different techniques for deriving an "integrated" record from the raw MUA activity. The present system employs analog elements which continuously compute the root-mean-square (RMS) value of the MUA signal (RMS-MUA). This is the preferred approach to integration because it yields a calibrated output signal, the technique can be reproduced easily by other investigators, and it conforms to the standard electrical engineering approach to the measurement of non-sinusoidal signals.

10.8 PITUITARY IMMUNOCYTOCHEMISTRY OF LHRH AND cAMP AFTER SYSTEMIC LHRH ADMINISTRATION. Jorge Pecci-Saavedra, J. Yuan Li*, Joan L. Greenwald* and Ludwig A. Sternberger. Center for Brain Research, University of Rochester School of Medicine, Rochester, NY 14642.

Male rats were injected I.V. with doses of LHRH ranging from 0.1 to 100μg and sacrificed by perfusion after 2-30 min. Vibratome sections of the pituitaries were processed for light and electron microscopy with the PAP technique using antibodies to cAMP, LHRH and LH. After injections of 1, 10, and 100μg of LHRH, cytoplasmic cAMP was visualized in large and small gonadotrophs with increasing intensities. Uninjected controls were unstained. A proportion of gonadotrophs, identified morphologically and immunocytochemically with anti-LH exhibited granular cytoplasmic staining for LHRH. Results allow for the identification of the gonadotrophs as responsible for the previously reported controversial increase of cAMP in whole pituitaries after stimulation by LHRH. The finding of intracytoplasmic LHRH in the present experiments gives further support to previous studies on intracellular LHRH receptors. These findings could suggest a biphasic action of LHRH on gonadotrophs: a) a releasing effect mediated through granule LHRH receptors; and b) a rapid, secondary activation of adenyl cyclase, that follows exocytosis and fusion of the LHRH-granule receptor complex with the plasma membrane. Results of this secondary effect may be up-regulation of LHRH receptors, gonadotropin replenishment and LHRH-receptor transport to GERL or Golgi regions.

Anti-cAMP antibodies were kindly supplied by A.L. Steiner. Supported by NIH grants HD 12932, NS 15843 and NS 15809 and by Conicet, Argentina.

10.9 HYPER-RESPONSIVENESS OF MEDIAN EMINENCE LHRH TERMINALS INDUCED BY NEONATAL MONOSODIUM GLUTAMATE TREATMENT. L.V. DePaolo* and A. Negro-Vilar. Department of Physiology, University of Texas Health Science Center at Dallas, Dallas, Texas 75235.

Monosodium glutamate (MSG) treated rats present atrophic gonads and pituitaries, low to normal gonadotropin levels, normal pituitary responsiveness to exogenous LHRH, but decreased responses to the negative and positive feedback effects of gonadal steroids. These disturbances, combined with the known neurotoxic effects of MSG on the arcuate nucleus (ARC) neurons, suggest a derangement in the LHRH releasing mechanism. The present experiments were designed to test this hypothesis. Neonatally MSG-treated male rats and their controls were sacrificed at 5 months of age. MSG-treated rats presented reduced body, anterior pituitary and ventral prostate weight. Interestingly, weights as well as protein content of the median eminence (ME) were also significantly reduced ($p<.01$) in MSG-rats. As reported by others, dopamine (DA) levels in the ARC were reduced by about 50% in MSG rats, whereas levels of the amine in other areas were not affected. LHRH concentrations (pg/μg protein) were slightly but significantly decreased in the ARC of MSG-rats, whereas LHRH in the ME in the same animals was two fold higher than control values. Release of LHRH from median eminence fragments was evaluated in vitro using a perifusion system. ME's from MSG-treated or control male rats were incubated in Krebs-Ringer medium for a control period of 50 min, after which the tissues were incubated for 50 min in a high K^+ medium at a concentration (30 mM) which induced half-maximal stimulation of LHRH release. This was followed by another 50 min period during which prostaglandin E_2 (PGE_2, 10 μg/chamber) an effective releaser of LHRH, was injected as a pulse superimposed on the high K^+ medium. Minor differences were seen in basal LHRH release between MSG and control rats. However, in response to K^+ stimulation, ME's from MSG rats released more LHRH ($p<.01$) than control rats. Similarly, after combined PGE_2 and high K^+ treatment, LHRH release in MSG rats was 2.5 fold higher ($p<.01$) than in controls. These results suggest that the hyper-responsiveness of the median eminence LHRH terminals in vitro may result from denervation of the LHRH neurons due, at least in part, to the loss of DA neurons in the ARC. Further, they indicate that the mechanism(s) that normally triggers LHRH release is defective in the MSG rat. Therefore, a reduced in vivo release of LHRH in MSG rats may be the primary defect causing the alterations in the hypothalamic-pituitary-gonadal axis of these animals. Supported by NIH Grants HD-09988-05-3 and HD-05776.

10.10 ROLE OF TUBEROINFUNDIBULAR DOPAMINE NEURONS IN THE BIPHASIC EFFECT OF 5-METHOXY-N,N-DIMETHYLTRYPTAMINE ON RAT PROLACTIN SECRETION, M. Simonovic, G.A.Gudelsky and H.Y. Meltzer, Dept. of Psychiatry, University of Chicago Pritzker School of Medicine,Chicago,IL 60637

The two best described influences on rat prolactin (PRL) secretion are an inhibitory effect of dopamine (DA) released by the tuberoinfundibular dopaminergic (TIDA) neurons and a stimulatory effect of serotonin (5HT) and 5HT agonists. Midbrain raphe nuclei are the major source of the hypothalamic 5HT input. Little is known about the interaction, if any, between the ascending 5HT neurons and TIDA neurons. We have investigated the effect of 5-methoxy-N,N-dimethyltryptamine (5MeODMT), a 5HT receptor agonist which inhibits raphe neurons, on the turnover of DA in the median eminence and on the increase in PRL produced by variety of drugs which affect the dopaminergic inhibition of PRL secretion.

5MeODMT (2.5 - 10 mg/kg,ip) produced a marked, dose-related but short-lasting (less than 30 min) stimulation of rat PRL secretion. Thirty minutes after 5MeODMT (10 mg/kg,ip) serum PRL concentration was not significantly different from those in vehicle-treated controls. The elevations in serum PRL concentration induced by a low dose of alpha-methylparatyrosine (AMPT, 50 mg/kg,ip), an inhibitor of DA synthesis, or haloperidol (0.15 mg/kg,ip),a potent DA receptor blocker, were significantly reduced in rats which received 5MeODMT (10 mg/kg,ip) 30 min before either of the two drugs. However, 5MeODMT pretreatment did not significantly alter the increase in serum PRL concentrations induced by a higher dose of AMPT (250 mg/kg,ip) or haloperidol (1.0 mg/kg,ip). 5MeODMT also failed to alter the increase in serum PRL following morphine (5, 10 or 20 mg/kg,sc) or gamma-butyrolactone (500 mg/kg,ip), two drugs which decrease the activity of TIDA neurons. In order to test the hypothesis that inhibition of raphe firing by 5MeODMT results in increased activity of TIDA neurons, the turnover of DA in the median eminence was estimated using AMPT-induced decline of DA concentration. Steady-state concentration of DA in the median eminence was not significantly altered by 5MeODMT (10 mg/kg,ip).However, 5MeODMT significantly enhanced AMPT-induced DA decline in this region.

These results demonstrate that 5MeODMT exerts a biphasic effect on rat PRL secretion.The initial stimulation of PRL release is probably due to its ability to activate postsynaptic 5HT receptors. The subsequent inhibitory effect of 5MeODMT appears to be due to increased activity of TIDA neurons. If the inhibitory effect of 5MeODMT on PRL secretion reflects its ability to inhibit the spontaneous firing of ascending 5HT neurons, then these results suggest that decreased 5HT transmission leads to increased activity of TIDA neurons.This research was supported by USPHS MH 30,938 and MH 29,206.

10.11 EFFECTS OF AGING ON THE RELEASE OF LUTEINIZING HORMONE IN FEMALE GOLDEN HAMSTERS. H.J. Chen. Division of Neurobiology, Barrow Neurological Institute, Phoenix, AZ 85013.

Release of luteinizing hormone (LH) as affected by aging, estrous cycle, luteinizing hormone releasing hormone (LHRH), castration and oestridiol benzoate, was studied in the female golden hamster (Mesocricetus auratus). About 80% of female golden hamsters cycled regularly when reaching 17-22 months of age until a terminal disease intervened. Age-induced irregularity of the estrous cycle included an absence of or a delay in proestrous vaginal discharge. Young female hamsters (3-5 months) had significantly (p 0.01) higher basal LH concentrations than old animals (17-22 months) in the morning of each stage of the oestrous cycle. LHRH elicited about 20-30 fold increase in serum LH concentrations in both young and old hamsters. No significant difference or definite pattern of LH release was observed between young and old hamsters in response to LHRH. However, in old hamsters that were not cycling, the peak of LH release in response to LHRH was delayed. The release of LH in response to exogenous LHRH was greater in the morning of proestrus than during other stages of the estrous cycle in both young and old hamsters. The rise in LH was significantly higher in the young than in old hamsters on the 13th and 15th days after castration. However,positive feedback stimulation of LH release by estridiol benzoate resulted in the same magnitude of LH release in both young and old hamsters.These results indicate that in the female hamster, LH release from the pituitary in response to acute stimuli such as LHRH and estrogens is the same in the young as in the old animals if they are cycling. The peak of LH release in response to LHRH was delayed in old hamsters with irregular estrous cycles. Basal LH concentrations as well as LH concentrations on the 13th and 15th days after ovariectomy were significantly higher in the young than in the old hamster,.suggesting that chronic release of LH may decrease or its degradation or clearance may increase during the aging process in female golden hamsters.

11.1 RESPONSES OF SENSORY RELAY NEURONS AND EXTRAOCULAR MOTONEURONS TO OPTOKINETIC STIMULATION IN THE FROG. S.L. Cochran, W. Precht* and N. Dieringer*. Brain Research Institute, Univ. of Zürich, CH-8029 Zürich, Switzerland.

Movement of the visual surround around an animal results in a compensatory following of the head and eyes. We have been attempting to determine the neuronal circuitry responsible for mediating this behavior in the frog, Rana temporaria. Neurons within two midbrain nuclei, innervated by the retina, are responsive to optokinetic stimulation of the contralateral eye. The basal optic nucleus (BON) contains neurons predominantly sensitive to vertically-moved patterns, while the pretectal (PT) neurons are sensitive to horizontally-moved patterns (Cochran et al., 1980). Additional recordings have been made from antidromically identified motoneurons within the oculomotor nucleus (innervating the ipsilateral, visually-stimulated eye) and the contralateral abducens nucleus. Oculomotor neurons have response properties identical to those found in the BON, both in terms of their direction specificity as well as their velocity sensitivity. In addition, some oculomotor neurons, presumably innervating the medial rectus musculature, and contralateral abducens neurons have response properties identical to PT neurons. These similarities in response properties suggests that a close relation exists between the sensory nuclei, the BON and PT nucleus, and the extraocular motoneurons. Indeed, in the frog the BON has been reported to project to the oculomotor nucleus in a monosynaptic fashion (Montgomery et al., 1979). One is thus led to the hypothesis that optokinetic reflexes of the ocular system are mediated by a three-neuronal chain from retina to midbrain sensory system to ocular motoneuron. Two findings are compatible with this hypothesis. First, the shortest EPSP latencies of BON, PT neurons, oculomotor neurons, and abducens neurons (3 msec; range 3-7 msec from optic nerve stimulation) are compatible with the proposed disynaptic innervation from the retina. However, sources other than BON or PT (e.g. the optic tectum) may also contribute to the excitation of the motoneurons. Second, PT neurons sensitive to horizontal optokinetic stimulation of the contralateral eye, can be activated at a short, fixed latency (approx. 1 msec) with electrical stimulation of the abducens nucleus. This presumed antidromic activation might indicate that PT cells terminate in the abducens. Further attempts are currently being undertaken to verify or nullify this hypothesis.

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11.2 METABOLIC MAPPING OF POSTNATAL DEVELOPMENT OF AVIAN BRAIN REGIONS RESPONSIVE TO RETINAL SLIP. Olivia C. McKenna and Josh Wallman. Dept. of Biology, City College of the City University of New York, New York, N.Y. 10031

Detection of retinal slip, movement of the visual world on the retina, is necessary for stabilizing eye movements. A previous metabolic mapping study demonstrated that in birds information regarding vertical retinal slip is transmitted to the nucleus of the basal optic root (nBOR), a portion of the accessory optic system, while information regarding horizontal retinal slip is transmitted to the lentiform nucleus of the mesencephalon (LM), a pretectal nucleus. We report here developmental differences between the two nuclei.

After injection of ^{14}C-2-deoxyglucose, chicks were placed in a striped drum rotating either vertically or horizontally at 2-4°/sec. for 45 min. Alternate brain sections were stained or placed against x-ray film to produce autoradiograms. Since the visual pathways in chicks are crossed, differential stimulation of the two eyes results in differential stimulation of the two sides of the brain. In all situations tested if the eye was covered, no label was seen in the contralateral brain. Results from 3 to 7 day old chicks were compared to those of 3 to 5 week old chicks.

We found that in the nBOR of older chicks upward moving stimuli resulted in labeling of the dorsal nBOR whereas downward moving stimuli resulted in labeling of the ventral nBOR. This functional separation was not detected in the nBOR of younger chicks. These results suggest the possibility of a postnatal reorganization of the synaptic afferents within the nBOR.

In the LM, postnatal changes were different. In young chicks labeling was produced only in the LM contralateral to the eye viewing temporal to nasal stimulus movement; in older birds both temporal to nasal and nasal to temporal movement produced labeling throughout the contralateral LM. These results suggest that the LM becomes responsive to nasal to temporal movement after the first postnatal week and that, unlike the nBOR, units responding to opposite directions are not separated anatomically, although we cannot exclude the possibility that developmental changes in oculomotor responses may account for these results as well.

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11.3 CELL TYPES IN THE CAT'S PARABIGEMINAL NUCLEUS. H. Sherk* (SPON: K. Fukada). Dept. of Neurobiology, Harvard Med. Sch., Boston, MA

The cat's parabigeminal nucleus has very limited connections with other parts of the brain. It receives input from the ipsilateral superior colliculus (SC), and projects to the ipsilateral and contralateral SC, and weakly to the ventral C laminae of the lateral geniculate nuclei (LGN) (A.M. Graybiel, Brain Res. 145: 365, 1978; H. Sherk, J. Neurophysiol. 42:1656, 1979). I have investigated whether all parabigeminal neurons have similar connections, or whether they form distinct subpopulations.

First, the possibility that there are interneurons in the nucleus was explored in 2 cats by making massive injections of horseradish peroxidase (HRP) into the left LGN and both SC's, with the intent of labeling all parabigeminal projection neurons. Coronal sections 2 μ thick were searched for unlabeled neurons. A few of these were present in every section, and made up 9% of the total population. They were small cells, averaging about 13 μ in diameter, compared to 19 μ for the labeled projection cells. In another experiment, scattered neurons throughout the nucleus that were similar in size to these unlabeled cells were heavily labeled autoradiographically by a local injection of 3H-GABA. At least some small parabigeminal neurons thus appear to take up GABA selectively.

Since parabigeminal cells send major outputs to both SC's, an obvious question is whether they do so by way of branching axons. To answer this, 125I-wheatgerm agglutinin (WGA) was injected in the right SC, and HRP was injected in the left SC. Many parabigeminal cells were labeled either with peroxidase or autoradiographically, but none contained both labels. WGA-tagged cells were larger than those labeled by peroxidase. It thus appears that in addition to a modest number of interneurons, the parabigeminal n. contains a population of large contralaterally-projecting cells, and a population of smaller, ipsilaterally-projecting neurons.

Observations on connectivity within the nucleus were made using the EM. The presence of Type II as well as Type I synapses gives some support to the hypothesis that the parabigeminal n. contains inhibitory interneurons. Of the terminals making Type I contacts, at least some appear to come from the SC, since they can be labeled by anterograde transport of HRP after tectal injections. Such labeled terminals are sometimes found as the first elements in axoaxonal synapses, which are fairly common in the nucleus. An unusual structure also present here is the crest synapse, and again a labeled tectal afferent sometimes forms the presynaptic element.

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11.4 NASO-TEMPORAL VERSUS TEMPORO-NASAL OKN IN THE CAT. K.P. HOFFMANN, BIO IV, UNIVERSITÄT ULM, D-79 ULM, GFR.

A model based on our anatomical and neurophysiolocical data from cells in the nucleus of the optic tract (NOT) is proposed to explain the difference in gain for optokinetic nystagmus (OKN) with temporo-nasal (nasal) or naso-temporal (temporal) stimulus movement in cats. The basic assumption in this model is that stimulus direction in visual space is coded by the activity in one NOT minus the activity in the other NOT. This signal, called the NOT-output, is then used to control OKN. Testing OKN of normal adult cats monocularly shows that the gain is higher with nasal stimulus movement than with temporal movement. With nasal stimulus movement each eye strongly activates all units in the contralateral NOT in two ways, via the direct retino-pretectal fibers and via the binocular cortico-pretectal axons. With temporal stimulus movement about 40 % of the units in the ipsilateral NOT are activated, because each eye reaches the ipsilateral NOT only via binocular cells in visual cortex. As a consequence of these pathways 60 % of the cells in NOT are monocularly driven by the contralateral eye and 40 % are binocular. This ratio of .6 : .4 is taken as the relative strength of retinal vs. cortical influence on NOT cells. The maximal output value which is reached with horizontal movement and binocular stimulation is set to 1. 0.8 of the NOT-output is supplied by the activation of one nucleus and .2 by the suppression of spontaneous activity in the other nucleus. The contribution to the NOT-output made up by activation is .8 x .6 from the retinal and .8 x .4 from the cortical input, the contribution from suppression is .2 x .6 retinal and .2 x .4 cortical. With monocular stimulation the NOT-output is dependent on the stimulus direction and becomes asymmetric. With nasal stimulus direction, the contralateral NOT is fully activated (.8), the ipsilateral NOT inhibited only via the cortex (.1). With temporal stimulus direction the contralateral NOT is fully inhibited (.2) and the ipsilateral NOT activated only via the cortex (.3). These output values of .9 in nasal direction and .5 in temporal direction are matched qualitatively by the gain of OKN in the same stimulus condition. Following from this model, cortical lesions in the adult or developmental manipulation in young kittens must increase the asymmetry between nasal and temporal OKN.

11.5 FROM LOCAL DIRECTION PREFERENCES TO GLOBAL ROTATION PREFERENCES IN THE ACCESSORY OPTIC SYSTEM. C. S. Leonard*, J.I. Simpson and R.E. Soodak*, Dept. Physiol. & Biophys., New York Univ. Med. Ctr. 550 First Avenue, New York, N.Y. 10016

Previous studies from this laboratory have shown that neurons of the medial terminal nucleus (MTN) of the rabbit accessory optic system respond preferentially to large, textured visual stimuli slowly moving in near vertical directions. In this study we have focused on the spatial distribution of preferred directions within the receptive field (RF) of MTN neurons and of neurons neighboring the MTN. Neurons from the latter group project to the dorsal cap (DC) of the inferior olive. Extracellular recordings were made in anesthetized, pigmented rabbits presented with stimuli provided either by rear projection onto a tangent screen or by a planetarium projector. Latencies of orthodromic and antidromic activation were obtained with electrical stimulation of the optic chiasm and the ipsilateral DC. Most neurons recorded within the MTN can be characterized by a single preferred direction (PD), either up and somewhat backward or down and somewhat backward, and have their RF in and above the visual streak. These neurons responded only to stimuli presented to the contralateral eye and were activated at short, presumably monosynaptic latencies (1.5-2.5msec) from the chiasm. Other neurons recorded within the MTN had two distinctly different PD's in separate regions of their monocular, contralateral RF. That is, in one part of the RF the preferred direction was up and somewhat backward whereas in the other part of the RF the preferred direction was down and somewhat backward. Neurons located outside the MTN, including those antidromically activated from the DC, were typically binocular, with one or the other eye dominant. Monocular neurons with one or two PD's, as well as most contralateral dominant binocular neurons were activated from the chiasm at latencies in the monosynaptic range, thus excluding a purely serial development of RF organization in which retinal fibers synapse only with neurons having a single PD. The direction selectivity of neurons with more than one PD can be characterized by a preferred axis of rotation. For example, if the PD in the RF region extending from 0° (the nose) to 45° posterior is up and backward, the PD in the region from 45° to > 160° posterior is down and backward, resulting in a preferred axis of rotation at approximately 45° to the midline. In short, transformations occurring in the accessory optic system put retinal signals of local direction preference into a global form compatible with semicircular canal signals of rotational self-motion. Supported by USPHS Grant #NS13742.

11.6 RESPONSES OF CELLS IN THE MONKEY LATERAL GENICULATE NUCLEUS (LGN) TO LUMINANCE AND CHROMATICALLY-MODULATED GRATINGS. B.B. Lee, T.R. Vidyasagar* and T.P. Hicks. Max Planck Institute for biophysical Chemistry, D-3400 Göttingen, FRG.

The activity of cells in the parvocellular layers (PCL) of the LGN may be increased or suppressed according to the colour of a stimulus. They might thus be expected to respond to both luminance and chromatic gratings. Responses to such gratings have been recorded from the LGN of the anaesthetised macaque. Sinusoidal grating stimuli were displayed on a tangent screen, colours being generated with interference filters in the light beams of the projector system.

PCL cells showed no low spatial frequency attenuation in their responses to either luminance- or chromatically-modulated gratings. For most chromatic gratings, responses of wide-band cells (WS,+G-R; WL,+Y-B; Creutzfeldt, O.D. et al., Exptl. Brain Res., 35: 527-545, 1979) were less vigorous and such cells resolved fine gratings less well. With narrow-band cells (NL,+R-G; NS,+B-Y) responses were enhanced and gratings better resolved. Magnocellular layer (MCL) cells responded scarcely or not at all to chromatic gratings. These results may be explained with reference to the cells' spectral response characteristics. When gratings of two wavelengths which both caused neuronal excitation were alternated in a grating, the cell's responses moved between two excitatory points on the spectral response curve and modulation of discharge was less than in the luminance-modulated case. If one of the colours used suppressed the cell's activity, responses were enhanced in comparison with the luminance-modulated case.

Wide-band and MCL cells resolved much finer gratings than narrow-band cells and it was the resolution of these cell types which was impaired with chromatic gratings. The decrease in acuity with chromatic gratings in human psychophysics thus acquires a physiological basis. The results are also of interest in relation to colour contrast phenomena.

11.7 INFLUENCE OF STIMULUS VELOCITY ON LGN NEURONS. G.A. Orban, K.-P. Hoffmann and J. Duysens, Lab. Neuroen Psychofysiologie, KULeuven, Campus Gasthuisberg, Herestraat, B-3000 Leuven, Belgium and Universität Ulm, Abt. Vergleichende Neurobiologie, Oberer Eselsberg M25, D-79 Ulm (Donau), West Germany.

Neurons in the dorsal lateral geniculate nucleus (LGN) were recorded in paralyzed and anesthetized (N_2O/O_2) cats. The influence of stimulus (light or dark bars) velocity (.18° to 900°/sec) was tested quantitatively with a multihistogram technique. Geniculate cells were classified into X and Y types according to their conduction velocity, center size, grating resolution and upper cut-off velocity for stimuli appropriate for center and surround. Velocity-response (VR) curves were compared to VR curves obtained under similar circumstances for cortical cells (Orban et al., J. Neurophysiol., in press and Duysens et al., Neurosci. Abstr., this volume). When tested with the center stimulus (i.e. a light slit for ON center cells) geniculate neurons responded over a wider range of velocities than cortical cells. More LGN cells responded to the fastest movement tested (900°/sec) while less responded to the slowest movement (.18°/sec) which in many cortical cells elicits nearly maximal response. Consequently hardly any geniculate cell showed a low pass VR curve, characterized by a general preference for slow movement. Comparison of X and Y cells showed that on average X cells responded to slower movements than Y cells. In particular all velocity high pass curves belonged to Y cells. Many X cells had a tuned curve with optimum between 40 and 70°/sec.

In order to evaluate the latency of responses, spatial lag velocity curves were prepared. These curves often showed a nonlinearity in Y cells. Such nonlinearities are also seen in many area 18 cells and in some cells of areas 17 & 19 subserving peripheral vision. Center stimuli evoked two responses : a main response with short latency (37-76 msec) and a second with long latency (80-178 msec). Surround stimuli (i.e. a dark bar in ON center cell) usually evoked two responses as well but these had longer latencies and a different origin than the responses to center stimuli. The differentiation between X and Y cells on the basis of velocity characteristics was enhanced when only the short latency center response was considered.

11.8 EFFECT OF BLOCKING RETINAL ON-CHANNELS WITH AMINOPHOSPHONOBUTYRIC ACID (APB) ON THE CAT VISUAL SYSTEM. J.C. Horton*(SPON:E.T. Hedley-Whyte) Dept. Neurobiology, Harvard Medical School, Boston, Mass.

Several months ago Slaughter and Miller reported that APB selectively blocks the on-channel in the perfused retina-eyecup preparation from the mudpuppy (Science, 211:182-85, 1981). I have tested the effect of this drug in the cat by injecting it into the eye, in the hope of studying the contributions of separate on and off channels to receptive field properties of cells in central visual nuclei.

Physiological recordings were made in 6 anesthetized, paralyzed cats. After the lateral geniculate body was located with a tungsten electrode, APB was injected into the vitreous of the contralateral eye and response properties in lamina A and A1 were compared every 10 minutes for any effect produced by the drug. Within a few hours a striking change in geniculate properties was observed. The on-response in lamina A was completely abolished: only off-center cells could be driven by visual stimulation; cells and multiunit activity responded to a stationary flashing spot only at light off. Center-surround receptive field organization of the remaining off-center cells was preserved, in the sense that a small spot of light evoked a more vigorous response than a large spot. More subtle changes in response properties may have been present. In two cats, after onset of the drug's action in the geniculate body, penetrations were made through the optic tract and recordings obtained from 27 retinal ganglion cell axons; no obvious abnormalities in receptive field properties were found. Of the 19 fibers from the normal eye, 7 were on-center and 12 were off-center; all 8 fibers from the APB-injected eye were off-center. This result was not unexpected since on-center geniculate cells receive their main excitatory input from on-center retinal ganglion cells (Hubel & Wiesel, J. Physiol., 155:385-98, 1961 and Cleland, Dubin, & Levick, J. Physiol., 217:473-96, 1971)

In a preliminary experiment the effect of APB on the striate cortex has been tested. Cells were less responsive to stimulation through the APB-injected eye; orientation selectivity did not seem to be affected. Some changes were noted in organization of receptive fields. In another cat APB was injected into both eyes and the animal observed closely for the next 14 hours. No obvious changes in visually guided behavior were apparent; careful testing will be necessary to reveal any possible deficit.

11.9 TERMINAL MORPHOLOGY OF PHYSIOLOGICALLY IDENTIFIED X AND Y OPTIC TRACT AXONS WITHIN THE LATERAL GENICULATE NUCLEUS OF THE CAT. L.R. Stanford, M. Sur* and S.M. Sherman. Dept. of Neurobiology and Behavior, SUNY at Stony Brook, Stony Brook, NY 11794.

We have correlated the physiological properties of retinal ganglion cells in the cat with their morphological pattern of projection into the lateral geniculate nucleus (LGN). Ganglion cell axons were recorded within the LGN and in the portion of the optic tract lying ventral to the LGN. Axons were classified as X or Y according to standard criteria, including conduction velocity and spatial summation properties. Physiologically characterized axons were impaled and iontophoretically injected with horseradish peroxidase. Brain sections were reacted with diaminobenzidine, using cobalt chloride intensification, permitting clear visualization of anterogradely-filled terminal processes and retrogradely-filled axon trunks.

To date, 10 X and 12 Y axons have been filled and recovered. Within the optic tract, each X and Y axon has a parent trunk that issues one or more major branches terminating within the LGN. Individual Y axons terminate in the main laminae and the medial interlaminar nucleus (MIN) of the LGN. They then continue medially and posteriorally to the superior colliculus, in accordance with a previous description (Bowling and Michael, Nature, 286:899, 1980). Within the LGN, contralateral Y axons terminate in lamina A and in the C laminae.

There are several morphological differences between X and Y axons. Within the optic tract ventral to the LGN, X axons are thinner and tend to occupy a more dorsal position than Y axons. X axons have relatively narrow terminal fields. Many X axons have long cylindrical fields, 100-150 μm in width, oriented perpendicular to the lamination. These lie centrally within one of the A laminae. Other X axons have shorter terminal fields that lie predominantly dorsally within a lamina. Y axons have broader terminal fields. Some Y axon terminations in lamina A or A1 are relatively wide (≤400 μm) at the base of the lamina. Other Y axons have terminal fields that are more cylindrical in shape and are oriented perpendicular to the geniculate laminar borders. Within the MIN, Y axon terminations spread predominantly in a dorsoventral direction. In the terminal field, both X and Y axons become extremely thin, and there is no clear difference in their width.

Both X and Y axons have small, medium, and large (crenulated) terminal boutons (Mason and Robson, Neuroscience, 4:79, 1979). Terminal boutons of X axons tend to occur in clusters more often than do those of Y axons.

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11.10 TERMINATION PATTERNS OF SINGLE OPTIC TRACT AXONS OF DIFFERENT PHYSIOLOGICAL TYPES. Douglas B. Bowling* and Charles R. Michael. Dept. of Physiology, Yale Univ. Sch. of Med., New Haven, CT 06510.

By recording from single axons in the optic tract of the cat and injecting them with the marker enzyme horseradish peroxidase, we have examined their physiological responses and central structure. Serial reconstructions reveal the courses and destinations of the axons' collaterals, their patterns of arborization and the three-dimensional distributions of their synaptic boutons. The projection sites and terminal distributions show consistent patterns that are related to three factors: 1) eye of origin (ipsilateral vs. contralateral), 2) physiological classification as Y or X (based primarily on differences in latency to electrical stimulation) and 3) sign of response to changing contrast (ON vs. OFF).

The Y axons send collaterals to LGNd (dorsal lateral geniculate nucleus), MIN (medial interlaminar nucleus) and SC (superior colliculus). Those from the ipsilateral eye terminate in lamina A_1 of the LGNd and in a single layer in the MIN. Those from the contralateral eye terminate in both laminae A and C of the LGNd and in two areas of the MIN (medially and laterally). In A and A_1 the terminal boutons are distributed across the full width of a lamina with about 75% of them in the lower half. The distributions, which contain from 600 to 2,000 boutons, tend to be conical in shape (base at the bottom of the lamina, apex at the top) for the OFF axons and hourglass shaped for the ON axons. The boutons are distributed laterally (in the plane of the retinotopic map) over 200 to 400 μm.

The X axons project to the LGNd and may or may not send a collateral into the MIN. Those from the ipsilateral eye terminate in lamina A_1 and those from the contralateral eye, in A. The terminal distributions, which span the width of a lamina, contain from 300 to 1,000 boutons, about 65% of which are in the upper half. In A the distributions tend to be conical (base at the top of the lamina, apex at the bottom). In A_1 the distributions are basically cylindrical in shape. The lateral spread of the terminals is 100 to 150 μm.

In some experiments injection of an axon results in stained cell bodies in the LGNd. The axons' terminal boutons are closely apposed to the proximal dendrites of the stained cells, suggesting that the staining is transsynaptic and specific for target cells of the injected axons. We have seen up to eleven geniculate cells contacted in this way by a single Y afferent.

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11.11 W-CELLS IN THE C LAMINAE OF THE CAT'S LATERAL GENICULATE NUCLEUS: CONTRAST SENSITIVITY AND OTHER RESPONSE MEASURES. M. Sur*, L.R. Stanford and S.M. Sherman. Dept. of Neurobiology and Behavior, SUNY at Stony Brook, Stony Brook, NY 11794

W-cells were recorded in the C laminae of the lateral geniculate nucleus of cats. Parameters studied were: the latency to optic chiasm, cortical and visual stimulation; response rate; variability in response; and contrast sensitivity as a function of spatial and temporal frequency. All cells have latencies to optic chiasm stimulation >2.0 msec, and most have latencies >2.5 msec. Some cells can be driven antidromically from visual cortex with latencies ≥2.0 msec. About a third of the neurons cannot be reliably driven by any form of visual stimulation, but they respond to chiasm shock. Those that can be visually driven have receptive fields larger than X- or many Y-cell fields at similar eccentricities. Contrast sensitivity functions were derived using phase-reversing sinusoidal gratings. W-cells show either linear or non-linear spatial summation. Linear and non-linear W-cells are found in roughly equal numbers. Linear W-cells respond only to the fundamental temporal frequency of stimulus, have well-defined "null" positions, and display an approximately sinusoidal dependence of sensitivity on spatial phase. Non-linear W-cells show phase-independent second harmonic responses. A major characteristic of W-cells is their very poor contrast sensitivity compared to that of X- or Y-cells. For most visually responsive W-cells, at 0.85 contrast, spatial resolution is 0.5-1.5 cycles/degree, and temporal resolution is 8-24 Hz. For non-linear cells, the spatial resolution of the fundamental component is lower and the temporal resolution higher than the second harmonic component of response. Our sample of linear W-cells includes both "sustained" and "transient" neurons, while the non-linear W-cells are "transient". Otherwise, there are no obvious differences between linear and non-linear cells in receptive field size, latency to optic chiasm stimulation, response rate, spatial and temporal resolution, and peak sensitivity. Cells unresponsive to visual stimulation tend to have longer latencies to optic chiasm stimulation than do visually responsive cells.

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11.12 RETINAL Y-CELL INPUTS TO THE DEEP LAYERS OF THE CAT'S SUPERIOR COLLICULUS. David M. Berson* and James T. McIlwain, Division of Biology and Medicine, Brown University, Providence, RI, 02912.

In the cat, retinal W-cell axons terminate near the surface of the superior colliculus (SC), whereas axons of Y-cells terminate more deeply in the superficial layers. Both of these direct retinal pathways, as well as a polysynaptic "Y-indirect" pathway (Hoffmann, '73), have been shown to make excitatory contact with cells in the upper tectal layers. We now present evidence that retinal Y-cell pathways also contribute substantial excitatory inputs to the deep collicular strata.

In ketamine anesthetized cats, bipolar stimulating electrodes were placed in the left optic disc (OD), right optic tract (OT) and left predorsal bundle. Extracellular activity was recorded from 163 deep layer cells in the right SC. In most cells (85%), single shocks to the OD or OT (50 μsec, 1-5 mA) elicited a burst of 1-3 spikes beginning 3-10 msec ($\bar{X}$=6.6) after OD and 2-9 msec ($\bar{X}$=5.7) after OT shock. The small latency difference appears to reflect mediation by rapidly-conducting Y-cell axons ($\bar{X}$=42 m/sec), but the absolute latencies indicate that these axons do not synapse directly upon the recorded cells (Y-indirect pathway). In a smaller number of deep cells (27%), there appeared, in addition, a single "early" spike, evoked intermittently but at a stable latency following OD shock (1.1-3.9 msec, $\bar{X}$=2.1) and OT shock (0.9-2.1 msec, $\bar{X}$=1.4). These latencies imply the existence of a sparse "Y-direct" input (mean conduction velocity 54 m/sec) making monosynaptic contact with the recorded cells. This short-latency excitation of deep tectal cells survived transection of the IIIrd, IVth, ophthalmic Vth and VIth cranial nerves as well as midpontine pretrigeminal brainstem transection. Hence, it is unlikely that the occurrence of the early spike following OD stimulation resulted from inadvertent activation of non-visual fibers in the orbit.

Cells with apparent Y-direct and Y-indirect input were found throughout the deep layers (laminae IV-VII of Kanaseki and Sprague, '74). Most of these cells could also be antidromically driven from the predorsal bundle. Thus, the present findings demonstrate a major retinal Y-cell influence on output cells in the deep layers of the cat's superior colliculus. Part of this influence may be mediated by a direct, monosynaptic projection from the retina.

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11.13 TRACING RETINOFUGAL FIBERS IN MAN: PARAPHENYLENE DIAMINE (PPD) AS A FIBER DEGENERATING STAIN. Alfredo A. Sadun, Lois E. Smith*, and Kenneth R. Kenyon*. Massachusetts Eye and Ear Infirmary, Harvard Medical School, Boston, MA 02114.

Previous investigations of the human visual pathways have been largely limited by the infeasibility of certain techniques in man. Single unit electrophysiology and neuron tracer studies are clearly inapplicable. Most significantly, silver impregnation of degenerating fibers has, for technical reasons, not been fruitful in man. Compounding the problem has been the lack of clinically supported autopsy material, poor fixation techniques, and the prolonged survival times inherent in human studies. Accordingly, the present view of the neuroanatomy of the human visual system has been formulated from classical observations of gross pathology, clinical deduction, and inference from animal studies.

We here demonstrate the use of paraphenylene diamine (PPD) in conjunction with standard transmission electron microscope (EM) tissue preparation methods. Osmium precipitates on degenerating neural processes resulting in their dark profile when examined by EM. PPD chelates osmium and thus becomes a light opaque marker of degenerating neural processes. Semi-thin epon embedded sections stained with PPD provide sufficient resolution, at the light microscope level, for the identification of degenerating axons and axon terminals (Sadun, Brain Res. 1975). The stain identifies degenerating axons even after survival times in excess of one year.

Post-mortem case studies are presented of patients with documented optic nerve lesions. Included is a specimen from a young woman who died one year after complete transsection of her left optic nerve. The degenerating ganglion nerve fibers are followed with PPD and confirmed with EM. Previously proposed primary visual projections are confirmed and new retinofugal pathways (Smith et al., Soc. Neurosci. 1981) are demonstrated with this method. It is now possible to study, in man, the many retinofugal projections and visual nuclei described in animal models.

12.1 CHEMORECEPTOR CHANNEL KINETICS INVESTIGATED IN FEEDING.
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A method of channel analysis in chemoreceptor activation is presented, and is associated with behavioral regulation in feeding. The analysis is based on the recording of membrane noise which may give information about chemoreceptor action at a molecular level. In this chemoreceptor preparation (fly, Musca), the specific action of a stimulant molecular species (sugar, salt or water) may be elucidated as a temporary opening of specific channel populations. A microcapillary (100μ tip diameter) containing the stimulus solution, also records the receptor response which is viewed on an oscillograph. The recorded response consists of the receptor potential, spike discharge and potential oscillations. We observed oscillations in non-spiking potentials which apparently correspond to the phenomenon of membrane or synaptic noise as observed in end-plate recordings such as the locust flight muscle. These oscillations in chemoreceptor nerves had a direct relationship with exposure to food deprivation or a normal light cycle. For instance, the oscillations increased with food deprivation, and they were diminished in darkness. By recording simultaneously DC at low gain and AC (TC = 1 sec.) at high gain we could visualize the corresponding DC correlate of these oscillations. In correspondence with membrane noise, we might expect these oscillations to represent step-function potential changes corresponding to opening or closing of channels. This is supported by our observations relating AC and DC recordings. Further, the step-like potentials are directly proportional to the rise-time and magnitude of the oscillations. Also, the oscillations exhibit both positive and negative polarities which reflect the polarity of the step-like potential change. In the course of long term stimulation, periods of oscillation alternate with those periods which are oscillation free. So that the oscillations appear to represent fluctuations in the number of open channels involved in the generation of the receptor potential. Thus, in the presence of a stimulant molecule, channels continue to be transiently opened, and a steady membrane potential change obtains. The receptor potential changes due to fluctuations in the number of open channels which appear to represent a rectangular conductance pulse. A model is described linking receptor activation (A) and channel dynamics: $A = \int_{1}^{n} \frac{dE}{dZ} \cdot \left[(Z_1) \oplus (Z_2) \oplus \ldots \oplus (Z_n)\right] dZ$

Where: E = the excitability state as measured as a function of the receptor potential; Z = receptor-channel equivalence; and ⊕ is an equivalence operator. Supported by NIH Grant NS14209

12.2 INCREASED BRAIN SEROTONERGIC ACTIVITY ASSOCIATED WITH CANCER ANOREXIA.
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Since increased brain serotonergic (5-HT) activity has been associated with reduced eating, the role of 5-HT in cancer anorexia was investigated. Previously, we observed elevated brain tryptophan (Trp) and 5-hydroxyindoleacetic acid (5-HIAA) in immature female rats that became anorectic 6 days after the injection (im.) of 5×10^4 Walker (W) 256 carcinosarcoma cells. To provide a more general demonstration of this phenomenon, we fluorometrically assayed brain indoles in 8 adult male rats injected (sc.) with 2×10^6 methycholanthrene (MCA)-induced sarcoma cells. Saline-injected control rats were divided into freely-feeding (FF, n=8) and pair-fed (PF, n=8) groups. Food intake was significantly decreased in tumor-bearing (TB) rats by day 23 post injection, with all rats being sacrificed on day 33. As in the W 256 model, plasma total Trp and albumin were reduced, while plasma free (unbound) Trp was elevated in TB rats. Brain Trp, 5-HT and 5-HIAA were also significantly increased in TB rats. To determine which brain areas exhibit the greatest changes in indole activity, we next assayed levels of Trp, 5-HT and 5-HIAA within both W 256 (n=8) and MCA (n=8) sarcoma lines as well as in FF (n=16) and PF (n=16) control rats in the following regions: hypothalamus, cortex, corpus striatum, hippocampus, mesencephalon, pons-medulla, cerebellum and remaining diencephalon-telencephalon. Tumors were induced as in previous experiments, with W 256 rats being sacrificed 1 day prior to their normal spontaneous death on day 10 and MCA rats killed after a TB rat exhibited 2 consecutive days of ad lib. food intake less than 2 g. In the W 256 model brain Trp was significantly increased in diencephalon-telencephalon, mesencephalon, cerebellum, cortex, hippocampus, hypothalamus and corpus striatum of TB rats. 5-HT was elevated in TB rats in the diencephalon-telencephalon and cerebellum, while increases in 5-HIAA were observed in the cortex, hippocampus, diencephalon-telencephalon, pons-medulla and cerebellum. In the MCA rats, Trp was elevated only in the diencephalon-telencephalon and corpus striatum of TB rats. Significantly increased 5-HT was observed in all areas except the hypothalamus and mesencephalon, while 5-HIAA was elevated in all areas except the hypothalamus. Thus, these data suggest that increased brain 5-HT activity in cancer anorexia is a general phenomenon. The absence of 5-HT changes in PF rats indicates that the biochemical alterations are not resultant from decreased food intake. Finally, the greater CNS regional changes in 5-HT with MCA tumors may reflect the more prolonged anorexia and greater cachexia suffered by these rats. Supported by USPHS grant CA 25786.

12.3 LESIONS OF THE DORSAL BRAIN STEM DISRUPTS FOOD INTAKE AND BODY WEIGHT IN RATS: PARALLELS TO THE LATERAL HYPOTHALAMIC SYNDROME.
Robert J. Contreras, Ellen E. Fox* and Margaret L. Drugovich*. Dept. of Psychology, Yale University, Box 11A Yale Station, New Haven, Ct 06520.

Research on the mechanisms of hunger has focused mostly on the lateral hypothalamus (LH) principally because bilateral lesions of the LH result in aphagia, anorexia and body weight loss (Anand & Brobeck, 1951). This is followed by the recovery of ingestive behaviors (Teitlebaum & Epstein, 1962), although LH lesioned rats maintain their body weights at subnormal levels. Powley and Keesey (1970) demonstrated that food depriving rats to 80% of their normal body weights prior to surgery shortened the period of aphagia and anorexia, although they still maintained a lowered body weight maintenance level similar to that of nondeprived lesioned rats. Since lesions to the dorsal brain stem also lead to chronic reductions in body weights (Contreras & Stetson, 1981), we examined the effects of preoperative food deprivation on food intake and body weight levels after brain stem lesions.

Of the 18 rats that were to receive lesions, 9 were reduced to 80% of their initial body weights by food deprivation, and the other 9 were fed ad libitum. Of the 12 rats that were to receive sham lesions, half were similarly food deprived and the other half fed ad libitum. Lesions were effected by a 28 gauge aspiration tube. After the operations body weight and food and water intakes were monitored for one month.

Lesions of the dorsal brain stem resulted in dramatic reductions in body weight that were never recovered. The nondeprived lesioned rats were anorexic for 10±2 days postoperatively during which time they lost an average of 80 g. The food deprived rats did not display postlesion anorexia and they gained an average of 20 g in the 10 days after surgery. The average body weights of these 2 lesion groups converged 5 days after surgery and were maintained at a constant percentage (76%) of the body weight levels of nondeprived sham rats for the last 20 days of the observation period. Furthermore, 6 days after surgery the average body weights of the two sham groups were not significantly different from each other.

These findings suggest that rats with lesions to the dorsal brain stem, which includes the area postrema and medial parts of the nucleus of the solitary tract, adjust their food intakes to maintain their body weights at a lower level, findings like those obtained following LH lesions. Our results do not challenge necessarily the preeminence of the LH in feeding behavior, but suggest that there may be redundancy in controlling mechanisms reminiscent of Jackson's principle of hierarchial organization.

12.4 REVERSAL OF HYPOTHALAMIC HYPERPHAGIA AND OBESITY FOLLOWING LESIONS OF THE AP-cmNTS.
Thomas Hyde*, Ricardo Eng, and Richard R. Miselis. (Spon.: R.O. Davies), Inst. Neur. Sci., Anim. Bio. Sch. Vet. Med., Univ. Penn., Philadelphia, PA, 19104.

The area postrema (AP) lies at the caudal end of the fourth ventricle on the dorsal medulla. The caudal medial zone of the nucleus of the solitary tract (cmNTS) lies ventral and lateral to the AP. Recent anatomical studies (Coil and Norgren, 1978) have shown that the AP and the cmNTS receive an intense afferent input from the subdiaphragmatic vagus. We have previously reported (Hyde and Miselis, 1980) that AP-cmNTS lesions cause a transient hypophagia and permanently reduced body weight in adult male albino rats. Furthermore, the lesions cause a polydipsia and enhanced salt appetite secondary to polyuria and urinary sodium loss. The disruption in food and water intake and body weight loss support the hypothesis that the sensory input to this region is vital for normal fluid and energy balance. Discrete lesions of the AP-cmNTS provide a "central vagotomy" preparation in which the role of visceral sensory input in ingestive behaviors can be assessed without the confounding motor deficits inherent in the subdiaphragmatic vagotomy preparation. We tested this hypothesis using the hypothalamic knife-cut induced hyperphagia and obesity model (Gold et al., 1977). Hypothalamic hyperphagia and obesity is reversed by subdiaphragmatic vagotomy (Powley and Opsahl, 1974; Sawchenko and Gold, 1981). Adult male Sprague-Dawley rats with parasaggital hypothalamic knife cuts became hyperphagic and obese. The knife-cut rats increased their weights by 44.5% over an 8 week period while the shams only increased their weights by 31.0%. AP-cmNTS lesions reversed the hyperphagia and obesity caused by the knife cuts, with body weights falling 32% over 5 weeks, back to the pre-knife cut level. Conversely, rats given AP-cmNTS lesions first never became hyperphagic or obese after knife cuts. These findings are similar to the subdiaphragmatic vagotomy effects on knife-cut obesity and VMH obesity. This supports the hypothesis that sensory input into the AP-cmNTS is vital for the expression and maintenance of hypothalamic hyperphagia and obesity. Alternatively, this lesion could be interrupting GI motor reflex arcs passing through the AP-cmNTS and out through the subdiaphragmatic vagus. Such loops could be vital to normal GI motor function. However, AP-cmNTS rats respond to food deprivation with a transient hyperphagia and gain weight at a heightened rate on a high fat diet. Studies using a "supermarket diet" to induce obesity, as well as an assessment of GI function in rats with an AP-cmNTS lesion are in progress to evaluate these possibilities. Thus, the AP-cmNTS lesion preparation may allow the study of visceral sensory function in ingestion without motor deficits.

12.5 TASTE REACTIVITY OF LATERAL HYPOTHALAMIC LESIONED RATS: EFFECTS OF DEPRIVATION AND TUBE FEEDING. Steven J. Fluharty* and Harvey J. Grill (SPON: C.R. Gallistel). Dept. of Psych. and Inst. of Neurol. Sci., Univ. of Penn., Phila., PA 19104.

Large bilateral lesions of the lateral hypothalamus (LHX) result in total cessation of ingestive behavior (Stage I). The specific nature of the ingestive deficits has not been systematically analyzed because existing tests require spontaneous ingestion. By utilizing a methodology that directly presents stimuli to the oral cavity we have examined the consumatory behavior of Stage I LHX-rats. We report that the taste reactivity (TR) and ingestion of orally infused substances appears normal in LHX-rats after food and water deprivation. Conversely, while tube feeding has no effect on these measures in intact rats, it results in a dramatic disruption of ingestion in LHX-rats.

Eighteen male rats received bilateral LH lesions. Pellets, a moist cereal, and H_2O were available ad lib and intakes were monitored daily. LHX-rats also received an average of 1 daily 12ml intragastric meal (sweetened condensed milk + water). Assessment of TR and intake of orally infused H_2O or sucrose (1.0M) began on the second post-surgical day. Slow motion analysis of videotapes was used to categorize the orofacial components elicited by intraoral injections of sucrose, NaCl, quinine, and water. Consumption of H_2O and 1.0M sucrose (1.0ml orally infused over 1 min) was also determined.

The average duration of adipsia and aphagia following lesions was 18.7±4.6 and 7.8±0.9 days, respectively. During this time and under conditions of 24hr food and water deprivation, LHX-rats exhibited normal TR and ingested 0.73±0.10 ml of H_2O and 0.98±0.01 ml of 1.0M sucrose. Conversely, when tested 90 mins after a tube fed meal, all LHX-rats passively or actively rejected H_2O and 13 of 18 animals demonstrated components of rejection sequences to all tastes examined. Further, ingestion of water was eliminated (0.05±.02ml) and 1.0M sucrose substantially reduced (0.36±0.08ml). This tube feeding effect does not appear to result from 1) the tubing procedure, or 2) GI distension because "dry-tubed" LHX-rats and those tubed 0.2M mannitol exhibit normal TR and ingestion. Collectively, these results suggest that the rejection of orally infused H_2O and taste stimuli that results from intragastric feeding in Stage I LHX-rats may result from exaggerated sensitivity to some post-absorptive consequence(s) of the meal. As such, these data further suggest that aphagic and adipsic LHX-rats may possess greater regulatory capacity than originally assumed.

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12.6 HYPERPHAGIA AND OBESITY PRODUCED BY UNILATERAL HYPOTHALAMIC KNIFE CUTS IN THE RAT. Anthony Sclafani and Paul F. Aravich*. Dept. of Psychology, Brooklyn College, Brooklyn, N.Y. 11210.

Unilateral parasagittal knife cuts between the medial and lateral hypothalamus did not significantly alter food intake or body weight in chow-fed female rats, whereas bilateral knife cuts induced a robust hyperphagia and obesity. When switched to a high fat chow, however, the unilateral cut rats (n=10) overate and outgained control subjects (n=8), although their food intake and weight gain was still considerably less than that of the bilateral cut rats (n=8). The unilateral cut rats further increased their food intake and weight gain when given a mixed palatable diet (MPD: chocolate-chip cookies, sweetened milk, high fat chow), and they eventually reached a level of obesity (728 g) only slightly less than that of the bilateral cut rats (761 g), and significanly higher than that of the controls (572 g). Although initially their caloric intake on the MPD was similar (198 vs 206 kcal/day), the unilateral and bilateral cut rats differed in their selection of foods. Compared to controls, the unilateral cut rats overate the MPD, but the food selections of the two groups were similar. When switched from the MPD back to the high fat chow, the unilateral cut and control groups lost weight (69 vs 44 g/30 days, n.s.), whereas the bilateral cut group gained weight (62 g/30 days). Therefore, although the unilateral cut rats became as obese as did the bilateral cut rats on the MPD, the two groups differed in several respects. As previously observed, control rats gained excessive weight on the high fat chow and especially on the MPD, and the effect of the unilateral knife cuts was to potentiate this diet-induced obesity.

12.7 HYPERDIPSIA AND ENHANCED SODIUM APPETITE AFTER AV3V LESIONS IN RATS. Edward M. Stricker and Thomas W. Gardiner*. Dept. of Psychology, Univ. of Pittsburgh, Pittsburgh, PA, 15260.

Large ablations of the anteroventral third ventricle (AV3V) region of the forebrain result in a transient adipsia and permanent loss of the drinking response of rats to a salt load (Buggy and Johnson, AJP 233:R44, 1977). With more discrete lesions centered near the ventral portion of nucleus medianus, we find that some animals become adipsic and later exhibit drinking deficits, while other rats with similar lesions become markedly hyperdipsic and usually do not show drinking deficits. Of 53 lesioned rats examined thus far, 18 exhibited hyperdipsia, 7 after a period of adipsia. Their mean daily water intakes ranged from 90 to 148 ml, 2-3 times those of control animals, throughout the 90 day period of observation. Almost all of this excess consumption occurred at night. Preliminary experiments suggest that the hyperdipsic rats do not have diabetes insipidus because urine volumes during overnight fluid deprivation were comparable to control values. Furthermore, hyperdipsic rats given hypertonic NaCl solution by sc injection excreted a concentrated urine. They excreted this sodium load much more slowly than control animals, however, and the degree of sodium retention correlated highly with the magnitude of hyperdipsia. These findings suggest that the hyperdipsia may be provoked by a prolonged retention of the sodium ingested in chow. However, rats remained hyperdipsic whether deprived of food overnight or maintained on a sodium deficient diet.

Another phenomenon observed in several lesioned animals was an unusually large consumption of hypertonic (.5M) NaCl solution. Control rats usually ingest less than 5 ml of this unpalatable fluid daily, whereas 6 of 8 rats that were hyperdipsic when given water alone consumed between 10 and 35 ml of saline each day. Although these intakes are comparable to those of adrenalectomized rats, renal sodium losses during 24 -hr sodium deprivation were normal, and both renin and aldosterone values were not elevated. Also, preliminary measures of plasma sodium, plasma protein, and hematocrit yielded values comparable to those for controls. The basis for this excessive drinking of saline, as well as the hyperdipsia, remains to be investigated.

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12.8 CONSUMMATORY BEHAVIORS AND WHEEL RUNNING ACTIVITY IN THE RAT FOLLOWING INTRAHYPOTHALAMIC INJECTIONS OF KAINIC ACID. M. Kalamatizadeh*, K. Sharifi Hossaini and M.S. Shahid Salles. Department of Physiology and Pharmacology, Shiraz University School of Medicine Shiraz, Iran.

Electrolytic lesions of the lateral hypothalamus (LH) produce aphagia, adipsia and akinesia. These have been interpreted as indicating the presence of eating, drinking centers and cells controlling locomotion within the lateral hypothalamus. We have previously shown that selective destruction of dopaminergic cells in or outside of the "mid lateral" hypothalamus reproduce the same syndrome, while a non-selective chemical lesion of monoaminergic cells did not reproduce the lateral hypothalamic syndrome. This finding supported the regulatory role of cells in the LH area in the control of ingestive behavior and locomotor activity. In order to further examine this hypothesis we have studied the behavior of rats following intrahypothalamic injections of kainic acid (KA). Kainic acid was dissolved in 0.9% NaCl (10 nM/1 µl) and administered over 240 sec in 25 rats. Rats given KA in the "mid lateral" hypothalamus showed lasting aphagia, adipsia and akinesia which invariably was lethal. Injections in the posterolateral hypothalamus encroaching on pars reticularis of substantia nigra produced permanent hyperdipsia without a significant change in locomotor activity or in food intake. Hyperdipsia disappeared during food deprivation and was absent when hypertonic saline was injected intraperitoneally. The fact that hyperdipsia was obtained in the absence of change in food intake and locomotion suggests that water intake is modulated by neurons in subthalamic region projecting to the LH area.

12.9 GLUCOPRIVIC FEEDING: PREVENTION BY PERIPHERAL BUT NOT BY CENTRAL SUGAR INFUSIONS. Robert C. Ritter. Department of Veterinary and Comparative Anatomy, Pharmacology and Physiology, College of Veterinary Medicine, Washington State University, Pullman, WA 99164 and WOI Regional Program in Veterinary Medicine, University of Idaho, Moscow, ID 83843.

Peripheral injections of antimetabolic glucose analogues, such as 2-deoxyglucose (2DG) or 5-thioglucose (5TG) cause increased feeding in a variety of mammals. The fact that intracerebroventricular (ICV) glucose analogue infusions also elicit feeding suggests that at least some of the glucoreceptors which mediate glucoprivic feeding are in the brain. Recent findings from my laboratory indicate that the receptors which mediate feeding in response to central glucoprivation are in the caudal brainstem (Slusser, Stone and Ritter, Neuroscience Abst 49.4, 1980). However, our findings do not rule out the participation of non-brain glucoreceptors in the control of glucoprivic feeding. Neither do our results prove that brain glucoreceptors are responsible for mediation of feeding in response to whole body glucoprivation.

In order to determine whether feeding in response to cerebral glucoprivation could be antagonized by elevation of plasma sugar concentrations, we examined the effects of intravenous infusions of 1.2 M glucose, 1.2 M fructose or 0.6 N NaCl upon feeding elicited by ICV 5TG. We found that feeding elicited by ICV 5TG is abolished by IV glucose but not by fructose or NaCl. The differential effects of glucose and fructose upon feeding elicited by central 5TG are similar to those reported by Rowland and Stricker for feeding elicited by peripheral 2DG. However, we also found that plasma catecholamine elevation elicited by ICV 5TG was not suppressed by intravenous glucose or fructose. This result may mean that glucose-induced prevention of feeding is affected at a site more accessible to blood born glucose (e.g. hepatic receptors) than that which contains the receptors mediating sympathoadrenal discharge. In fact we have found that ICV infusions of glucose (0.125 or 1.2 M) or fructose (0.25 M) fail to suppress feeding in response to peripherally administered 2DG. This result might support the existance of peripheral glucoreceptors for feeding. However, ICV sugar infusions also failed to prevent sympathoadrenal hyperglycemia. Therefore, it is also possible that ICV sugars do not reach the brain glucoreceptors in concentrations sufficient to antagonize the highly competitive glucose analogue. Experiments now in progress will differentiate between these alternative interpretations.

12.10 OROGASTRICALLY-MEDIATED INSULIN RELEASE MAY BE REQUIRED FOR TERMINATION OF DELAYED GLUCOPRIVIC FEEDING BY GLUCOSE. Sue Ritter and Nancy L. Pelzer*. Coll. Vet. Med., Wash. State Univ., Pullman, WA 99164.

Enhanced feeding in response to glucoprivation can still be observed from 6-8 hr after insulin or 2-deoxy-D-glucose (2DG), even though blood glucose has returned to normal levels. In fact, when food is withheld for 6 hr after injection of insulin or 2DG, rats consume as much in the 2 hr postglucoprivic feeding test as they do when food is continuously available during ongoing glucoprivation. This phenomenon is now referred to as delayed glucoprivic feeding (DGF). Recently, we reported that intravenous infusion of glucose from 1 1/2-2 hr after insulin fails to prevent DGF. Nevertheless, ingestion of an equivalent amount of glucose (7.9 Kcal) over the same time period abolishes DGF. DGF is also abolished when glucose infusion is accompanied by the ingestion of a saccharin solution, even though each substance by itself is without effect. Thus, it appears that glucorestoration must occur in combination with some orogastrically-mediated signal in order to terminate DGF. We have hypothesized that orogastrically-mediated insulin release may be the required signal, since other investigators have shown that postglucoprivic release of endogenous insulin is stimulated more effectively by ingested than by infused glucose. As a partial test of this hypothesis, we generated tolerance curves for ingested and infused glucose in normal rats and in rats exposed to insulin-induced glucoprivation. Glucose was administered 3 1/2-4 hr after insulin injection when exogenous insulin had disappeared from blood, as determined by radioimmunoassay. We found that after prior glucoprivation, clearance of both ingested and infused glucose was significantly impaired. After glucose infusion, however, the impairment was more pronounced. Moreover, infusion of glucose in combination with epinephrine, which is known to be elevated by glucoprivation and to inhibit glucose-stimulated insulin release mimicked the effect of prior glucoprivation. The differential clearance of infused and ingested glucose from blood would be compatible with a greater stimulation of insulin release by ingested glucose. If this hypothesis is born out by additional experiments, we might speculate that insulin suppresses DGF by making glucose available to peripheral tissues. Data we have recently reported indeed suggests that DGF is abolished only by restoration of nutrients to both brain and peripheral tissues. The fact that DGF can be abolished by β-hydroxybutyrate infusion, while infused glucose is ineffective, is compatible with this view as well, since β-hydroxybutyrate can be utilized by brain tissue and, in addition, does not require insulin for its uptake by peripheral tissues.

12.11 FACTORS INVOLVED IN THE CONTROL OF DELAYED GLUCOPRIVIC FEEDING AND POSTGLUCOPRIVIC HYPOTHALAMIC NOREPINEPHRINE TURNOVER. Steven I. Bellin and Sue Ritter, College of Veterinary Medicine, Washington State Univ., Pullman, WA 99164.

In rats, delayed glucoprivic feeding (DGF) can be demonstrated from 6-8 hr after a large insulin dose (Ritter et al, Am. J. Physiol., 234:E617-E621, 1978) when other signs of glucoprivation are no longer in evidence. Elevated rates of hypothalamic norepinephrine (NE) turnover are also associated with glucoprivation and, like enhanced feeding behavior, persist into the postglucoprivic period. The ingestion of isocaloric (7.9 Kcal) pelleted rodent chow or D-glucose (10.5 ml, 18% w/v solution), as well as the intravenous infusion of caloricallly equivalent glucose (4.4 ml, 2.4M) or DL-β-hydroxybutyrate (BOH, 5.1 ml, 2.4M) solution, from 1 1/2-2 hr following 2.5 U/kg regular insulin (s.c.), normalize apparent rates of hypothalamic NE turnover. However, isocaloric fructose solutions, [ingested (10.5 ml, 18% w/v) or infused (4.4 ml, 2.4M)] or ingestion of fat (7.9 Kcal lard) or saccharin, do not. These data suggest that restoration of a fuel available to brain tissue is both necessary and sufficient to normalize NE turnover rates after insulin. Similarly, DGF is abolished by ingested chow, ingested glucose or infused BOH. However, glucose infusion has no effect on DGF. Moreover, ingestion or infusion of fructose or ingestion of lard (7.9-47 Kcal) significantly attenuates, but does not abolish, DGF. Thus, in contrast to postglucoprivic NE turnover, it would appear that the abolition of DGF requires cooperative action of signals arising from receptors on both sides of the blood-brain barrier. If so, the effectiveness of ingested pellets or glucose and of infused BOH in abolishing DGF would reflect their access to receptors on both sides of the blood-brain barrier. The failure of fructose and fat to totally abolish DGF may reflect the exclusion of these nutrients from brain. However, their partial effectiveness supports the hypothesis that a signal of peripheral origin contributes to the termination of DGF. The differential effects of ingested and infused glucose on DGF are puzzling and may indicate that preabsorptive signals are required for termination of DGF by glucose. We hypothesize that orogastrically-mediated insulin release is a likely candidate for such a signal. Finally, the dissociation of feeding and enhanced NE turnover in these experiments suggests that NE neurons may not play a causal role in the termination of DGF.

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12.12 CHRONIC DECEREBRATE RATS DEMONSTRATE PREABSORPTIVE INSULIN SECRETION AND HYPERINSULINEMIA. Harvey J. Grill and Kent Berridge*, Dept. Psychol. and Inst. Neurol. Sci., U. Pennsylvania, Philadelphia, Pa. 19104.

The role of the forebrain in regulating tonic insulin secretion, and of the caudal brainstem in analyzing and relaying gustatory information to the pancreas was examined in 15 chronic decerebrate rats. Supracollicular decerebration resulted in a dramatic and persistent rise in plasma insulin levels, which lasted throughout the 2 week period examined after transection. Baseline levels rose from 1-2 ng/ml to 4-8 ng/ml. This hyperinsulinemia was accompanied by intermittent glycosuria and was abolished by a 22 hr fast. In contrast, hemidecerebration produced only an occasional and transient rise in insulin secretion, which disappeared within 2 weeks, and was not accompanied by glycosuria. In spite of the metabolic disturbance displayed, evidence was obtained that the 22 h fasted decerebrate retains the capacity to respond to an oral glucose infusion (2.76 M) by rapidly increasing its plasma insulin levels by 1-3 ng/ml, prior to glucose absorption. These data suggest that a mechanism for analyzing taste information and neurally activating insulin secretion is complete within the caudal brainstem. Procedure: 15 male rats were maintained on a sweetened condensed milk diet for 3 days before duplicate 200 ul tail-blood baseline samples were taken, and urine was collected. Rats were then hemidecerebrated at midbrain level by blunt spatula transection. Urine and blood samples were taken at days 2,7,and 14 after hemitransection. Decerebration was then completed and rats were subsequently maintained by intubation. Urine and blood samples were taken at days 2,7, and 14 after this second stage transection. After 2 weeks, surviving rats were implanted with oral and jugular cannulae, and given 2 days to recover before being run in our standard preabsorptive insulin response test (Berridge, Grill, & Norgren, 1981): a 200ul baseline blood sample was taken via jugular cannula, and 1 ml of a 2.76 M glucose solution was infused into the mouth over 1 min. Blood samples were taken each min for the first 5 min, and every other min for the next 4. A no-stimulus control test was run 2 days later. All blood samples were centrifuged, and the plasma radioimmunoassayed for insulin and assayed for glucose. Urine was tested for glucose using Multistix test strips.
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13.1 VASOPRESSIN POTENTIATION IN THE PERFORMANCE OF A LEARNED APPETITIVE TASK. G.F. Koob, A. Ettenberg, M. Le Moal, and F.E. Bloom. A.V. Davis Center for Behavioral Neurobiology, The Salk Institute, San Diego, California 92138.

Recent research has provided evidence of a role for the neurohypophyseal hormone vasopressin (AVP) in memory consolidation. However, the putative involvement of AVP in memory is based almost entirely on data drawn from paradigms employing aversively motivated tasks (e.g. conditioned avoidance paradigms). If AVP has general memory-enhancing properties, this should be demonstrable using a positively motivated (appetitive) task.

Naive male albino rats were individually placed into a large rectangular open box for 5 min. each. Recessed into the middle of one of the long walls of the box was an alcove that contained a standard metal drinking tube filled with water. While the rats all found and inspected the tube, none consumed any measurable amount of water during the 5 min. exposure. Upon removal from the box, the rats were treated with one of the following four injection procedures: a saline injection followed, 2 min. later, by another injection of saline (SAL/SAL); saline followed by a 1.0 µg injection of AVP (SAL/AVP); a 5.0 µg dose of the AVP receptor antagonist [1-deaminopenicillamine, 2-(o-methyl) tyrosine] arginine vasopressin, followed by a 1.0 µg injection of AVP ($AAVP_5$/AVP) or a 25.0 µg dose of the antagonist followed by 1.0 µg of AVP ($AAVP_{25}$/AVP). Immediately after this injection regimen, animals in all four groups were water deprived for 48h. The rats were then returned to the test box where the latency to make initial contact with a dry drinking tube was recorded for each animal.

When tested in this "latent learning" paradigm, AVP during training produced reliable improvement (faster latencies) in finding the water tube during a memory test conducted two days later. This effect was blocked by 25.0 µg of the AVP receptor antagonist but not by 5.0 µg. These results are, therefore, consistent with the view of an AVP involvement in memory consolidation.

This research was supported by federal grants NIDA 01785 and NIAAA 03504.

13.2 BRATTLEBORO RATS, WHICH CONGENITALLY LACK VASOPRESSIN, ARE HYPERRESPONSIVE TO ELECTRICAL SHOCK AND ACOUSTICAL STIMULATION. D.M. Gash and P.H. Warren.* (Spon. by Dr. Wayne Hoss). Depts. of Anatomy, Center for Brain Research, and Psychology, University of Rochester, Rochester, NY 14642.

Studies over the past decade from several laboratories have demonstrated that the neuropeptide hormone vasopressin (VP) has significant behavioral effects and may be involved in memory and learning processes. Since these studies have largely relied upon shock motivated behavior, an important issue to be addressed is the role of VP in an animal's response to painful or stressful stimuli.

In the present study we employed a sensitive quantitative test, based upon the startle response, to assess responsiveness to an electric shock and an acoustic stimulus. We compared the responsiveness of animals lacking the ability to synthesize VP (homozygous Brattleboro rats) with animals that do synthesize VP (heterozygous Brattleboro rats). Thirty homozygous and 10 age-matched heterozygous males were used as subjects. The homozygous rats responded to shock at 800 and 1000 µA at a greater amplitude then the heterozygotes ($p \leq 0.005$). The homozygotes similarly had a higher startle response to acoustic stimulation (20 ma, 110 DBA, 10 KHz; $p \leq 0.001$).

These data indicate that homozygous Brattleboro rats should have a better retention of the punishment effect in an approach-avoidance test than heterozygotes. Indeed, in recent studies in our laboratory (Brito et al. Brain Res. Bull. 6: 71-75, 1981) we have observed this effect. The present study suggests that at least part of the influence VP exerts on behavior may be due to modification of an animal's responsiveness to pain and stress.

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13.3 ANTINOCICEPTIVE EFFECTS OF CENTRAL AND SYSTEMIC ADMINISTRATION OF LYSINE VASOPRESSIN AND STRUCTURAL ANALOGS. J. H. Kordower, V. Sikorszky,* J. Cort* and R. J. Bodnar. Dept. of Psychology, Queens College, CUNY, and Dept. of Physiology, Mount Sinai Medical School, New York, NY.

The magnocellular neurosecretory peptide vasopressin is also distributed in such extrahypothalamic regions as the periaqueductal gray and substantia gelatinosa, suggesting a role in pain perception.[1, 2] This notion is supported by the observation that Brattleboro rats, deficient in vasopressin, display hyperalgesia and selective analgesic deficits.[3] Moreover, Berntson and Berson[4] found that systemic and central injections of vasopressin at doses of 16-100 ug increased tail-flick latencies. The present study extended these findings to physiological doses and sought to establish structure-activity relationships of vasopressin's anti-nociceptive effects. Pain thresholds were determined over six 2-trial sessions spaced 5 min apart as measured by the tail-flick and flinch-jump tests. Following baseline, nanogram doses of lysine vasopressin (LVP) or its prolonged analog was administered either intracerebroventricularly (0, 150, 500 ng/5 ul) or subcutaneously (0, 150, 500, 1500 ng/5 ul) 5 min prior to the fourth session. Injection order was incompletely counterbalanced and administered on alternate days. Tail flick latencies of 8 rats were significantly increased by central LVP 10 and 15 min following the 150 ng dose, and at 5, 10 and 15 min following the 500 ng dose. Systemic LVP increased tail-flick latencies 15 min following the 1500 ng dose. The prolonged LVP analog increased tail-flick latencies of 8 other rats only after central administration. However, it was effective at both doses across all post-injection intervals. Vehicle injections failed to alter latencies. By contrast, neither systemic nor central injections of LVP or its prolonged analog changed flinch-jump thresholds of 10 rats at any dose or post-injection test time. These results indicate that the anti-nociceptive effects of LVP are modality-specific and selective for noxious thermal, as opposed to electrical, stimuli. Given the dose and time course of vasopressin's effects, it appears that the antinociception is mediated by central mechanisms. Ongoing research with LVP analogs that selectively inactivate the acetyl or carboxyl terminals of the peptide chain will further define vasopressin's antinociceptive effect. (Supported by NIH Grants NS 14449 and 5S05RR07064.)

[1]Swanson, L. W., Brain Res. 128 (1977) 346-353.
[2]Nilaver et al., Neuroendocrin. 30 (1980) 150-158.
[3]Bodnar et al., Life Sciences 26 (1980) 1581-1590.
[4]Berntson and Berson, Life Sciences 26 (1980) 455-459.

13.4 VAGOTOMY ABOLISHES THE INHIBITORY ACTION OF CHOLECYSTOKININ ON EXPLORATORY BEHAVIOR IN RATS. J. N. Crawley, S. E. Hays*, S. M. Paul* and F. K. Goodwin. Clinical Psychobiology Branch, NIMH, Bethesda, MD 20205.

Cholecystokinin (CCK_8) inhibits feeding behavior in fasted animals (Antin et al., 1975). Controversy exists over possible aversive abdominal responses to systemic CCK_8 administration (Deutsch and Hardy, 1977). Lesioning of sensory pathways from the abdomen to the brain by bilateral vagotomy attenuates or abolishes CCK_8-induced satiety in rats (Simansky, Jerome and Smith, 1980).

We have shown that CCK_8 reduces exploratory investigation of a wide variety of environmental objects (Crawley et al., 1981). In mice and rats, intraperitoneal CCK_8 reduced parameters of exploratory behavior, including duration of pauses, number of movements, and time spent in the corners of an open field, as well as approaches to novel objects and encounters with a female. Intraperitoneal and intraventricular routes of CCK_8 administration produced identical profiles of inhibition of exploratory behaviors. The intraventricular dose required for this effect was similar to the intraperitoneal dose, suggesting a lack of central specificity.

Unsulfated CCK_8 did not significantly reduce exploratory behaviors. Since sulfated CCK_8 binds with much greater potency than unsulfated CCK_8 to the peripheral CCK_8 receptor (Sankaran et al., 1980) but these two analogs have relatively similar potencies at the central CCK_8 receptor (Saito et al., 1980), these results provide a second indication for a peripheral site of the CCK inhibition of exploration.

Bilateral abdominal vagotomy blocked the inhibitory effects of CCK_8 on exploratory behavior. Vagotomized rats treated with CCK_8 either intraperitoneally or intraventricularly showed normal exploratory behaviors as compared to saline-treated controls. Sham-operated rats treated with CCK_8 replicated the previously reported reduction in exploratory parameters.

These results provide several further lines of evidence implicating peripheral CCK receptors in a sensory feedback pathway from the digestive organs to central sites regulating attention to environmental stimuli.

13.5 CEREBRAL VENTRICULAR TRANSPORT AND UPTAKE: IMPORTANCE FOR THE CCK SATIETY EFFECT. CA Baile and MA Della-Fera, Sch Vet Med, U Pa, Kennett Square, PA 19348.

CCK-8 is a potent and specific suppressor of food intake when injected into the lateral cerebral ventricles(LV) of sheep. Studies showing that feeding is increased during injection of specific CCK antibody (CCK-AB) into LV provide strong support for a role for endogenous brain CCK-8 in satiety. It is unlikely that the large antibody molecules entered the brain from CSF, thus the increased feeding was due to sequestration of CSF CCK by CCK-AB. These results, therefore, also suggest that CCK-8 must first be secreted into CSF before it reaches site(s) of action for satiety. Support for this hypothesis was obtained from preliminary studies indicating a change with feeding and fasting in immunoactive CCK concentration (iCCK) in LV CSF of sheep. However, in a repeat study in which CSF was collected from the cisterna magna(CM), iCCK concentrations were too low to measure. Apparently, loss of CCK occurred as it traversed the ventricular system.

Because of the very low endogenous levels of CCK in CM CSF, two proposals were made: 1) Exposure of CCK-8 to ventricular sites more rostral than CM is necessary for its satiety effect; 2) CCK-8 is either enzymatically degraded in CSF as it traverses the ventricles or CCK-8 is taken up from CSF by specialized ependymal cells (as shown for other CSF substances). To test the first proposal, we compared the effects on food intake of 75 min continuous injections into LV or CM of synthetic CSF or .64 pmole/min CCK-8 in 2 hr fasted sheep (N=5). LV CCK-8 injections decreased food intake 40-50% ($p<.05$), but CM injection of CCK-8 had no effect. In another study 2.55 pm/min CCK-8 injected into CM also had no effect.

To test the second proposal, two experiments were carried out: 1) CCK-8 was added to CSF and incubated for up to 24 hr at 37°C. There was no diminution in iCCK concentration over time, thus, enzymatic degradation of CCK-8 in CSF is probably not responsible for the low CM CCK concentrations. 2) To determine whether disappearance of CCK-8 did indeed occur as it traveled thru the ventricular system, .64 pm/min CCK-8 was injected into LV of sheep (N=4) for 3 hr (.03 ml/min) and CM CSF was collected every 30 min for measurement of iCCK. Inulin (2.5 mg/ml) was added as a marker for measurement of bulk absorption of CSF. Only about 5% of the CCK-8 was recovered from CM, thus about 95% of the CCK-8 was removed from more rostral ventricular areas by means other than enzymatic degradation or bulk absorption. In a repeat study using LV injection of .16 pm/min CCK-8 , the same results were obtained.

We propose that during feeding, CCK-8 is secreted into CSF and travels via CSF to ventricular sites at which it is actively taken up and transported to receptors responsible for its satiety action. Supported in part by NIH Postdoctoral Fellowship NS06595.

13.6 ROLE OF PEPTIDES IN THE CONTROL OF FOOD INTAKE OF OBESE RODENTS. C. L. McLaughlin and C.A. Baile, Dept. Clin. Studies, School Vet Med., Univ. of Penn., Kennett Square, PA 19348

The increased meal size characteristic of adult Zucker obese rats was shown to be present in weanling rats as early as 4 wks of age (.97 vs .70 g, $p<.02$) by using an automated feeding behavior data collection system. Food intake 60 min after a 5.5-hr fast was 67% greater in 4-wk old obese rats than lean rats (2.5 vs 1.5 g, $p<.01$) and 100% greater in 5-6 wk old obese Bar Harbor mice than lean mice (.71 vs .36 g, $p<.001$). The responses of obese rats and mice were compared with those of lean rats and mice to several peptides thought to play a role in the control of food intake. Serum concentrations of 3 of the peptides increase during a meal - cholecystokinin (CCK) and bombesin (BBS), released primarily from the duodenum, and pancreatic polypeptide (PP), released primarily from the pancreas. Food intake has been shown to be decreased in rats by exogenous administration of these peptides and by naloxone, a specific antagonist of opiates, which can stimulate food intake. As much as 4.0 µg/kg CCK-8, which decreased 60 min food intake of 4-wk old lean rats by 70%, had no effect on food intake of obese rats ($p<.01$). In obese mice 5-6 wks old 2 µg/kg CCK-8 also had no effect on food intake while decreasing that of lean rats 22% (.28 vs .36 g, $p<.05$). Adult Zucker obese rats were less sensitive to satiety elicited by a dose-range of concentrations of CCK-8 (.06 to 1.0 µg/kg) injected before meals scheduled 2 hrs apart and adult obese mice were less sensitive than lean mice to 1.0 µg/kg CCK-8 after a 3.0 hr fast (100 vs 50% of control, $p<.03$). BBS affected food intake similarly in both 4-wk old and adult obese and lean rats. However, obese mice were less sensitive than lean mice when 5-6 wks old and fasted 5.5 hrs, but were equally sensitive when they were adults. In adult mice 8 µg/kg PP decreased food intake less in obese than lean mice (94 vs 62% of control, $p<.04$). In adult rats, however, up to 128 µg/kg PP did not affect food intake of Zucker obese rats more than lean rats when injected during the initiation of the dark cycle (47 vs 97% of control, $p<.003$), but not when injected during the light (95 vs 68% of control, $p<.08$). Thus, while only obese mice are less sensitive to BBS and PP, both obese mice and rats are less sensitive to satiety elicited by CCK. This decreased responsivity in obese animals could result in increased average meal size and daily food intake.

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13.7 STIMULANT EFFECTS OF NEUROTENSIN MICORINJECTION INTO THE VENTRAL TEGMENTAL AREA. P.W. Kalivas*, S.K. Burgess, C.B. Nemeroff and A.J. Prange, Jr., Biological Sciences Research Center, Univ. of North Carolina, School of Medicine, Chapel Hill, NC 27514

Neurotensin (NT) is an endogenous tridecapeptide which has a heterogenous distribution in the central nervous system (CNS), including high concentration in the ventral tegmental area (VTA). The fact that dopaminergic (DA) perikarya are also found in the VTA, and that these perikarya have limbic terminal fields which have been implicated in modulating spontaneous motor activity, raises the possibility that NT release in the VTA may alter spontaneous motor activity. To test this postulate, NT was bilaterally microinjected into the VTA and motor behavior quantified.

Male S.D. rats were chronically implanted with bilateral guide cannulae 1 mm above the VTA. One week following surgery, animals received a microinjection of NT or saline vehicle (0.6 µl/side/0.6 min). All cannulae placements were verified via light microscopic examination of cresyl violet stained brain sections. Automated measurements of motor activity were obtained using a Stoelting electromagnetic apparatus. Animals were adapted to the apparatus for 120 min prior to microinjection and behavior quantified for at least 120 min post-injection.

Administration of 5 µg NT (total dose/brain) resulted in a significant increase in motor behavior vs. saline controls. Using a standard ethogram, an observer ignorant of the treatment found that NT induced significant increases in exploratory behaviors (sniffing, rearing, locomotion) at the expense of resting or sleeping. The chemical specificity of this NT-induced effect was shown by demonstrating that in doses equimolar to 5 µg NT, neither TRH, LHRH nor D-Pro^{10}-NT altered spontaneous motor activity. In contrast, D-Tyr^{11}-NT was at least as potent as NT. The involvement of mesolimbic DA was investigated by pretreating animals in the lateral ventricle with 10 µg haloperidol. While this treatment did not alter baseline motor activity, it significantly decreased NT-induced motor activity. Further, 60 min following intra-VTA administration of 5 µg NT, animals were sacrificed and DA and DOPAC content determined using HPLC. As compared to saline, NT treatment caused a significant increase in the DOPAC/DA ratio in the n. accumbens, but no change in the striatum. These findings demonstrate that intra-VTA administration of NT results in an increase in spontaneous motor activity, probably via activation of the mesolimbic DA system. The existence of immunoreactive NT in the VTA suggests that these findings may reflect a physiological function of NT in the CNS. This work was supported by NIMH MH-32316, MH-22536, MH-34121, MH-33127 and NICHHD HD-03110.

13.8 CYTOPROTECTION BY INTRACISTERNALLY ADMINISTERED NEUROTENSIN AGAINST COLD-RESTRAINT STRESS-INDUCED GASTRIC ULCERS: CORRELATION OF TIME COURSE WITH EFFECTS ON GASTRIC PH AND ANTERIOR PITUITARY HORMONE LEVELS Charles B. Nemeroff, Daniel E. Hernandez*, Roy C. Orlando*, George A. Mason* and Arthur J. Prange, Jr., Biol. Sci. Res. Ctr., Depts. Psychiatry and Medicine, Univ. North Carolina Sch. Med., Chapel Hill, NC 27514.

Since central nervous system (CNS) administration of neurotensin (NT), an endogenous peptide, was reported to inhibit gastric acid secretion, we previously compared the effect of intracisternally (IC) administered NT and systematically administered cimetidine on the development of cold-restraint stress (CRS)-induced gastric ulcers in rats. After three hr of CRS, IC NT, but not IP cimetidine (an inhibitor of acid secretion), was cytoprotective in this model. In this study the relationship between cytoprotection and acid secretion was further investigated by simultaneous monitoring of gastric mucosal pathology and gastric pH at 30 min intervals over the three hr stress period. Since the gastric mucosa may be altered by pituitary hormone secretion, serum levels of growth hormone (GH), thyrotropin (TSH) and prolactin (PRL) were assayed. Groups of adult male S-D rats (200-250 g) ($n \geq 5$/group) were food deprived (24 hr), treated with IC saline (controls), IC NT (30 µg) or IP cimetidine (100 mg/kg), then immediately restrained with wire mesh and placed supine in a cold room (4°C) for 30,60,90,120,150, or 180 min. After CRS, the rats were decapitated and blood collected for hormone assays. The pH of the gastric contents was measured and the gastric mucosa examined for lesions with dissecting microscope. The incidence of gastric lesions was scored without knowledge of the treatment regimen. After 30 and 60 min of CRS, no gastric lesions developed in any treatment group. However from 90-180 min, saline-treated controls showed a significantly increased incidence of gastric ulcers; an increase paralleled in the cimetidine-treated group for all time periods except 90 min. In contrast, for the entire 180 min period, NT-treated rats showed a marked reduction in ulcer incidence. Despite the failure of cimetidine to remain cytoprotective, for each time period the pH of the gastric contents was approximately 6, a value significantly higher than either NT-treated (pH=4) or saline-treated rats (pH=4). Cimetidine did not significantly alter GH, TSH, or PRL levels when compared to saline-treated rats; NT-treated rats exhibited a smaller TSH response at 30 min, followed by a rebound rise in TSH after 90 min of CRS. No change in PRL or GH was noted after IC NT. These data indicate that the anti-ulcerogenic effect of NT is neither mediated by inhibition of gastric acid secretion nor by altered secretion of GH or PRL. The relationship between changes in TSH and cytoprotection following NT remains obscure (Supported by NIMH MH-32316, MH-34121, MH-33127, MH-22536 and NICHHD HD-03110).

13.9 THE EFFECTS OF D-AMPHETAMINE, METHYLPHENIDATE AND APOMORPHINE ON NEUROTENSIN-INDUCED HYPOTHERMIA IN MICE. G. Bissette*, D. Luttinger, C.B. Nemeroff and A.J. Prange, Jr., Biol. Sci. Res. Ctr., Univ. North Carolina Sch. Med., Chapel Hill, NC 27514

Neurotensin (NT), an endogenous tridecapeptide, produces hypothermia, muscle relaxation and analgesia after intracisternal (IC) or direct central nervous system (CNS) injection in rodents (Proc. Natl. Acad. Sci. 76:5368, 1979). Our group has also demonstrated that destruction of brain dopamine (DA), but not norepinephrine, containing systems with 6-hydroxydopamine significantly potentiates NT-induced hypothermia, as does pretreatment with the DA receptor antagonist, haloperidol (Brain Res. 195:69, 1980). To further characterize the interaction between NT and brain DA systems, the effect of 3 stimulant drugs (d-amphetamine, methylphenidate and apomorphine) on NT-induced hypothermia was studied. These pharmacological agents are generally acknowledged to act primarily on CNS DA circuits. Adult, male Swiss-Webster mice were lightly anesthetized with ether and injected IC with either NT (0.1-30 µg) or vehicle (0.9% NaCl, 10 µl). The mice were then immediately treated with intraperitoneal (IP) injections of d-amphetamine (0.5-2.0 mg/kg), methylphenidate (10 mg/kg), apomorphine (2.5-5.0 mg/kg) or vehicle. Thus each experiment included 4 groups of mice (n=8/group): (1) Vehicle IC + vehicle IP; (2) NT IC + vehicle IP; (3) Vehicle IC + Stimulant IP; (4) NT IC + Stimulant IP. Rectal temperatures were assessed at 0,30,60,90 and 120 minutes post-IC injection with the use of a digital display thermocouple; all experiments except otherwise noted were conducted at ambient temperatures of 25°C. D-amphetamine (2 mg/kg) completely blocked the hypothermic effect of IC NT (10 and 30 µg) whereas a lower dose of this stimulant (1 mg/kg) partially but significantly antagonized the hypothermic effects of both 1 and 10 µg NT. Although d-amphetamine produces a significant hypothermic effect in cold-exposed rodents, mice treated with NT and d-amphetamine and placed at 8°C showed less hypothermia than mice treated with NT alone. Like d-amphetamine, methylphenidate (10 mg/kg) significantly blocked the hypothermia observed after IC NT (10 µg). As previously reported, apomorphine (2.5 or 5.0 mg/kg) produced a significant hypothermia; mice treated with both apomorphine and NT did not show a greater decline in body temperature than mice treated with either agent alone. These data, taken together with previous findings, support the hypothesis that NT and indirect DA agonists (e.g. d-amphetamine and methylphenidate) are antagonists in the CNS. (Supported by NIMH MH-32316, MH-34121, MH-33127, MH-22536 and NICHHD HD-03110).

13.10 SEASONAL VARIATION IN TRH CONTENT OF DIFFERENT BRAIN REGIONS IN THE HIBERNATOR, CITELLUS LATERALIS. T.L. Stanton, A. Winokur and A.L. Beckman. A.I. duPont Institute, Wilmington, DE, 19899 and Univ. of Pennsylvania, Sch. of Med., Phila., PA, 19104.

Studies in our laboratory have shown that central administration of thyrotropin releasing hormone (TRH) produces state-dependent changes in body temperature, metabolic rate, motor activity and general arousal level in the ground squirrel (Citellus lateralis). These observations suggest that TRH may modulate the level of activity within neuronal systems. In the present study, we have investigated the possibility that changes in TRH concentration in some brain areas might be associated with naturally-occurring changes in the physiology and behavior of this seasonal hibernator. The variation of endogenous TRH content in 10 regions of the brain was measured between seasons and across the euthermic (i.e., not hibernating) vs. hibernation state.

Ground squirrels were wild-trapped in late spring and housed individually under natural light conditions. Euthermic animals were sacrificed in the mid-portion of each season: early August (Summer), early November (Fall), early February (Winter), early May (Spring). Hibernating animals, housed at 5°C throughout the winter, were sacrificed in early February. Brains were dissected into: pineal, olfactory bulb, hypothalamus (HYP), septum, preoptic area (POA), forebrain, hippocampus, cerebellum, midbrain, and brainstem. Each region was weighed, homogenized, methanol extracted and assayed for TRH content by radioimmunoassay.

Statistically significant decreases in TRH content occurred during hibernation when compared to one or more euthermic groups in the HYP, septum, POA, midbrain, and olfactory bulb. Only in the HYP was TRH lower than in all of the euthermic groups. Significant fluctuation in TRH during hibernation also occurred in the pineal, but the level of TRH in this region depended upon elapsed time in the hibernation bout. TRH content was considerably greater in the last quarter of the bout than in the first quarter ($p < .001$). Seasonal variation within euthermic groups was also evident in the olf. bulb, POA, midbrain, and pineal, in which TRH levels fell significantly in the winter. The greatest variation was observed in the pineal; TRH content was lower in winter compared to fall ($p < .02$) and spring ($p < .001$) and increased in fall relative to summer ($p < .01$). These results suggest that TRH may be involved in the control processes attributed to these brain areas, and support a role for TRH in the neural control of hibernation. (Supported by the A.I. duPont Institute, NSF Grant BNS 78-19002, and Research Scientist Development Award K01-MH00044).

13.11 THYROTROPIN RELEASING HORMONE (TRH) AND THE TRH ANALOG MK-771 REVERSE ENDOTOXIN SHOCK VIA AN ACTION AT CENTRAL EFFECTOR SITES. John W. Holaday and Alan I. Faden. Dept. Med. Neurosci., Walter Reed Army Institute of Research, Washington D.C. 20012.

In previous reports, we have demonstrated the therapeutic efficacy of naloxone in reversing shock due to endotoxemia, hemorrhage, or spinal transection. Our concern was that this opiate antagonist would intensify traumatic pain even as it improved shock pathophysiology and survival. Recently, we have shown that intravenous (iv) TRH reverses endotoxic and hemorrhagic shock in rats without affecting nociceptive latencies (Science, in press). The purpose of the present study was to determine if TRH or the TRH analog MK-771 (pyro-2-aminoadipyl-histidyl-thiazolidine-4-carboxamide) exerted therapeutic effects in endotoxic shock via an action at neuroeffector sites within the central nervous system. Male Sprague-Dawley rats (250-300 g) were affixed with a transcranial guide tube, an external jugular-vein cannula, and a tail-artery cannula. On the day following surgery, conscious rats in their home cages were connected to microtransducers; cardiovascular and respiratory variables were monitored. Endotoxic shock was induced by iv injection of E. coli lipopolysaccharide endotoxin (15 mg/kg, Difco). Within minutes, arterial pressure fell 25-30 mmHg. At that time, TRH (10.0 µg, n=7), MK-771 (1.0 µg, n=6), or saline (n=6) were injected through the guide tube into the right-lateral ventricle in a volume of 20 µl over 20 sec. Within seconds, TRH and MK-771 resulted in large increases in mean arterial pressure (MAP), pulse pressure (PP), heart rate (HR), and respiratory rate (RR). Peak effects were observed 15 min post injection. At that time, TRH and MK-771 produced significant elevations in cardiorespiratory variables; saline had little effect.

	MAP(mmHg)	PP(mmHg)	HR(beats/min)	RR(breaths/min)
saline	9.7±3.8	11.7±5.1	25.3±34.3	38.0± 8.6
TRH (10 µg)	36.0±7.6	33.6±7.3	53.9±17.6	126.1±35.2
MK-771 (1 µg)	35.4±2.6	18.3±1.0	137.0±15.7	143.3±39.2

Although MK-771 produced a similar increase in MAP as TRH, it resulted in lesser increases in PP and greater increases in HR. These effects of TRH were centrally mediated since this small dose was without effect following iv injection in endotoxic rats.

Thus, the therapeutic effects of TRH and MK-771 in endotoxic shock appear to be centrally mediated, possibly expressed through autonomic mechanisms which affect cardiorespiratory function. Since intracerebroventricular TRH is without effect on nociceptive latencies (Holaday et al, Life Sci. 22, 1537, 1978), these results further suggest that TRH may have therapeutic utility in treating shock or acute hypotension similar to naloxone but without the adverse effect of intensifying pain.

13.12 CORRELATED BEHAVIORAL EXCITATIONS FOLLOWING MICROINJECTIONS IN RAT PERIAQUEDUCTAL GRAY OF MORPHINE AND ACTH(1-24). Y. F. Jacquet, Center for Neurochemistry, Rockland Research Institute, Ward's Island, New York City, NY 10035.

Excitatory effects (fearful hyper-reactivity and explosive motor behavior) were significantly correlated (Chi-square = 9.69; $p<0.01$) following separate microinjections of morphine sulphate (52 nmol) and ACTH(1-24) (17-34 nmol) into the periaqueductal gray of rats, indicating that the ACTH-elicited, and morphine-elicited behavioral excitations are mediated by the same sites in the periaqueductal gray. Subsequent microscopic study of 100µ cressyl-violet stained brain sections obtained from all the subjects showed that the microinjections were more effective when the sites were located within rather than below the periaqueductal gray. Analgesia was observed following morphine but not ACTH microinjections. When rats were microinjected with ACTH (6.8 nmol) 10 min prior to a microinjection of B-endorphin (2.9 nmol) in the same periaqueductal gray site, no antagonism of the B-endorphin-induced analgesia was observed, indicating that different neural mechanisms mediate the effects of the two neuropeptides. These results confirm that many of morphine's behavioral effects are mediated by specific sites within the periaqueductal gray, and add further support for the proposal (Jacquet, Y.F. et al, Science, 198:842, 1977) that morphine's dual actions - excitatory and inhibitory - are mediated by two classes of receptors, one, a stereospecific, naloxone-sensitive receptor that mediates the inhibitory (e.g., analgesic) action (the endogenous ligand of which was proposed to be B-endorphin), the other, a nonstereospecific, naloxone-insensitive receptor that mediates the excitatory (e.g., fearful hyper-reactivity and explosive motor behavior) action (the endogenous ligand of which was proposed to be ACTH (Jacquet, Y.F., Science, 201:1032, 1978)). In addition, these results are consistent with our proposal (1977, 1978) that the opiate abstinence syndrome may be due in part to the unmasking of the excitatory action of morphine (at the "ACTH" receptor) following weakening of the inhibitory action of morphine (at the "endorphin" receptor) due to selective tolerance development or selective naloxone blockade.

13.13 CHOLECYSTOKININ OCTAPEPTIDE (CCK-8) INJECTED IN CEREBROSPINAL FLUID (CSF) DECREASES PLASMA INSULIN CONCENTRATION IN SHEEP.

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We have shown CCK-8 to be a potent and specific suppressor of feeding when administered as a continuous injection into the lateral cerebral ventricles(LV) of sheep. Strong support for a physiological role for endogenous brain CCK peptides and specific CCK receptors in satiety was obtained from studies showing that significant increases in food intake occurred with LV injections of either a specific CCK receptor blocker or CCK antibody. Because CCK-8 is present in several CNS sites, though, it is likely to be involved in a variety of physiological functions controlled by the brain.

The present studies were carried out to determine whether brain CCK-8 could influence secretion of 3 hormones important in control of peripheral metabolism: GH, Insulin(I) and glucagon. First, the effect of 60 min continuous injections of 0(sCSF), .04, .16 and .64 pmole/min CCK-8 on plasma I and glucose(G) concentrations was determined (N=6). LV CCK-8 at .64 pm/min caused significant decrease in plasma I at 30 and 60 min after starting injection: by 60 min plasma I had decreased by 1.95±.1 ng/ml from preinjection levels with .64 pm/min CCK-8 ($p<.01$); sCSF had no effect on I. Lower doses of CCK-8 caused slight, but nonsignificant decreases in plasma I. Plasma G was not affected by any treatment. In other experiments the effect of continuous LV injection of sCSF or .64 pm/min CCK-8 on plasma GH and glucagon concentrations was studied. CCK-8 had no effect on plasma levels of either hormone, thus the decrease in I is not due to generalized suppression of hormonal secretion (eg, as caused by somatostatin secretion).

Desulfated CCK-8 (desCCK-8) binds less strongly to CCK receptors and is much less potent in decreasing feeding during LV injections in sheep. To determine whether the effect on I was also a chemically specific effect, plasma I and G concentrations were measured during 60 min LV injections of sCSF, .64 pm/min desCCK-8 and .64 pm/min CCK-8 . Plasma I was decreased by LV CCK-8 at .64 pm/min ($p<.05$), but desCCK-8 had no effect (60 min I, ng/ml: sCSF 2.2±.3, desCCK-8, 2.5±.3, CCK-8 1.2±.1). No treatment affected plasma G concentrations.

Finally, to determine the importance of the more rostral brain areas, we compared the effects on plasma I and G of LV and cisterna magna(CM) injections of sCSF or .64 pm/min CCK-8. LV CCK-8 decreased plasma I ($p<.05$), but CM injections had no effect (60 min I, ng/ml: LV sCSF 2.6±.4, LV CCK-8 1.6±.1, CM sCSF 2.8±.3, CM CCK-8 2.3±.4); no treatment affected plasma G.

These results suggest that brain CCK-8 acts on CNS sites involved in the control of insulin secretion, possibly via vagal efferents to the pancreas. Supported in part by NIH Postdoctoral Fellowship NS06595.

14.1 TRIGEMINAL EVOKED POTENTIALS IN UNANESTHETIZED CATS. E. H. Chudler*, W. K. Dong (SPON: K. Y. Chan). Dept. of Anesthesiology, Univ. of Washington, Seattle, WA. 98195.

We found that tooth pulp evoked far-field and near-field potentials were differentially affected by barbiturate and inhalation anesthetics; the latter potentials were markedly attenuated. Comparison of near-field potentials among anesthetized cats was impossible due to variability in their waveform and latency. Thus, a behavioral method was developed to study near-field evoked potentials in unanesthetized cats.

Under anesthesia, two channels were drilled into the dentin of a mandibular canine tooth. Wire electrodes for stimulation were secured in these cavities with dental amalgam and composite resins. Interelectrode impedances varied from 7kΩ to 16kΩ. Evoked potentials were recorded from an array of wire electrodes implanted in the skull. EMG leads were implanted into deep neck muscles.

A behavioral paradigm was established to allow the animal to terminate the stimuli presentation. Cats were trained to remain still for a period of 10 to 15 sec. During this time, electrical stimuli were delivered to the tooth and evoked potentials were recorded and computer averaged. If the cats held still, a food reward was automatically delivered. However, if the cat moved and the EMG exceeded a preset voltage, the shock was terminated and no reward was given.

Acute animal studies have demonstrated that activation of large myelinated (Aß) fibers by tooth pulp stimulation was sufficient to evoke far-field potentials (designated I, IIa, IIb, IIIa, IIIb with peak latencies of 0.9, 1.6, 2.3, 3.5 and 4.1ms) and early near-field potentials (IV, P_1, N_1 with peak latencies of 5.2, 6.2 and 9.9ms; P_2, N_2, P_3, N_3 with latencies from 25 to 160ms). Delivery of stimulus intensities to chronic animals that were below levels for escape evoked the same far-field and early near-field potentials.

Evidence from acute lesion studies and from comparing latency and refractoriness of surface recorded potentials to those of in-depth recorded potentials indicates the following main sources: I-trigeminal nerve/ganglion; IIa,b-principal sensory nucleus of V; IIIa,b-thalamus (VPM); IV, P_1, N_1-primary sensorimotor cortex. Additional lesion and mapping studies are needed to identify the origins of the other near-field potentials. With the behavioral paradigm described, evoked potentials can be recorded without contamination from drugs. It may also be possible to record evoked potentials associated with escape behavior and activation of high threshold afferents in the alert unanesthetized, unrestrained animal.

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14.2 FREQUENCY DEPENDENCE OF LATENCIES IN THE VISUAL EVOKED RESPONSE. J. M. Flinn* (SPON: I. Weiss). Depts. of Physics and Psychology, George Mason University, Fairfax, Va. 22030.

Phase characteristics of transient evoked potentials have received little attention, although they can be used to obtain information about the group delay, i.e., latency, as a function of frequency via the relationship $\tau_g = (2\pi)^{-1}\, d\phi/df$, where τ_g is the group delay or latency, f is the frequency, and ϕ the phase of the Fourier coefficients of the signal. In this experiment, latencies were obtained from the visual evoked potentials (VEPs) of 64 twelve-year-old girls. The recording was made using bipolar electrodes 6 cm apart astride C_4. Responses to 100 light flashes of 4×10^5 lumens/steradian were recorded; two runs were recorded with the light flash (runs 1 and 2) together with a control run (run 3). The slope of the phase as a function of frequency varied with frequency for runs 1 and 2 but not for run 3. Analysis of variance showed that there was a significant variation of latency with frequency, calculated over 5 Hz bands, for runs 1 and 2, but not for run 3. For run 1: $F(7,406) = 5.7$, $p < .01$; for run 2: $F(7,406) = 4.7$, $p < .01$; for run 3: $F(7,406) = 1.4$. For runs 1 and 2, the latency had a maximum of 201 ± 12 msec; this occurred in the frequency range 5-10 Hz. The minimum was 104 ± 11 msec, in the range 20-35 Hz. These results are similar to those obtained from the phase analysis of steady-state VEPs (Spekreisje, H., Estevez, O., and Reits, D. In: J. Desmedt (Ed.), Visual Evoked Potentials in Man. Oxford, Clarendon Press, 1977). This shows that similar phase and latency information is obtained by analysis of either steady-state or transient VEPs. Previous experiments have shown that harmonics of the stimulating frequency are observed in the steady-state VEP; thus the system is nonlinear. However, the fact that the flash stimulus, which acts as an impulse, produces the same phase data as the steady-state VEP shows that this nonlinearity is weak. Thus it should be possible to obtain latency as a function of frequency by using a transient stimulus without the necessity of sweeping through a range of modulating frequencies. This would be particularly useful in the analysis of VEPs due to patterned stimuli, where the latency may depend on both spatial and temporal frequencies.

14.3 VISUAL EVOKED POTENTIALS EVOKED BY LIGHT-EMITTING DIODES MOUNTED IN GOGGLES, R. P. Lesser*, H. Lueders*, N. Donovan*, G. Klem*, (SPON: K. L. Barnes). Department of Neurology, Cleveland Clinic Foundation, 9500 Euclid Avenue, Cleveland, OH 44106.

Visual evoked potentials to pattern reversal stimulation are accepted to be of more stable morphology than those evoked by flash via a photic stimulator. These potentials require, however, that the patient fixate on the field of stimulation and in certain circumstances, including functional visual deficits, refractive disorders or surgical monitoring a patient may be unwilling or unable to attend carefully to the patterned field.

We have compared the potentials following pattern reversal stimulation to those evoked by light-emitting diodes (LED) separated by 5mm and mounted in 4x4 arrays inside of goggles worn by the patient. Pattern reversal was delivered by a television system in which each check subtended 2^o of visual arc within a total field subtending 8^o of visual arc. The patient sat 1 meter from the television screen. For both stimulus modalities filter settings at half-amplitude were 1 and 100 Hz; 256 trials were taken for each average with at least two averages for each modality to each eye. Stimulus rate was 2/second. The montage utilized was O1-Cz, O2-Cz, Oz-Cz, A1A2-Cz (international 10-20 system).

Following goggle stimulation a series of positive and negative deflections occurred. There was more inter-individual variability in these deflections, compared to thosed evoked by pattern reversal, but within individuals the potentials evoked by right and left eye stimulation were relatively similar. This suggests that potentials evoked by LED-goggle stimulation may be of value in certain clinical states to define the presence or absence of an evoked potential as well as to assess the symmetry of conduction from the two eyes.

14.4 COMPARISON OF PATTERN ELICITED MASS POTENTIALS FROM THE RETINA, LATERAL GENICULATE NUCLEUS (LGN), AND THE VISUAL CORTEX OF THE CAT I. Ohzawa* and R. D. Freeman (SPON: T. E. Cohn), School of Optometry, University of California, Berkeley, CA 94720

Pattern-elicited electroretinograms (ERG), LGN and cortical evoked potentials (EP) from cats were measured to compare mass response properties at different stages of the visual pathways. The stimuli were sine-wave gratings phase reversed in either of two temporal modes, square- or sine-wave. Patterns were presented in pseudo-random order, and responses were averaged and Fourier analyzed by a computer. ERGs were recorded via stainless steel wires in the vitreous and the orbit behind the globe. LGN and cortical EPs were recorded via bipolar and steel screw electrodes, respectively.

For square-wave temporal modulation, the ERG consisted mainly of a vitreous negative peak at 120-200 mS after reversal. The a- and b-waves seen in flash ERGs were missing in these pattern ERGs. To show that the absence of a- and b-waves was not due to retinal damage, mean luminance modulation was added to grating patterns. A progressive recovery of a- and b-waves was observed with increased luminance modulation. ERG spatial frequency response did not show any fall-off at low frequencies for square-wave temporal modulation, whereas LGN and cortical EPs clearly showed a band-pass characteristic for the same conditions. To determine whether low-frequency fall-off, which implies lateral inhibition, was obscured by temporal transients, sine-wave temporal modulation was used in some recordings. Under this condition, ERGs showed a clear fall-off at low frequencies. This suggests that the generating mechanism of the pattern ERG involves lateral inhibition.

The amplitudes of cortical EPs were approximately proportional to log contrast. We found this relationship also in the LGN, but not in the retina. ERGs showed high contrast thresholds, and often a low saturation contrast with a log-linear region in between. Contrast thresholds for ERGs were variable, ranging from 3% to 10%. LGN and cortical EPs simultaneously recorded in interleaved fashion with ERGs showed much lower thresholds than ERGs, and had a clear response at the thresholds of the corresponding ERGs.

These contrast results are somewhat paradoxical in the sense that central structures show clear responses when the retina shows no response, and that LGN and cortical EPs do not saturate far beyond the retinal saturation contrast. One hypothesis for the former finding is that the local ERG is cancelled out by summation across the retina at low contrasts, and only appears when a nonlinearity becomes manifest at higher contrast.

(EY01175)

14.5 PATTERN AND FLASH EVOKED POTENTIALS IN THE HOODED RAT.
M. Onofrj* and I. Bodis-Wollner. Dept. Neurology, Mt. Sinai School of Medicine, New York, NY 10029.

Thirty-two male adult hooded rats had evoked potentials (EPs) recorded with chronically implanted electrodes placed over area 17, the postbregmatic region, the nasion and the earlobe. The transient flash EP of the rat has 4 consistent components: N_1 at 31 ± 4 msec, P_2 at 46 ± 3 msec, N_2 at 65 ± 3 msec, and P_3 at 114 ± 9 msec. Reducing the maximum luminance of the Grass flash stimulator by a factor of 16 delayed P_2 by 10 ± 3 (S.D.) msec. Using 8 and 10 flashes per second following luminance reduction the phase shift of the steady-state EP was 30^{o}.

Pattern EPs to counterphase modulation of .3 cpd checks and .1 cpd square wave gratings were obtained in 14 out of 20 rats. The typical triphasic EP waveform was most consistent over the hemisphere contralateral to the eye. The measured components were P at 85 ± 5 msec, and N at 113 ± 13 msec. To 5 Hz counterphase modulation a clear 2nd harmonic EP could be recorded in 6 out of 13 rats. In 4 tested rats a +2 diopter lens consistently doubled the P and N amplitude. A +4 diopter lens increased it by only 10%.

Thus both the pattern and flash EP of the hooded rat are comparable in latencies and waveforms to EPs of higher mammalians. There has been controversy surrounding the acuity of the rat based on electrophysiological (Shaw 1974,1975) and refractive studies (Massof 1972; Block 1969). Using EP methodology we find agreement with Meyer and concur with the visual acuity limits established with behavioral (Wiesenfeld 1976, Birch 1979), refractive (Hughes 1979), and single cell (Brown 1965; Montero 1973; Wiesenfeld 1975) data.

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14.6 DOPAMINERGIC DEFICIENCY CAUSES VEP DELAYS IN THE MALE HOODED RAT.
I. Bodis-Wollner and M. Onofrj* (SPON: M. Yahr). Dept. Neurology, Mt. Sinai School of Medicine, New York, NY 10029.

The hooded rat has reliable visual evoked potentials (VEPs) to both flash and patterned stimuli (Onofrj and Bodis-Wollner 1981). Now we studied in 8 rats the effects of alphamethylparatyrosine (AMPT), a dopamine (DA) depletor, and haloperidol, a DA blocker, on flash and pattern EPs. The latency of the various components of the transient flash EP started to shift 90 min following i.p. administration of 250 mg/kg AMPT. The delay peaked in 220 min: 19 min for P_2 (normal 46 ± 3 msec), 23 msec for N_2 (65 ± 3 msec), and 23 msec for P_3 (114 ± 9 msec), while the steady-state EP shifted by 145^{o}. Recovery was complete in 24 hours. In 4 rats the pattern EP to .1 cpd gratings showed a delay in P of 24 ± 4 msec (normal 86 ± 5 msec). The maximum effect of i.p. haloperidol 3 mg/kg occurred in 35-60 min. On the average, P_2 was delayed by 15 msec, and N_2 by 18 msec. The mean delay was 21 msec for the P component of the pattern EP.

Our flash EP data agree with those obtained by Dyer using similar pharmacological agents. Comparing VEP effects due to pharmacological manipulations to those obtained by luminance reductions, it appears that miosis is unlikely to explain these delays. Our study using patterns suggests that dopaminergic deficiency has a significant, reversible effect on the VEP and therefore implies a role of this neurotransmitter in the visual pathway of the hooded rat. We conclude that besides the known mechanism of VEP delays, retinal synaptic malfunction must also be considered. The relationship between these data and VEP changes in humans suffering from Parkinson's disease and those treated with phenothiazines (Bodis-Wollner and Yahr 1978,1980) require further study.

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14.7 CONTRAST AND COUNTERPHASE-FREQUENCY EFFECTS OF THE THREE PRIMARY COLORS ON HUMAN VISUAL EVOKED RESPONSE.
W. R. Klemm, R. A. Goodson* and R. G. Allen*. USAF School of Aerospace Medicine, Brooks AFB, TX 78235.

Using a counterphasing checkerboard stimulus, we evaluated the visual evoked potential (VEP) in 10 subjects for the response to different contrast levels. For each color the highest contrast setting was used to test the effects of varying the reversal rate of the stimulus.

Overall mean luminance of each color was photometrically equated and kept constant. The VEP was computer averaged for 90 consecutive 1-sec epochs and the power (PSD) determined at the appropriate stimulus frequencies (plus their first two harmonics).

For each color and each of the stimulus frequencies (6, 11, 16 Hz) the magnitude of the VEP did not seem to be influenced by the amount of simultaneously occurring alpha activity. The magnitude of response did not always correspond to a person's impressions of how well he was perceiving the stimulus.

For each color, the contrast-response curves revealed small PSDs at low contrast (0.1) and larger PSDs at the 3 high-contrast settings (0.3 - 0.5). Magnitude of PSD varied markedly by color and by subject. The shape of the curves, depending on color and subject, often indicated a nonlinear saturation of responses. A given subject commonly had a physiologically "preferred" color which produced larger responses.

Most subjects responded best at 6 Hz, poorest at 11 Hz, with intermediate responses at 16 Hz. As in the contrast-response tests, there were differential VEP responses at each color.

Similar contrast- and reversal-rate effects occurred at lateral electrode sites, but unusual lateralization effects occurred in some subjects.

We conclude that these VEPs seemed to be independent of alpha. Also there seems to be considerable variation among subjects in the response to color, contrast, and reversal rate. Finally, the results suggest that white-light VEP responses may not necessarily reflect the response characteristics at specific colors.

14.8 MAGNETIC AUDITORY EVOKED FIELDS:TONE AND CLICK DIFFERENCES
M. Reite, J.T. Zimmerman* and J.E. Zimmerman*. Univ. of Colo. School of Medicine, Denver, CO 80262 and Nat. Bureau of Standards Boulder, CO 80302.

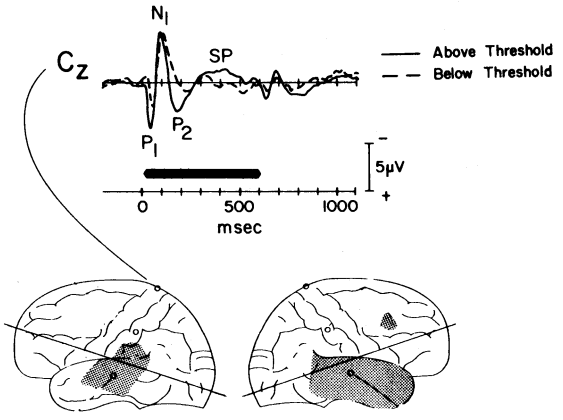
Previous studies in our laboratory have demonstrated that human magnetic auditory evoked fields (MAEFs) to click demonstrate interhemispheric asymmetry, being of greater amplitude contralateral to the stimulated ear, and that the current dipole responsible for click evoked fields is approximately perpendicular to the Sylvian fissure in the area of the primary auditory cortex. In this study we examined whether auditory fields evoked in response to tone stimuli could be differentiated from those evoked in response to click stimuli, and whether magnetic recordings could do this better than EEG recordings.

Ten normal adults served as subjects. Magnetoencephalographic (MEG) recordings were made using a figure-eight SQUID gradiometer in an aluminum shielded room. The sensor was placed 1/4 of the distance from T3 to C3 for left hemisphere recordings, and 1/4 of the distance from T4 to C4 for right hemisphere recordings. EEG recordings were obtained simultaneously from these positions in 7 subjects. MEG and EEG data, bandpassed between 1 and 40 Hz, was averaged for 500 msec after each of 128 aperiodic 92dB alternating clicks and 90msec long 2000 Hz tones. Recordings were made from both hemispheres in response to both ipsilateral and contralateral stimulation. MAEFs and AEPs were plotted and the maximum amplitude of each response occuring within 250 msec of stimulus onset was measured.

A repeated measures t test was performed for differences between tone and click stimulation for each hemisphere and for both contralateral and ipsilateral stimulation. Such comparisons were made for both MAEFs and AEPs. The results indicated MAEFs were of significantly higher amplitude for tones than for clicks for contralateral stimulation of the left hemisphere, and for both contralateral and ipsilateral stimulation of the right hemisphere. In general, EEG AEPs to tones were of lesser amplitude than for clicks; this effect was statistically significant for ipsilateral stimulation of the left hemisphere only. The larger MAEFs to tone may reflect a larger net current flow in auditory cortex as a result of the longer duration tone burst.(Supported by Office of Naval Research Contract No. N00014-79-C-0383).

14.9 *LONG-LATENCY AUDITORY EVOKED POTENTIALS IN CORTICAL DEAFNESS*
D. L. Woods, R. T. Knight *, and H. J. Neville Neuropsychology Laboratory, The Salk Institute, La Jolla, CA 92037

We recorded long-latency auditory evoked potentials (AEPs) to stimuli presented above and below perceptual threshold in a patient who became deaf as a result of the bilateral destruction of auditory cortex. The AEPs were similar in amplitude, latency, scalp distribution and refractory properties to those recorded in subjects with normal hearing. The differential effects of brain lesions on auditory-perceptual functioning and AEPs suggests that they are subserved by different neural systems.



14.10 *AUDITORY AND SOMESTHETIC 40HZ ERPS FROM THE HUMAN SCALP.*
R. Galambos. Speech, Hearing & Neurosensory Center, and Department of Neurosciences, Univ. of Calif., San Diego, La Jolla, CA 92093

Relatively little attention has been paid to human event related potentials (ERPs) initiated by stimuli repeated at high rates (beyond 20 per sec). However, as we have reported (Proc. Nat. Acad. Sci., Apr. 1981), clicks or tones generate large, stable nearly-sinusoidal waves when repeated at around 40 per sec; these auditory 40Hz ERPs evidently represents a consolidation, or superimposition of the 3 or 4 approximately sinusoidal waves called the middle (80-100 msec) latency auditory ERP components. We can now add that vibratory stimuli to finger or toe generate similar large stable ERPs when repeated at around 40Hz. The somesthetic response can be subdivided into two types: a large event localized to the scalp overlying the cortical projection of the limb stimulated, and a (usually) smaller one distributed widely over the scalp which more closely resembles the auditory ERP in amplitude. Dissimilarities do, however, exist in the optimal frequencies (tested: 20 to 60 Hz) and particular scalp sites where auditory and somesthetic 40Hz ERP response maxima occur.

When separate clicks are delivered to the ears the 40Hz ERPs they produce add approximately algebraically, i.e., the ERP wave response to the two clicks is larger when they are in phase and approaches zero when they are out of phase. A similar algebraic summation is seen when tactile and auditory stimuli are intermixed with appropriate control of the time interval separating them. These interactions within and across modalities may occur because extralemniscal generators with similar properties spread their effects widely and symmetrically through the cortex. This possibility, and others, will be discussed.

14.11 NEURAL RECOVERY FROM SEVERE HUMAN HEAD INJURY ASSESSED WITH MULTIMODALITY EVOKED POTENTIALS (MEPS): FUNCTIONAL CONSEQUENCES OF SECONDARY INSULT. P. G. Newlon*, R. P. Greenberg and D.P. Becker Div. of Neurosurgery, Med. Coll. of Va., Richmond, VA., 23298

Multimodality Evoked Potentials (MEPs; visual, auditory and somatosensory) recorded early after injury have been shown to aid assessment of brain dysfunction and outcome prediction in comatose head injury patients. Those who exhibit normal or mildly abnormal MEPs in the first 72 hrs. post-trauma generally reach a good/moderate outcome by 1 year, while a majority of patients with severely abnormal or absent MEPs have a poor outcome. Errors in outcome prediction made from early MEP tests may occur if patients suffer secondary insults later in their hospital course. We have examined changes in MEPs over time in these patients as they relate to occurrence of complication and resultant outcome, to determine whether serial MEP studies reflect the neurological effects of secondary insult. 109 patients who received multiple MEP studies up to one yr. post-trauma were studied and were assigned to the following groups, based on the most severe MEP abnormality exhibited in an early study ($\bar{X}$ Day 3): N=Normal, MA=Mildly abnormal, SA=Severely abnormal, A=Absent. These groups were subdivided into those with (C) and without (NC) complication. Insults included were pulmonary infection/edema, GI bleeding, ventriculitis, meningitis, inappropriate ADH levels, seizure, sepsis, gram(-) shock, respiratory/cardiac arrest and delayed hematoma. Outcome was defined as Good/Moderately disabled (G/M), Severely disabled/Vegetative (S/V) or Dead (D). Data analysis bore the following results: 1) Outcomes of patients in the N (n=22) and A (n=13) groups were not altered appreciably by complication. 2) When MEPs of patients in the MA(C) group (n=32) returned to normal (50% of cases) or remained MA (30% of cases), 90% of the patients made a G/M recovery despite complication. However, 6 MA(C) patients (20%) showed MEP deterioration over time and all had poor outcomes (2 S/V, 4D). 3) MEPs of the SA(C) group (n=16) improved in only 5 patients (31%). 2 patients made a G/M recovery, 2 were S/V and 1 D. MEPs of 4 patients (25%) remained SA and all did poorly (1 S/V, 3 D). 7 patients (44%) showed MEP deterioration with complication and had poor outcomes (2 S/V, 5 D). 4) Of the patients in the MA(NC) and SA(NC) groups 79% and 71% respectively showed MEP improvement and all had G/M outcomes. MEPs remained MA or SA in 4 patients who made good recoveries and 1 patient with SA(NC) which remained so, died. Although secondary insults may worsen outcome for patients who initially show MEP abnormalities, MEPs are sensitive to neurological change. Serial studies may help to determine which patients have suffered further brain compromise and which have not. Supported by NINCDS Grant 5-20627 and T.I. Award 5K07NS00346-03

14.12 EVOKED POTENTIAL ABNORMALITIES IN PATIENTS WITH INHERITED ATAXIAS. M. Nuwer, S. Perlman* and J. Packwood*. Dept. Neurology, Reed Neurological Research Center, U.C.L.A., Los Angeles, Calif. 90024.

Evoked potential tests have been shown to be sensitive tools for studying central nervous system abnormalities. We have investigated visual, brainstem auditory, and somatosensory evoked potentials in 20 patients with a variety of inherited ataxias. Such tests in these patients can help demonstrate subclinical neurological impairments and better define the physiological abnormalities underlying clinical symptoms and signs.

Visual evoked potential (VEP) tests were performed one eye at a time with checkerboard pattern-reversals, using 30' checks projected onto a square screen 13 degrees along each side, reversing 2/second, recording OZ-CZ. Brainstem auditory evoked potential (BAEP) tests were performed one ear at a time with 100 microsecond condensation clicks 65 dB above threshold, 10/second, recording from the ipsilateral mastoid to CZ. Somatosensory evoked potential (SEP) tests were performed with sufficient electrical stimulation of the median nerve to cause a 1 cm movement of the thumb, 2/second, recording from scalp over the contralateral hand region of somatosensory cortex to a posterior parietal reference, and often also recording both from the seventh cervical spine, and from Erb's point, to a mid-frontal reference.

VEPs were slow in most cases independent of the diagnosis. SEPs were slow in most patients with Friedreich's Ataxia, Combined Cerebral and Cerebellar Atrophy, and Ataxia Telangectasia. However, SEPs were normal in most patients with Olivopontocerebellar Atrophy (OPCA). BAEPs were present and normal in most conditions, including Friedreich's. However, BAEPs were abnormal in all of the OPCA patients. Left-right symmetry was preserved in SEP and VEP abnormalities, but BAEPs were often asymmetric when abnormal.

These findings agree with previous reports, extend the diseases and modalities tested, but disagree with a previous report of absent BAEPs in most Friedreich's patients.

15.1 SPROUTING OF DORSAL ROOT AXONS FOLLOWING NEURAL INJURY. Richard E. Coggeshall and Claire E. Hulsebosch, Depts. of Anatomy and of Physiology and Biophysics and the Marine Biomedical Institute, The University of Texas Medical Branch, Galveston TX 77550

Sprouting of dorsal root axons that follows partial denervation of the mammalian spinal cord has been indicated by several investigators using the silver staining method. However, this method is not adequate for quantifying the increase in axonal numbers which is called sprouting. Accordingly, the present ultastructural study was undertaken to quantify the numbers of myelinated (M) and unmyelinated (UN) axons in the dorsal roots on the operated (OP) and normal (N) side of the rat spinal cord after hemisection (HS) at birth or after cutting 3 consecutive dorsal roots above and below a spared root (SR) in rats one month old. Data from the pair of dorsal roots just cranial and caudal to the lesions as well as data from the spared roots are shown in the following table in which each number is a mean value from at least 4 rats allowed to survive 1 month or 3 months, as indicated, after surgery.

		HS 1 MONTH POST-OP		HS 3 MONTHS POST-OP		SR 1 MONTH POST-OP	
		OP	N	OP	N	OP	N
CRANIAL	MY	1340	1298	1353	1282	1398	1411
ROOTS	UN	3751	3183	4029	3371	4316	3541
SPARED		LESION		LESION	MY	1629	1641
ROOTS					UN	5226	4448
CAUDAL	MY	1346	1339	1473	1459	2191	2133
ROOTS	UN	4061	3237	5336	3975	5671	5610

The data indicate 1) there is no significant difference in the myelinated axon population in each pair, 2) there is a significant increase in the unmyelinated axon population of dorsal roots on the operated side except in the SR caudal roots, and 3) the increase in the unmyelinated population persists 3 months after hemisection. Therefore, the major conclusions are 1) that the unmyelinated axons are the sprouting population and 2) that survival time after the initial insult does not change the amount of sprouting. These data confirm that dorsal root axons sprout in response to spinal cord denervation and show that this sprouting can be quantified. Further work relating the amount of sprouting to age is underway. Supported by NIH grants NS 10161, NS 06246, NS 07377 and NS 11255.

15.2 SPROUTING AND SYNAPSE FORMATION BY AXONS WHILE SEPARATED FROM THEIR CELL BODIES. S. Rotshenker, Dept. of Anat. & Embryol. Hebrew Univ. Med. Sch., Jerusalem, Israel.

In previous studies (Rotshenker, J. Physiol. 292:535, 1979; Rotshenker and Reichert, J. Comp. Neurol. 193:413, 1980) we observed that axotomy of the nerve to one cutaneous-pectoris muscle of the frog induced sprouting and synapse formation in the intact contralateral muscle, thus producing a pattern of supernumerary innervation. In a recent study (Rotshenker, Neurosci. Abs. 10, 1980) I examined the ability of colchicine to imitate axotomy in producing contralateral sprouting and synapse formation after the drug was applied to nerves of left muscles and found that supernumerary innervation developed in both left and right muscles. The appearance of new synapses occured after about 2.5 weeks in right muscles but as early as 2 days in left muscles. The short latency response in left muscles raised the possibility that colchicine induced the sprouting of axons that were exposed to it directly independent of their cell bodies. To test this hypothesis I examined the ability of motor axons to sprout and form synapses while being separated from their cell bodies. Cutting the left 2nd spinal nerve that contains the axons innervating the muscle close to its exit from the spinal cord resulted in a failure to record end plate potentials (e.p.p.s) by the 4th day after the operation. E.p.p.s. were recorded throughout the first 3 days as faithfully as in intact muscle. The incidence of polyneuronal innervation detected electrophysiologically 2 and 3 days after the operation was 17.5±1.0%(SEM) in left muscles and 19.0±1.6%(SEM) in right muscles. In a second group of frogs the 2nd left spinal nerve was cut and 0.01-0.015 M of colchicine applied to the nerve to left muscles at axilla (about mid way between the spinal cord and muscle). The incidence of polyneuronal innervation detected electrophysiologically 2 and 3 days after the operation was 30.8±2.0%(SEM) in left muscles and 18.2±1.9%(SEM) in right muscles. This reflects a significant 1.7 fold increase over normal in the incidence of polyneuronal innervation in muscles whose axons were treated with colchicine while being separated from their cell body. In such muscles morphological studies produced anatomical evidence for sprouting and synapse formation. Our previous observations on the transneuronal induction of sprouting and synapse formation and the present finding that the same motor axons can respond in a similar way after being exposed to low doses of colchicine while being separated from their cell body further suggest that motor neurons may be induced to sprout in more than one way, whether by presenting appropriate stimuli to their soma and central processes or peripheral extensions.

15.3 DENDRITIC OUTGROWTH FROM AN ADULT INSECT MOTONEURONE AFTER NEURAL LESIONS. R.M. Pitman and K.A. Rand. Department of Physiology & Pharmacology, University of St Andrews, Fife, Scotland KY16 9TS.

Alterations in dendritic field following neural damage may reflect the ability of a neurone to undergo adaptive changes. However, the factors controlling dendritic modification are unclear. Deafferentation of interneurones in immature insects may be followed in different instances either by reduced growth or sprouting of specific dendrites (Murphey et al. J. comp. Neurol., 159: 407, 1975; Hoy et al. Soc. Neurosci. Abstr., 4:115, 1978), while similar lesions may produce no detectable change in dendritic field of adult insect interneurones (Tweedle et al. Brain Res., 60: 471, 1973). We report here experiments in which dendritic sprouting has been observed in an identified adult insect motoneurone following neural lesions.

We have studied the metathoracic 'fast' coxal depressor motoneurone of the cockroach, Periplaneta americana, by intracellular cobalt injection and silver intensification at various intervals after a number of nerve trunks to the metathoracic ganglion had been severed.

We have made the following observations: (1) Axotomy is not a potent stimulus for dendritic growth; although supernumerary processes may occasionally develop after severing the nerve trunk in which the axon of this neurone travels (nerve 5), the extent of this sprouting is limited. (2) Sprouting is usually more extensive if a number of nerve trunks are severed. (3) Extensive dendritic sprouting may follow lesions which damage neither the axon nor the dendrites of the neurone under study. (4) Processes may grow well beyond the normal dendritic field of the neurone. Dendrites of this motoneurone are normally restricted to the ganglionic neuropile ipsilateral to its cell body and axon. However, after nerve trunk lesions, processes may extend into anterior or posterior connectives, into the contralateral neuropile or into inappropriate segmental nerve trunks. (5) New processes frequently have varicosities both along their length and at their ends.

We conclude that dendrites of adult insect neurones may have a greater capability for outgrowth than has been previously thought; direct physical damage to neurone is not a pre-requisite for dendritic sprouting to occur.

15.4 EXPANSION OF THE RECEPTIVE FIELDS OF LEECH NOCICEPTIVE CELLS FOLLOWING DELETION OF SINGLE NEURONES. Susanna Blackshaw[1], John Nicholls[2] and Itzhak Parnas[3]. 1. Institute of Physiology, the University, Glasgow G12 8QQ, Scotland; 2. Neurobiology Department, Stanford University Medical School, Stanford CA 94305; 3. Neurobiology Department, Hebrew University, Jerusalem.

In the leech, the cell bodies of nociceptive neurones or "N" cells lie within the central nervous system. They are easily identified and much is known about their electrical properties, their arborization within the CNS, their synaptic connections with segmental motorneurones, and the way in which they sprout and regenerate these central connections after injury. In confirmation of earlier work, N cells do respond selectively to noxious mechanical stimulation of the skin, but not to touch, light pressure or stretch applied to the body wall (Nicholls, J.G. and Baylor, D.A., J. Neurophysiol. 31: 740-756, 1968). The skin fields of the 2 cells on one side of a ganglion overlap - each neurone innervates a well defined area extending from the ventral midline to the dorsal midline and covering the ipsilateral half of the body segment. In addition, and in keeping with its nociceptive role, one of the 2 cells is also driven by noxious stimulation of connective tissue lining internal viscera.

After 3 of the 4 N cells in a ganglion of a living animal have been killed by intracellular injection of Pronase, the receptive field of the remaining N cell spreads into the denervated area. Some enlargement of the skin field is apparent by 3 weeks. After 12 weeks the neurone has expanded its field to cover the entire area on the other side of the animal, a region it does not normally supply. The spread of N cell receptive fields is modality specific. When N cells have been killed, the fields of mechanosensory neurones responding to touch (T) or pressure (P) are unchanged.

15.5 TRANSPORT OF CYTOSKELETAL PROTEINS IN NEWLY-FORMED AXONS OF REGENERATING RAT SCIATIC NERVE MOTONEURONS. I. G. McQuarrie, M. King* and R. J. Lasek. Department of Anatomy, Case Western Reserve University School of Medicine, Cleveland, OH 44106.

The polypeptide composition of the two slowest rate components of axonal transport has been determined for guinea pig retinal ganglion cells by using gel electrophoresis of optic nerve segments following intra-ocular injection of labeled amino acids (Black & Lasek, J. Cell Biol. 86:616-623, 1980). Each component has a unique composition; the faster component moves at 2-3 mm/d (SCb), and the slower at 0.25 mm/d (SCa). The microfilament network of the axon apparently advances with SCb, and the microtubule-neurofilament network with SCa. In rat retinal ganglion cells, SCb and SCa have the same rates and compositions (McQuarrie et al., Soc. Neurosci. Abstr. 6:501, 1980).

In similar studies on rat sciatic nerve motoneurons, SCb advanced at 3.4 $\pm$ 0.6 mm/d (clathrin peak) and SCa advanced at 1.2 $\pm$ 0.2 mm/d (neurofilament peak), when animals were 5-8 weeks old at the time of injection (400-750 uCi ^{35}S-methionine into the ventral horn at the T13-L1 interspace). When the L4 spinal nerve was crushed at one week after injection, the waves of labeled slow component proteins were trapped between the crush and the spinal cord. By 2 days later, when sprouting began, labeled SCb proteins had accumulated proximal to the crush. Subsequently, at axonal outgrowth times of 5-15 days, a peak of labeled SCb proteins advanced distal to the crush at a rate of 3.3 $\pm$ 0.2 mm/d. This rate was comparable to the axonal outgrowth rate of 3.6 $\pm$ 0.4 mm/d, arrived at by using two methods that yielded identical mean rates: the nerve pinch test, and the farthest advance of labeled neurofilament proteins. The SCb peak was enriched in actin, tubulin, and 32 kilodalton proteins; other SCb proteins, including clathrin and the 70 kilodalton group, did not have increased radioactivity levels at the site of the peak.

In summary, newly-formed axons of regenerating rat sciatic nerve motoneurons have an SCb rate that is unchanged from normal and is similar to the axonal outgrowth rate. Actin and tubulin appear to enter axonal sprouts preferentially. In these neurons, actin and tubulin are normally found in both SCb and SCa (McQuarrie et al., Soc. Neurosci. Abstr. 6:501, 1980). Since protein components of the two cytoskeletal networks are transported together and preferentially enter axonal sprouts in these vigorously regenerating neurons, we conclude that the presence of a dynamic association between the two networks may be a requirement for optimal axonal regeneration.

15.6 TRANSNEURONAL REGULATION OF SYMPATHOHIPPOCAMPAL SPROUTING. R. Madison* and J.N. Davis. (SPON: E. Olender). Depts. of Anatomy, Pharmacology and Neurology, Neurology Research Laboratory, Duke Univ. Med. Ctr. Durham, NC 27705.

Peripheral noradrenergic fibers from the superior cervical ganglion (SCG) appear in the rat hippocampal formation (HF) after damage to the fimbria or medial septum (MS). Unilateral section of the preganglionic trunk of the SCG (decentralization) prior to a MS lesion results in apparently less peripheral sprouting in the ipsilateral HF as judged by fluorescent microscopy. In order to extend and quantitate the effects of decentralization radiochemical measurements of norepinephrine (NE) were carried out bilaterally in the HF of adult male Sprague-Dawley rats after unilateral decentralization of the SCG and bilateral electrolytic MS lesions. Animals were sacrificed 4, 7, 9 or 12 weeks later and processed either for fluorescent microscopy or for radiochemical determinations of NE levels for the dentate and hippocampal gyri.

Normal (unoperated) animal dentate NE was 0.63 $\pm$ 0.10 µg/gm. Histochemical sprouting of peripheral sympathetics in the dentate on the side contralateral to SCG decentralization appeared vigorous and continued to increase over time. Dentate NE levels were 0.89 $\pm$ 0.17 at 9 weeks ($p < 0.05$ vs. normal dentate) and 1.39 $\pm$ 0.32 at 12 weeks ($p < 0.001$). By contrast fewer fibers were present in the dentate ipsilateral to the decentralization at 4 and 7 weeks. NE levels were 0.43 $\pm$ 0.06 ipsilateral and 0.82 $\pm$ 0.12 contralateral ($p < 0.05$) to the decentralization at 7 weeks after the lesion. Unexpectedly no difference in the density of peripheral fibers could be seen at 9 weeks or thereafter. NE levels also dramatically increased at 9 weeks ipsilateral to the decentralization (0.96 $\pm$ 0.06) and continued to increase at 12 weeks (1.22 $\pm$ 0.28). No differences in NE levels were noted in the hippocampal gyri at all times studied.

These data show 1) that peripheral noradrenergic sprouting continues to increase for up to 12 weeks after MS lesions and 2) that loss of afferent input reduces the extent of peripheral noradrenergic spouting. It seems likely that the increase in sprouting ipsilateral to the decentralization after 9 weeks represents reinnervation of the SCG by preganglionic fibers. These experiments demonstrate the regulation of a neuronal rearrangement by afferent input due either to nerve impulse flow or transneuronal transfer of a factor or factors. This unusual model of sprouting allows quantitation of regulatory mechanisms and should be useful in understanding some of the principles underlying neuronal rearrangements in the adult rat brain.

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16.1 VESTIBULAR NERVE ACTIVITY INDUCED BY OFF VERTICAL AXIS ROTATION. Theodore Raphan, Walter Waespe*, Bernard Cohen, Dept. of Neurology, Mount Sinai School of Medicine, New York, N.Y. 10029.

Semicircular canal and otolith afferents were recorded in the vestibular nerve while monkeys were given steps of velocity about off vertical axes to determine the role of the peripheral labyrinth in generating the continuous nystagmus induced by this stimulus. Average frequency of firing was obtained by a computer program that counted the number of spikes within a 192 msec window and updated the count every 1.6 msec. The algorithm had a resolution of 5 Hz, a frequency response of a zero order hold system and a linear range up to 625 Hz. Neurons were tested using velocities from 3.5°-180°/sec and tilt angles of 30°, 60°, and 90°. For a step in velocity, semicircular canal units had an increase in frequency which decayed to a steady state with a time constant of 3-6 sec. These units did not show a direction specific steady state response to off vertical axis rotation (Goldberg & Fernandez, 1981). The average frequency of firing was either not affected or increased, and there was little or no modulation in relation to head position. Otolith units on the other hand modulated their activity in relation to head position. The firing rates of "regular" otolith neurons were approximately sinusoidal and the peak firing rates occurred at head positions that were approximately independent of the velocity of rotation, direction of rotation or angle of tilt. Phase differences between these modes of excitation were within 20° of each other. The depth of modulation was related to tilt angle and presumably was dependent on the neuron's polarization angle. Peak frequencies of irregular neurons, including those that had no steady state response to static head tilts, occurred at specific head positions while rotating. The phase of the peak frequency was not significantly altered with speed of rotation or phase of tilt. Differences in phase were within 20-30° for specific directions of rotation. However, there was a phase change in peak frequency for oppositely-directed rotation. It was close to 100° in some instances. The data indicate that the semicircular canals play a subordinate role in the generation of continuous nystagmus during off vertical axis rotation. The unchanging phase relation of peak frequency relative to given head positions for regular as well as irregular units during unidirectional rotation is consistent with the theory that sequential activation of otolith units induces traveling waves in the cellular structure of the maculae. The velocity of this wave is detected centrally and converted to a signal that activates the velocity storage integrator to generate the continuous nystagmus.

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16.2 EFFECT OF STATIC HEAD POSITION ON THE DURATION OF VERTICAL OPTOKINETIC AFTER NYSTAGMUS (OKAN) IN MONKEYS. V. Matsuo*and B. Cohen. Depts. of Neurology and Opthalmology, Mt. Sinai School of Medicine, New York, N.Y. 10029., Spon.: H. Krieger, M.D.

Although horizontal OKAN is generally left-right symmetrical with regard to peak velocities and time constants, vertical OKAN is asymmetrical. When animals are tested on their sides, downward OKAN reaches higher velocities and is longer-lasting than upward OKAN. A testing apparatus recently became available that permitted us to induce OKN in various planes with respect to the animal and to the earth. An unexpected finding was that the time constant of vertical OKAN was modified by the attitude of the head.

Vertical OKAN was induced in 5 monkeys using full field stimulation. Eye movements were recorded using d.c. electro-oculography. Downward OKAN (re: the animals's longitudinal axis) was induced by rotating the optokinetic drum with the animals in various angles of tilt in the roll axis. One animal was also placed in the prone, supine and 25° pitch forward positions. Steady state slow phase velocities of OKN were similar regardless of head orientation, and peak velocities of OKAN were not markedly affected. However, time constants (TC's) of downward OKAN were much shorter when the animal was upright. Mean TC's were 14.7 sec, ± 6.9 when the animals were horizontal. In contrast, mean TC's were 1.9 sec, ± 1.1 when animals were upright. Downward OKAN TC's varied as a function of the angle of tilt, decreasing at a rate of approximately 10% of the maximum value every 10° of deviation from the lateral position. With the animal in the prone and 25° pitch forward positions, the time constants of downward OKAN were reduced to the range observed when animals were upright. When supine, time constant values were intermediate. Upward OKAN is weak or absent in normal animals (Matsuo et al., 1979). When present, upward OKAN also was most vigorous when the animal was in the lateral position and weakest when upright.

The results are consistent with psychophysical findings of Young et al. (1975) who reported that the sensations of pitching and linear vection during vertical OKN were strongest when subjects were in the lateral position and weakest when subjects were upright or 25° pitched forward. Activity responsible for the changes in time constant of downward OKAN presumably originates in the otolith organs since it was preserved in animals with their semicircular canals disabled.

Supported by NS 00294, Training Grant EY 07014-06 and Core Center Grant EY-01867.

16.3 DIFFERENTIAL PROJECTIONS OF REGULARLY AND IRREGULARLY DISCHARGING VESTIBULAR-NERVE AFFERENTS ONTO INDIVIDUAL SECONDARY NEURONS OF THE SUPERIOR VESTIBULAR NUCLEUS IN THE BARBITURATE-ANESTHETISED SQUIRREL MONKEY. J.M. Goldberg, C. Fernández* and S.M. Highstein. Depts. of Pharmacol. Physiol. Sci. and of Surgery (Otolaryngol.), University of Chicago, Chicago, IL 60637 and Dept. Neuroscience, Albert Einstein College of Medicine, Bronx, NY 10461.

Vestibular-nerve fibers, in responding to brief electric shocks delivered via the perilymphatic space, show large variations in their firing thresholds; irregularly discharging (I) afferents have lower thresholds than do regularly discharging (R) afferents (Neurosci. Abstr., 6:224). The two afferent groups also differ in their conduction times (Brain Res., 122:545). These findings have been exploited to study the central projections of I and R afferents. Intracellular recordings were made from secondary neurons in the superior vestibular nucleus (SVN). Monosynaptic EPSPs were evoked by shocks delivered to the ipsilateral labyrinth. The difference in afferent thresholds depends on synchronizing the shock to the last occurrence of an action potential. For this reason, a two-shock paradigm was used. Shock strength is expressed in multiples of the extracellular field threshold (T) recorded in the SVN. The first shock was kept at a high intensity (16-32xT) so as to synchronize vestibular-nerve activity. The second shock was presented 4 msec later and was varied from 1xT to 16-32xT. Our vestibular-nerve data indicate that I afferents are recruited at 1-4xT, R afferents at 4-32xT. SVN neurons differ in the ways their EPSPs grow as the intensity of the second shock increases. Three broad and probably overlapping groups can be recognized. Some neurons have low EPSP thresholds (1-2xT) and show near-maximal EPSPs for shocks of 4xT or less. Other neurons have EPSP thresholds greater than 4xT and maximal EPSPs are evoked only at 16-32xT. Still other neurons have low EPSP thresholds (1-2xT) and their EPSPs continue to grow up to 16-32xT. The respective groups are likely to represent neurons receiving a predominantly I-fiber input, a predominantly R-fiber input, and a mixed (M) innervation of I and R inputs. The latent periods of EPSPs are consistent with this interpretation and the established differences in vestibular-nerve conduction times. I-input neurons have shorter latent periods than do R-input neurons. In M-input neurons, it is the later portions of the EPSPs which grow as shock intensity is raised and as the more slowly conducting R inputs are presumably recruited. Our results imply that the diversity of afferent information carried by I and R afferents is preserved at the level of the vestibular nuclei.

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16.4 VERTICAL SEMICIRCULAR CANAL AND OTOLITH INTERACTIONS IN THE VESTIBULO-NECK REFLEXES OF THE ALERT CAT. John H. Anderson and C. Pappas*, Dept. Otolaryn. and Physiol., U. Minn., Mpls., MN. 55455

Because of the linkage of head with neck, disturbances of posture are greatest in the vertical planes and are likely to stimulate both the vertical semicircular canals and otolith organs. The aim of the present experiments was to determine the relative contributions to the neck muscle responses by the otolith and canal inputs and to provide evidence for participation by both the utricule and saccule. Alert, blindfolded cats were used. Each animal was restrained and subjected to sinusoidal roll rotations from 0.04 to 4.0 Hz with displacements of up to ±40 deg. The EMG response of the sternomastoid muscle, a rotator and ventral flexor of the head, was recorded.

When the animals were positioned in the normal, right side up position, the right and left muscles responded in a reciprocal manner and relative to contralateral angular acceleration, the EMG's showed phase lags of 130-150 deg at the low frequencies. Above 1.0 Hz the phase lag became significantly less, e.g., 80-110 deg at 3.0 Hz, and is presumably due to the canal inputs. At the lower frequencies the otolith inputs become relatively larger and would be expected to have a significant effect on the muscle activity. Some evidence for this was obtained by the vectorial addition of two independent sets of data. For this, one set of recordings was made while rotating the animal to either side after it had been placed upside down. This was done to selectively change the otolith input: It had the opposite sign compared to normal while the canal input remained exactly the same. The results showed a significant difference: The phase lag was much less at the lower frequencies (e.g., about 70-90 deg at 0.1 Hz) up to about 0.2 to 0.4 Hz, indicating that the otolith inputs (e.g., utricular) were, in fact, contributing to the EMG responses. A second set of recordings was made while rotating the animal after it had been turned 90 deg onto its side. In this situation the component of gravity along the animal's z-axis was modulated such that the otoliths (e.g., saccules) on the right and left were simultaneously stimulated instead of reciprocally so as for the normal, right side up position, although the canal input was exactly the same. The EMG of the muscle on the top side showed much less of a phase lag compared to normal, whereas the EMG of the muscle on the underside showed a phase similar to normal. Thus, the right and left EMG's were neither in phase nor 180 deg out of phase with each other. These results indicate that during vertical rotations, the canal inputs predominate at high frequencies whereas at lower frequencies the canal, utricular, and saccular inputs each make significant contributions.

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16.5 MORPHOLOGY OF SECONDARY VESTIBULAR NEURONS LINKED TO THE POSTERIOR CANAL IN RABBIT AND CAT. W. Graf*, R. A. McCrea and R. Baker. Dept. of Physiol. and Biophys., New York Univ. Med. Ctr., 500 First Avenue, New York, N.Y. 10016.

The vertical vestibulo-ocular reflexes have been subject to behavioral and electrophysiological studies, yet less information has been contributed about the morphology of the neuronal network. The present study investigated the morphology of secondary vertical vestibular neurons by injecting HRP into cells connected to the posterior canal. Bipolar electrodes were implanted into the ampullae of the anterior and posterior (PC) semicircular canals of pigmented rabbits while vestibular neurons in cats were identified by natural and electrical stimulation. Axons monosynaptically activated by PC stimulation were injected in the contralateral rostral MLF (excitatory pathway) and later reconstructed after processing the brain with a DAB-$CoCl_2$ method. The cell bodies of these neurons were located in either the medial or ventro-lateral vestibular nuclei. In the rabbit, all of the axons bifurcated after crossing the midline, with one branch ascending and the other descending in the MLF. The ascending branches gave rise to collaterals which terminated in the trochlear and inferior rectus subdivision of the oculomotor nuclei. In addition a few of these axons also sent collaterals into the interstitial nucleus of Cajal, the paramedian pontine reticular formation and the periaqueductal grey. The descending branches were followed to the caudal part of the medulla in the MLF. These branches gave rise to collaterals which terminated in the vestibular nuclei, the reticular formation, the nucleus prepositus hypoglossi and the abducens nucleus. In the cat, we observed similar termination patterns with collaterals to both trochlear and oculomotor nuclei. However, axon collaterals of some neurons crossed the midline after terminating in the inferior rectus subdivision and reached a similar part of the contralateral oculomotor nucleus. Collaterals of these axons also terminated in the supraoculomotor region between trochlear and oculomotor nuclei, but no descending axonal branches to the spinal cord have yet been observed. Collaterals to the archicerebellum were not seen in either species. Our data indicate strong similarities in the morphology of PC linked secondary vestibular axons in the two species. Slight differences in termination pattern probably reflect the different kinematic properties of extraocular muscles required for compensatory eye movements in lateral- versus frontal-eyed animals. Supported by USPHS Grants NS05857, EY02007 and DFG Grant Gr 688/1.

16.6 GABAergic TERMINALS IN THE LATERAL VESTIBULAR NUCLEUS: AN IMMUNOCYTOCHEMICAL DEMONSTRATION OF THE CEREBELLAR CORTICOVESTIBULAR PATHWAY. C.R. Houser, J.E. Vaughn and R.P. Barber*. Division of Neurosciences, City of Hope Research Institute, Duarte, CA 91010.

Physiological studies have demonstrated that Purkinje cells in the anterior lobe of the cerebellar cortex mediate a GABAergic inhibition of neurons in the dorsal part of the lateral vestibular nucleus (dLVN). Since the anatomical substrates of this pathway have not been completely elucidated, we have used an immunocytochemical method for the localization of the GABA synthesizing enzyme, glutamic acid decarboxylase (GAD), to describe the relative number, location and ultrastructural characteristics of GAD-positive (GAD+) axon terminals in the dLVN and to examine the effects of cerebellar cortex ablation on these terminals.

Specimens containing the dLVN were obtained from normal rats and from rats in which the vermis of the anterior cerebellar lobe was ablated 12 days prior to sacrifice. The tissue was processed for GAD immunocytochemistry and examined by both light and electron microscopy. In normal rats, GAD+ axon terminals were numerous in the dLVN, and were concentrated around the somata and proximal dendrites of large and medium sized neurons. A large number of GAD+ terminals also were present in neuropil regions, suggesting that GABAergic synapses may be located on dendrites of many sizes. Ultrastructural observations confirmed the presence of GAD+ synapses at these sites and showed that GABAergic terminals frequently were located adjacent to one another on neuronal somata and proximal dendrites, where they occasionally displayed an en passant pattern of synaptic contacts. Most GAD+ terminals formed symmetric or intermediate type synaptic junctions and contained moderate numbers of synaptic vesicles and mitochondria. Following ablation of the anterior vermis, the number of GAD+ terminals in the dLVN decreased by 73%, the loss being slightly greater around neuronal somata and proximal dendrites than within adjacent neuropil regions.

These results indicate that neurons of the dLVN are contacted by numerous GABAergic synaptic terminals, and that a large majority of these terminals originate from the cerebellar cortex of the anterior vermis. The large number of GABAergic terminals and their proximal location on many of the neurons suggest that the function of the dLVN in the control of posture and movement may depend to a large extent upon the modulation of GABAergic inhibition. Supported by USPHS Grant NS12116 from the National Institute of Neurological and Communicative Disorders and Stroke.

17.1 NEONATAL 6-HYDROXYDOPAMINE ADMINISTRATION ELIMINATES SPARING OF FUNCTION AFTER NEONATAL FRONTAL CORTEX DAMAGE. R. J. Sutherland, B. Kolb, J. B. Becker, and I. Q. Whishaw, Dept. of Psychology, The Univ. of Lethbridge, Lethbridge, Alberta, Canada, T1K 3M4.

Bilateral removal of the frontal cortex in adult rats causes a disruption of behaviour requiring accurate spatial orientation and chronically reduces the body weight set-point. Previous work has shown that if similar damage is produced in neonatal rats, then adult spatial orientation and body weight set-point appear to be normal. This sparing of function after neonatal damage depends upon the integrity of the remaining posterior neocortex (Kolb & Whishaw, J. Comp. Physiol. Psychol., 1981, in press). Several lines of evidence indicate that neocortical and hippocampal synaptic plasticity are modulated by the local availability of noradrenaline (NA) (Kasamatsu, et al., Science, 1976, 194, 206-209, J. Neurophysiol., 1981, 45, 254-266; Goddard, et al., Neurosci. Abstr., 1980, 10, 89). The present experiment examined the possibility that the behavioural sparing associated with early brain damage depends upon the availability of cortical NA.

Sixty Long-Evans X Wistar rats received neonatal or adult frontal cortex removal, neonatal 6-hydroxydopamine (6-OHDA)(3 X 100 mg/kg, ip) or saline injections, or both neonatal frontal removal and neonatal 6-OHDA. All rats were tested as adults in the Morris water maze, in running wheels, in a straight swimming alley, chronic body weight was recorded, and these measures were correlated with levels of cortical NA.

The most significant findings were that: 1) frontal cortex removal in adults produced a severe spatial deficit in the Morris water maze, 2) neonatal frontal cortex removal was associated with significant sparing in this task, 3) neonatal frontal cortex removal in rats depleted of cortical NA produced as severe a spatial deficit in the water maze as adult frontal removal, 4) neonatal 6-OHDA alone did not affect spatial behaviour, 5) chronic body weight set-point was normal in male rats with only neonatal frontal cortex removal or only neonatal 6-OHDA, but was reduced in rats with adult frontal cortex removal or neonatal frontal cortex removal following NA depletion, and 6) in females only the neonatal frontal cortex removal in rats depleted of cortical NA produced a chronically reduced body weight set-point.

These findings are consistent with an important role for NA systems in permitting the sparing of behavioural functions associated with early brain damage and extend to the neural systems involved in feeding and cognitive behaviours in the rat the notion that NA modulates neuronal plasticity.

17.2 EVIDENCE FOR RECOVERY OF FUNCTION FOLLOWING 6-HYDROXYDOPAMINE LESIONS OF THE DORSAL TEGMENTAL NORADRENERGIC BUNDLE. D.C.S. Roberts, D.S. Segal†, G.J. Vickers* and B.A. Pappas. Dept. of Psychology, Carleton Univ., Ottawa, Canada, K1S 5B6 and †Dept. of Psychiatry, U.C.S.D., La Jolla. CA. 92093.

6-Hydroxydopamine (6-OHDA) injections into the region of the dorsal tegmental bundle (DB) have been used by several laboratories to investigate the involvement of noradrenaline (NA) in a variety of behaviours. This treatment causes degeneration of the ascending projection from the locus coeruleus to the forebrain, and reduces NA levels in the hippocampus and cortex to approximately 5% of control levels. Because this depletion requires 2-3 weeks, most behavioural studies using the DB-6OHDA treatment have not been initiated until several weeks post-lesion. Despite the severe depletion of NA, in general, no consistant or major deficits have been attributed to this NA loss; although some subtle or unreliable effects have been noted. Because recovery of function occurs to some extent after most brain lesions, we tested the hypothesis that recovery occurs several weeks following the DB-6OHDA treatment, and that major deficits or alterations in behaviour may be apparent at time points closer to the lesion. We now report that behavioural effects are observed several days after the lesion which disappear or are attenuated by 3 weeks.

D-amphetamine (2.5 mg/kg) was found to produce significantly more intense stereotyped behaviours (particularly oral movements) in DB-6OHDA treated animals when compared to control animals, when tested 3 days post-lesion. This increase in stereotypy was also reflected by a significant decrease in locomotor activity during the peak stereotypy phase. In contrast, when tested 3 weeks post-lesion, the DB-6OHDA group showed less stereotypy, compared to controls. The augmented sterotypy could be reinstated, however, after drug challenge of repeated daily injections of amphetamine. The facilitation of oral stereotypy seen at post-lesion day 3 agrees with previous reports using NA synthesis inhibitors and blocking agents.

Similarly, the DB-6OHDA treatment was found to have time-dependant effects on acquisition of a two way shuttle box. When tested 3 days post-lesion, the DB-6OHDA group required significantly more trials to reach criterion (18 avoidances /20) during a one day training session (DB-6OHDA=68± 14 trials; control=35± 4). No differences were found between groups when testing was delayed 3 weeks. While the nature of the deficit (associative vs non-associative) observed several days after the DB-6OHDA treatment is not yet established, the results demonstrate that recovery may occur following the lesion, and suggest the "true effects" of the lesion may be manifest soon after the lesion. (Supported by the Medical Research Council).

17.3 EFFECT OF 5HTP ON H-REFLEX RECOVERY CURVES. J. Metz, H.H. Holcomb, and H.Y. Meltzer. University of Chicago, Department of Psychiatry, Chicago, Illinois 60637.

The recovery curve of the H-reflex is abnormally high in about 25% of psychiatric patients (Metz et al., Psychol. Med. 10, 541-548). We have conducted pharmacological studies to understand the neural transmitter mechanisms which may underlie this abnormality. In this study, we found that d,l 5-hydroxytryptophan (5HTP) decreases the H-reflex recovery curve less frequently in patients with primary affective disorders than in normal controls.

We administered 200 mg 5HTP orally to 10 normal subjects and 29 patients with affective disorders (RDC diagnoses: 11 depressed, 8 manic, 7 schizoaffective depressed, 3 sa manic). Subjects were tested twice on the recovery curve: a baseline test and a test 2 hours after 5HTP. Some patients were tested both with and without other treatment.

The normal group had lowered recovery after 5HTP relative to the baseline condition ($t = 3.06$, $p < .02$). Unmedicated patients, however, showed no significant effect of 5HTP. Eight of 10 normal subjects had lowered recovery following 5HTP, but only 11 of 27 unmedicated patients had decreased recovery (Fisher exact probability = .038). No significant differences were found among different classes of patients. Patients receiving antidepressants (5 of 7 decreased after 5HTP) or lithium carbonate (4 of 7 decreased) more closely resembled normals in their response to 5HTP.

The decrease in the recovery curve following 5HTP in normal subjects suggests that a serotonergic mechanism influences the excitability of the alpha-motoneuron. We have found a similar decrease in the recovery curve in normal subjects and in psychotic patients following acute or chronic treatment with chlorpromazine. The 5HTP effect, therefore, may be mediated by a decrease in post-synaptic dopaminergic activity. These results suggest a reduced sensitivity to serotonin or an abnormal interaction between serotonin and dopamine in some patients with affective disorders. Our results also indicate that a serotonin deficiency may underlie the abnormally high recovery curves found in psychotic patients.

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17.4 6-HYDROXYDOPAMINE MODELS OF CHILDHOOD HYPERACTIVITY: EFFECTS OF AMPHETAMINE ON SWIMMING AND LOCOMOTOR ACTIVITY IN NEONATAL RATS. G. K. Hodge. Dept. of Psychology, University of New Mexico, Albuquerque, NM 87131.

Because stimulant medications are sometimes effective in attenuating the symptoms of childhood hyperactivity, and since drugs such as dextroamphetamine (DEX) are believed to act, in part, by facilitating dopamine (DA) function, it has been suggested that the symptoms of the disorder may be attributable to decreased DA activity. Support for this position stems from studies reporting that DEX attenuates hyperactivity of rat pups treated with 6-hydroxydopamine (6-OHDA).[1] At the opposite end of the developmental spectrum, DA agonists have been found to improve swimming behaviors in aged rats.[2] Normal swimming patterns in the rat emerge at about the same time in which 6-OHDA elicits hyperactivity. Since swimming requires complex coordination and integration of neural responses, this model of putative DA function may provide additional insight into the maturational dynamics of DA function and dysfunction in hyperactivity.

Following desipramine pretreatment, 5-day-old rats were injected intracisternally with either 100 µg/25 µl of 6-OHDA or 25 µl of the ascorbic acid/saline vehicle. Postoperatively, swimming was measured daily; open-field activity was measured on postpartum days 15, 19, and 22. Half of the animals in each group received exclusively either 1 mg/kg DEX or saline 30 min before behavioral observations (90 min for activity).

6-OHDA treatment retarded the normal course of development of swimming postures; swimming remained impaired until postpartum day 16. DEX impaired swimming in control animals through day 11; swimming tended to be facilitated thereafter until around the third week when there was no longer any difference compared to saline-injected controls. DEX reversed the swimming impairments of 6-OHDA-treated neonates; i.e., swimming developed normally. Although 6-OHDA induced hyperactivity at days 15, 19, and 22, DEX did *not* attenuate hyperactivity; in fact, it actually *increased* activity even more so on days 19 and 22 (results consistent with those of Pappas et al.[3]).

These data suggest the importance of DA systems in the acquisition and integration of certain motor behaviors. Whether the 6-OHDA preparation is a tenable model of childhood hyperactivity, however, remains more problematic.

(Supported by HEW grant no. 1-SO7-RR07185-01)

[1]Shaywitz et al. *Nature, 261:* 153-155, 1976.
[2]Marshall & Berrios. *Science, 206:* 477-479, 1979.
[3]Pappas et al. *Psychopharmacology, 70:* 41-46, 1980.

17.5 AMPHETAMINE-ELICITED ROTATIONAL BEHAVIOR: SEX DIFFERENCES AND ESTROUS CYCLE VARIATIONS ARE NOT DUE TO DIFFERENCES IN BRAIN LEVELS OF AMPHETAMINE. Jill B. Becker, Kimberly A. Lorenz* and Terry E. Robinson. Neuroscience Laboratory and Department of Psychology, University of Michigan, Ann Arbor, MI 48109.

Sex differences have been reported in amphetamine (AMPH)-elicited behaviors. However, metabolic differences result in different brain levels of AMPH in male and female rats given the same systemic dose. In this study, whole brain levels of AMPH were determined following the i.p. injection of ^{3}H-d-amphetamine sulfate and subsequent separation of AMPH from its metabolites. When male and female Holtzman rats were injected with the same dose of AMPH, males had significantly lower brain levels of AMPH than females. From dose-response curves we estimated doses which would yield equal brain levels of AMPH in the two sexes, then confirmed the prediction. We could then study AMPH-elicited rotational behavior in male and female rats which had the same brain concentrations of AMPH at 5, 30 and 60 min post-injection.

Rotational behavior was studied in intact male and female rats employing automated rotometers. After 15 min of habituation, animals were injected with AMPH and rotational behavior was recorded for 1 hour. At 1.25 mg/kg AMPH there were no differences in brain levels of AMPH during the female estrous cycle. A dose of 1.56 produced brain levels of AMPH in males equivalent to the females at 1.25 mg/kg. Thirty min after injection, there were significant differences in the number of net rotations (# 360° turns in predominant direction minus those made in the other direction) made by the 5 groups. The number of net rotations made by male rats during the first 30 min was significantly less than the number made by female rats during estrus (E), diestrus 2 (D_2) or proestrus (P). Female rats during diestrus 1 (D_1) were not significantly different from males. There were also significant differences in the number of net rotations females made during different stages of the estrous cycle. Females in E made significantly more net rotations than D_1, D_2 or P females. Rats tested during the day of D_1 made significantly fewer net rotations than any of the other female groups. It is interesting that estrous females exhibited the greatest number of net rotations, yet 24 hours later on D_1 there was a dramatic decrease.

In conclusion, we find sex differences in AMPH-elicited rotational behavior in rats known to have equivalent brain concentrations of AMPH. We also find estrous cycle dependent variations in rotational behavior. This suggests that in female rats fluctuations of hormones within a physiological range can significantly alter the activity of brain dopamine systems.

(Supported by Grants No. NS16437 and HD05997).

17.6 SEX DIFFERENCES AND ESTROUS CYCLE VARIATION IN ROTATIONAL BEHAVIOR ELICITED BY ELECTRICAL STIMULATION OF THE NIGROSTRIATAL DOPAMINE SYSTEM. Terry E. Robinson, Dianne M. Camp* and Jill B. Becker. Psychology Department and Neuroscience Laboratory, University of Michigan, Ann Arbor, MI 48109.

Electrical stimulation-induced rotational behavior is thought to provide a behavioral index of functional activity in the nigrostriatal dopamine (DA) system. We have used this behavioral model to investigate whether hormonal states influence the nigrostriatal DA system in male or female rats.

Sixty-five rats were implanted with bipolar stimulating electrodes aimed at the nigrostriatal DA bundle, as it courses through the lateral hypothalamus. Electrical stimulation of sites in or near these fibers produced vigorous contraversive rotational behavior. The most effective sites were located *within the DA fibers, while less effective sites were located* just dorsal to the bundle. These latter sites required higher current intensities to produce rotational behavior. The maximum current intensity used was 300μA (0.1 msec duration monophasic rectangular pulses). Ineffective sites were located either medial or ventral to the DA fibers.

Each animal was tested daily for rotational behavior for a 21 day baseline period, using the same current parameters each day. We found that the amount of rotational behavior observed in female rats varied across the estrous cycle. Vigorous rotational behavior on the day of estrus was followed by a significant drop on diestrus 1. The amount of rotational behavior then increased gradually, peaking on the next day of estrus. After 21 days of baseline testing half the females were ovariectomized (OVX) and half the males castrated (CAST). The remaining animals received a sham-operation. After one day of recovery, testing for rotational behavior continued for an additional 30 days. OVX of female rats resulted in a gradual and significant decline in electrical stimulation-induced rotational behavior relative to sham-operated females, males, or CAST males. These latter three groups did not show a decline in rotational behavior relative to baseline levels, and did not differ from each other. Thus, there are sex differences in the effects of gonadectomy on electrical stimulation-induced rotational behavior. OVX of female rats attenuates electrical stimulation-induced rotational behavior while CAST of male rats has no effect. The effect of OVX is not due to a generalized hypokinesia in OVX females since a time sample of spontaneous behavior failed to show group differences in the incidence of active behaviors (walking and rearing) or immobility in the test situation. We conclude that gonadal steroid hormones directly or indirectly modulate nigrostriatal DA activity in female, but not male, rats. *(Supported by Grant NS16437 to TER).*

17.7 NEUROBEHAVIORAL CONSEQUENCES OF MANGANESE CHLORIDE ADMINISTRATION IN RATS. Charles L. Kutscher* and Catherine M. Stoney* (SPON: J. A. Rosecrans). Psychology Research Lab., Syracuse University, 101 Stevens Place, Syracuse, NY 13210.

Manganese (Mn) toxicity in humans has been associated with a variety of extrapyramidal symptoms such as tremor, abnormal gait and loss of muscular control. Mn is ubiquitous in the environment because of its widespread use in manufacturing processes including gasoline distillation. Dietary administration of Mn produces changes in brain catecholamines (J. Neurochem. 36: 683, 1981) and serotonin levels (J.Tox. Environ. Health 4: 701, 1978), but no systematic studies of Mn intakes on behavior have been done. We studied various types of motor responses in male and female rats given 0, 0.5 or 1.0% $MnCl_2$ to drink from weaning (21 days) until day 180 of life. Mn produced no effects on latency of forepaw suspension (tested every 10 days from 40-90 days of age), wheel running, and rotorod performance (using two drum diameters and three speeds). No effect was detected in gait measured by walking animals up an inclined plane after inking the paws.

In males Mn produced a depression in body weight, but no weight changes were noted in females. Mn produced a dose-dependent reduction in both males and females in duration of cold water swimming (15±1°C). Effect of the amphetamine analogue phenylethylamine (PEA) on wheel turning was studied. In males the PEA-induced hyperactivity seen in the 0% Mn group was prevented by Mn administration in both the 0.5 and 1.0% groups. In females activity was not affected by either Mn or PEA. Brain levels of Mn, norepinephrine, dopamine and serotonin were noted and correlated with behavioral changes.

Mn dosages produced no overt clinical symptomatology other than weight deficit in males. The PEA challenge and swimming endurance tests were more sensitive than the conventional motor tasks used. Findings are consistent with the hypothesis that Mn administration alters motor function and provides the first comprehensive and systematic report of Mn-induced behavior changes in animals.

17.8 **DIFFERENTIAL BEHAVIORAL EFFECTS FOLLOWING REGIONAL DOPAMINE DEPLETION IN RAT NEOSTRIATUM.** **D.B. Neill*, M.R. Holdiness*, M.D. Rosen*, and J.B. Justice, Jr.* (SPON: H.D. Rees). Depts. of Psychology and Chemistry, Emory University, Atlanta, GA 30322.**

A number of studies have shown that near-total destruction of the dopaminergic innervation of the neostriatum in the rat produces a constellation of behavioral effects including hypokinesia, aphagia, and deficient sensorimotor function. It is difficult to account for all of these acute and chronic effects in terms of a single disrupted mechanism. In the present study, the hypothesis that spatially selective dopaminergic denervation of different neostriatal regions would produce distinct behavioral effects was tested. Adult male albino rats were bilaterally injected with 6-hydroxydopamine hydrobromide (4 ug as the salt in 2 ul of 0.5% ascorbic acid-isotonic saline vehicle) at one of three sites: just anterior to the globus pallidus (GP), in the body of GP, or in the tail of GP. Vehicle control groups were also prepared for each injection site. Upon completion of all behavioral testing, all rats were decapitated, their brains removed, blocked, and quickly frozen. Cylindrical punches (1.3 mm dia., 1 mm length) were taken through nuc. accumbens/olfactory tubercle and at 4 anterior-posterior levels of neostriatum. Dopamine concentration (ng DA/mg protein) was determined for each punch by a gas chromatographic/mass spectrometric assay.

The results showed that DA depletion in anterior neostriatum (in front of the ant. commissure) correlated with the post-operative reduction in spontaneous activity. Food intake in animals with anterior depletions appeared depressed because of a deficient ability to maintain any behavior, and recovered in parallel with activity. DA depletion in posterior neostriatum correlated with the reduction in food intake. Animals with posterior depletions often showed pronounced aphagia/hypophagia with little change in activity. The decreased food intake after posterior depletions seemed related to impaired sensorimotor coordination. DA levels were not significantly altered in nuc. accumbens/olf. tubercle by any injection.

Based on these and other findings, an hypothesis for the functional organization of the dopaminergic innervation of rat neostriatum will be proposed.

17.9 DOPAMINERGIC NATURE OF FEEDING INDUCED BEHAVIORAL STEREOTYPIES. Irving Goodman, James Zacny*, Augustine Osman*, Albert Azzaro & Carol Donovan*. Departments of Psychology & Neurology, West Virginia University, Morgantown, WV 26506.

Stereotyped behaviors, including pecking, locomotion, head shaking, mandibulating and swallowing can be induced by daily restricted feedings in weight-reduced, caged pigeons. These feeding-induced behavioral stereotypies (FIBS) occur with greatest frequency immediately following feeding and may last for hours thereafter. This study attempted to investigate the influence of dopamine agonists and antagonists on FIBS in the light of the established dopaminergic nature of another induced stereotyped behavior apomorphine pecking in avians.

Two groups of weight-reduced pigeons were fed their limited grain ration at the same time daily. Behavioral stereotypy was monitored on 3 hourly occasions preceding and following feeding. Individuals in one of the 2 groups received a single weekly injection of apomorphine (APO) (3 mg/kg, IP) over a 7 wk period. Statistical comparisons made overthis period between the 2 groups indicated a significantly greater number of animals engaging in FIBS and a greater number of FIBS in the APO group. Subsequently, the non-APO group received 7 weekly APO injections and exhibited a significant increase in FIBS, compared to its first 7 wk record. After several weeks, all animals were given the dopamine antagonist haloperidol (HAL)(1 mg/kg, IP). On the day of injection all FIBS ceased. A comparison of FIBS one day prior to HAL and on subsequent days revealed a significant reduction on days 1 and 2 following HAL, and a return to normal by day 5, the next tested day. Likewise, a lower dose of HAL (0.5 mg/kg) caused an absence FIBS on the injection day. A comparison with the pre-injection day FIBS showed a reduction on post-day 1 and a return to normal on post-day 2.

Three FIBS and 3 non-FIBS birds were sacrificed and levels of telencephalic catecholamines were fluorometrically determined. All 3 non-FIBS birds' brains contained normal levels of norepinephrine (NE) but low levels of dopamine (DA), whereas within the FIBS group 2 animals contained normal levels of NE and DA and a third animal contained a low amount of DA but also a low amount of NE.

These findings support the view that brain DA is significantly involved in the display and control of yet another type of stereotyped behavior, FIBS. This behavioral methodology appears to have some potential usefulness in assessing pharmacologically induced synaptic changes over time and their effect upon behavior.

17.10 QUIPAZINE-INDUCED BEHAVIORAL CHANGES IN SELECTED MEMBERS OF A PRIMATE SOCIAL COLONY. R.F. Schlemmer, Jr., and J.M. Davis. Illinois State Psychiatric Institute, Chicago, IL 60612

Quipazine (QUIP) is a potent centrally active serotonin (5-HT) agonist which has been used extensively to study 5-HT-mediated behavior in rodents. In this study, QUIP was administered to investigate the role of 5-HT systems in the mediation of social and solitary behavior in a primate species. In the 1st expt., 4 acute doses of QUIP (0.1-3.0(base)/kg) were administered to each member of a stable, adult Stumptail macaque social colony in a Latin-square design. Only 1 monkey received drug treatment per day & at least 2 wks. separated each QUIP treatment to the same monkey. In the 2nd expt., QUIP, 1.6 mg/kg was given once daily for 5 consecutive days to each member of the same colony in a crossover design. Only 2 monkeys received drug treatment at one time. Each experiment was preceded by a period of no less than 5 days where normal baseline behavior of the colony was observed. Saline was administered to all monkeys on baseline days & to those animals not receiving drug on treatment days. QUIP & saline were administered i.m. 15 minutes prior to behavioral observation. A daily 1 hr. behavioral observation was conducted by the same "blind" observer who quantified & recorded the bahavior of each monkey in the colony from a checklist of 48 social, solitary, & abnormal behaviors for this species. In general, QUIP reduced activity, induced several abnormal behaviors, & disrupted normal affiliative behavior. QUIP induced limb jerks (a myoclonic spasm of an extremity), body shakes & ptosis as abnormal behaviors. QUIP increased the distancing scores between the treated monkeys & other members of the colony & eliminated social grooming. QUIP also significantly reduced self grooming, food forage, & locomotion; but significantly increased checking (vigilance) & lying down. Emesis was also noted in some QUIP-treated monkeys. The threshold dose for most QUIP-induced behavioral changes was 0.3 mg/kg. Upon repeated administration, a rapid partial tolerance developed to many of the QUIP-induced behavioral changes including limb jerks, body shakes, ptosis, checking, & grooming; but tolerance did not develop to the reduction in general activity scores. Administration of the 5-HT antagonist cinanserin HCl, 5 mg/kg, 2½ hrs. prior to QUIP treatment antagonized all QUIP-induced abnormal behaviors, but failed to restore some affiliative behaviors to baseline levels. Although it has been suggested by some investigators that QUIP may affect dopamine as well as 5-HT systems, it appears from these results that the major behavioral changes induced in these monkeys by QUIP are mediated through 5-HT systems. Therefore, this study provides evidence for a role for 5-HT in the mediation of important affiliative, solitary & abnormal behaviors in primates. It is also interesting to note that several QUIP-induced behavioral changes resemble those induced in this species by hallucinogens such as LSD.

18.1 STRENGTH OF LOCOMOTOR RHYTHMICITY IN ONE- AND TWO-EYED APLYSIA. W. P. Jordan, M. E. Lickey, and S. O. Hiaasen*. Dept. Psychol., University of Oregon, Eugene, OR 97403.

Aplysia californica are diurnal with locomotor activity onsets near dawn in LD 12:12. In darkness the locomotor rhythm freeruns for several weeks. Each of the two eyes contains a circadian oscillator that freeruns in vitro, and these eye clocks are known to participate in the control of the circadian locomotor rhythm. The two eye clocks get out of phase (split) when Aplysia are left in darkness. Splitting does not occur immediately upon release into DD, but requires about 3 wk to develop (Hudson and Lickey, 1980). This suggests that the possession of two internal clocks may actually be a liability for long-term freerunning in DD, since split eyes may produce conflicting temporal signals. The experiments reported here test this prediction.

Forty-six Aplysia were entrained to LD 12:12 before release into DD. Prior to release, one eye was removed from 23 animals; the remaining 23 were two-eyed controls. Locomotor activity was recorded for at least 33 d in DD. At the end of locomotor recording the eyes from 15 Aplysia were removed and the phases of the eye rhythms were assessed in vitro.

Throughout DD the quality of the locomotor rhythm was better in one-eyed animals than in two-eyed ones. For days 4-13 in DD, 12 of the 23 two-eyed animals were arrhythmic by a standard adopted previously (Lickey and Wozniak, 1979), whereas, only 5 of 23 one-eyed Aplysia were arrhythmic ($p < .04$, Fisher exact test). These differential proportions of rhythmic versus arrhythmic animals were maintained throughout DD. Analysis of variance of the standardized peak standard deviations from periodogram analysis (Strumwasser, et al., 1967) found the one-eyed animals to be significantly better freerunners than were two-eyed Aplysia.

The phase of the eye rhythms from the 7 one-eyed animals tested were found to be aligned with the active portion of the locomotor rhythm recorded previously. The eyes from the 8 two-eyed animals tested had interocular phase differences of 6 hr or more. Thus, the relationship between eye phase and locomotion in these animals was ambiguous.

These results confirm the prediction that one-eyed Aplysia freerun more vigorously than do two-eyed animals. On the other hand, the results do not conform to the expectation that the superiority of the one-eyed animals should develop only after about 3 wk of DD when interocular splitting is known to develop. An explanation of this discrepancy will shed further light on the organization of the Aplysia circadian system. (Supported by PHS F32 NS06425 and NSF 77-28251)

18.2 CONTRIBUTION OF OPTIC NERVE TO PRC OF APLYSIA EYE CLOCK. R. G. Prichard and M. E. Lickey. Dept. of Psychology, University of Oregon, Eugene, OR 97403.

We have previously shown that the circadian oscillator in the Aplysia eye can be reset by LL/DD transitions in vitro. For eyes that are neurally attached to the brain only 12 h of LL are required as a precursor to resetting; for eyes that are detached from the brain at least 27 h of LL are required. This report concerns: (1) the response of the eye rhythm to DD/LL transitions, (2) for LL/DD, whether it is the duration of LL or the phase of LL/DD that is the cue for resetting, and (3) the PRC for attached and detached eyes for 6 hour light pulses.

In the first experiment, intact Aplysia were given DD/LL transitions after 18-72 h of preceding DD. The eyes and brain were then immediately removed for recording the eye rhythms. During recording in LL one eye was attached to the cerebral ganglion and the other was detached by cutting its optic nerve. The initial results indicate that the DD/LL does not cause appreciable phase shifts in either an attached or detached eye.

In the second experiment animals were switched from LD 12:12 to LL beginning at the time of dawn. LL/DD occurred 21 h later, 3 h before projected dawn. Just before LL/DD the eyes and brain were removed for recording, one eye attached and one detached. In various preparations various amounts of the initial portion of LL was replaced by darkness until a minimum duration of LL was found that would support resetting by LL/DD 3 h before projected dawn. As little as 6 h of light was a sufficient precursor for full resetting in attached eye; 3 h of light was insufficient. Full resetting did not occur in detached eyes even when the entire 21 h of LL was present. We conclude that it is the phase of LL/DD, not the duration of LL, that determines the magnitude of resetting by LL/DD.

In the third experiment animals were switched from LD 12:12 into DD beginning at the last dusk. In various preparations a 6 h light pulse was delivered beginning at each 3 hour interval of the first 2.5 days in DD. The eyes and brain were removed for recording just before the end of the light pulse. The PRC of detached eyes had small amplitude; maximum phase shifts of $\pm$ 2 to 3 h occurred in response to pulses during the projected night. Thus, six hour pulses gave a PRC with no higher amplitude than previously obtained by Jacklet (1974) with 1 h pulses. The PRC of attached eyes had a much higher amplitude; during the projected night 6 h pulses gave phase shifts of up to $\pm$ 12 hr. The difference in amplitude of the PRC's of attached and detached eyes is probably not due to a difference in photoreceptive ability, since both eyes received all but a few minutes of the light pulse while attached to the cerebral ganglion in vivo. NSF 28251 and PHS EY 05383.

18.3 OCULAR AND EXTRAOCULAR PHOTORECEPTORS GENERATE A RESPONSE IN THE APLYSIA CEREBRO-PEDAL CONNECTIVE. M. H. Roberts and G. D. Block. Dept. of Biology, University of Virginia, Charlottesville, VA. 22901.

Aplysia express a diurnal circadian rhythm in locomotor activity (Strumwasser 1967). Circadian pacemakers located within the retinae (Jacklet 1969) appear to play a role in timing the behavioral rhythm, although extraocular pacemakers and photoreceptors also participate (Strumwasser 1974, Lickey et al. 1977).

Previous work in our laboratory (Roberts and Block Neurosci. Abst., Vol. 6, 1980) has demonstrated that the cerebro-pedal connectives are critical pathways for the expression of the locomotor rhythm. Aplysia with these connectives severed continue to locomote, but the distribution of activity appears random even when the animals are exposed to light cycles.

The present study was undertaken with the aim of determining if light responsive units were present in the cerebro-pedal connectives. This would hopefully allow us to identify the extraocular photoreceptors controlling diurnal locomotor patterning. Our initial experiments employed an in vivo recording electrode (Block, Neurosci. Abst. Vol. 5. 1979) attached to the cerebro-pedal connective. Analysis of multiunit activity from this connective in unrestrained animals revealed the presence of a light evoked response which appeared as an increase in ongoing small unit activity and as a burst of large units. In an effort to determine the location of the receptors generating this response, a semi-intact preparation was employed. Electrical activity in the cerebro-pedal connective could be recorded while selectively illuminating parts of the body wall and central ganglia with a fiber optic probe. Results from these in vitro experiments revealed that illumination of the tentacles, rhinophores or eyes leads to a response in the cerebro-pedal connective. Furthermore, the response to illumination of any of the 3 structures appears identical, suggesting a common pathway. Experiments are currently underway to determine whether denervation of all 3 sensory structures blocks the diurnal locomotor rhythm. Supported by NS15264.

18.4 MECHANISM OF PHASE SHIFTING THE APLYSIA EYE BY SEROTONIN AND 8-BENZYLTHIO-cAMP: CHANGES IN K^+ CONDUCTANCE. A. Eskin, Dept. of Biology, Univ. of Houston, Houston, Texas 77004.

Serotonin (5-HT) treatments phase shift the circadian rhythm from the isolated eye of Aplysia. The characteristics of the phase shifts caused by 5-HT along with the transmitter-like properties of 5-HT indicate that 5-HT plays a role in the transmission of circadian information in the eye. The effect of 5-HT on the rhythm seems to be mediated by changes in cAMP since a variety of treatments which should elevate intracellular levels of cAMP precisely mimic the effects of 5-HT, phosphodiesterase inhibitors potentiate the effects of 5-HT, the effects of 5-HT and 8-benzylthio-cAMP are non-additive, and 5-HT causes large changes in cAMP in the eye. We have investigated the mechanisms whereby 5-HT and cAMP cause phase shifts and found that changes in K^+ conductance appear to be responsible for phase shifting by 5-HT and cAMP.

Previously, we found that membrane fluxes of Na^+, Cl^-, or Ca^{++} ions are not involved in the 5-HT effect. Since then we found that OK_o^+ treatment, which depolarizes membrane potentials of molluscan neurons by blocking Na-K pumps, phase shifts the rhythm. The phase response curve for this treatment is very similar to that of 5-HT instead of being like phase response curves of depolarizing treatments. This result led to the hypothesis that phase shifting by OK_o^+ was produced by release of 5-HT which in turn caused the phase shift. The fact that phase shifts produced by OK_o^+ and 5-HT are non-additive is consistent with this hypothesis. Another depolarizing treatment, hiK_o^+, did not produce phase shifts at the phase where OK_o^+ and 5-HT caused phase shifts, though this treatment should also have led to release of 5-HT. This result suggested that the phase shift of 5-HT resulted from a change in K^+ conductance since the effects of such a change in conductance would be reduced by the elevated K_o^+ of the hiK_o^+ solution (K_o^+=30mM). HiK_o^+ completely blocked the phase shift normally caused by 5-HT. The hiK_o^+ treatment by itself did not cause phase shifts. Since the 5-HT phase shift appears to be mediated by cAMP I investigated whether elevating K_o^+ would modify the effects of cAMP. The ability of 8-BT-cAMP to phase shift the rhythm was significantly reduced by an accompanying hiK_o^+ treatment. Thus both the effects of 5-HT and 8-BT-cAMP appear to involve changes in K^+ conductance. These agents are probably phase shifting by an increase in K^+ conductance and the resulting hyperpolarization of the membrane potential since depolarizing treatments cannot mimic the effects of either 5-HT or 8-BT-cAMP. These results provide additional evidence that cAMP mediates the effect of 5-HT since the effects of 5-HT and 8-BT-cAMP were both blocked by the same treatment.

18.5 ELECTROPHYSIOLOGY OF BULLA EYES: CIRCADIAN RHYTHM AND INTRACELLULAR RESPONSES TO ILLUMINATION. G.D. Block and W.O. Friesen. Department of Biology, University of Virginia, Charlottesville, Virginia 22901.

Circadian pacemakers have been identified in the eyes of several molluscan species including Aplysia (Jacklet, 1969), Navanax (Eskin & Harcombe, 1977) and Bursatella (Block and Roberts, 1981). We here report that the eyes of Bulla likewise express a circadian rhythm. In addition, the Bulla eye appears to be particularly favorable for neurophysiological studies by intracellular recording techniques.

Extracellular recording from the optic nerve of isolated eyes in continual darkness revealed a circadian rhythm in the frequency of spontaneously generated compound action potentials (CAPs). This frequency rhythm, whose maximum amplitude is 200 CAPs/hr, persisted for at least 3 cycles with a period ranging from 23-24 hr (Instant Ocean, HEPES 30 mM). Peak activity occurred within 1 hr of projected dawn.

Intracellular recording from the retina with the lens removed revealed several types of electrical activity in response to illumination. Photoreceptors, visible on the retinal surface, had a measured resting potential of -40 to -80 mV and responded to illumination with a transient depolarization of up to 110 mV. This depolarization resembles the response obtained from receptor cells (R-cells) in the Aplysia retina (Jacklet, 1969). Simultaneous recording from receptor pairs revealed electrical coupling between some, but not all receptors. Typically, coupled cells were adjacent, while no coupling was observed between distant cells.

Other retinal responses, observed during deeper penetrations, included: 1) a unit with small, spontaneously occurring spikes which hyperpolarized upon illumination, with a concomitant cessation of spiking activity; 2) a unit which depolarized upon illumination and simultaneously produced small spikes; and 3) a unit which depolarized upon illumination and produced large spikes. These were followed 1-1 by large unitary spikes in the optic nerve, independent of simultaneously occurring CAPs. Supported by NS15264.

18.6 SWITCH FROM NOCTURNAL TO DIURNAL LOCOMOTOR BEHAVIOR FOLLOWING EYE REMOVAL IN THE BUBBLE SNAIL, BULLA GOULDIANA. Philip A. Davenport* and Gene D. Block. (SPON: W. O. Friesen). Dept. of Biology, University of Virginia, Charlottesville, Virginia 22901.

We find that Bulla, like several other opisthobranchs, exhibit a circadian rhythm in locomotor activity. Bulla are nocturnal on light cycles (L:D,12:12) and when placed into darkness, most Bulla exhibit clear free-running locomotor rhythms (τ = 24-25 hr) for at least 1 month. Since the eye of Bulla expresses a circadian rhythm in the frequency of optic nerve impulses (Block & Friesen, this volume), we were interested in determining what role the eye plays in controlling locomotor behavior.

In order to assess ocular control of locomotor rhythmicity, the eyes of 12 Bulla were removed, either during locomotor free-runs in D:D or during entrainment to light cycles. Removal of the eyes during free-runs always resulted in major deterioration of the locomotor rhythm. Nevertheless, in most animals, rhythmicity persisted for several cycles following eye removal, suggesting the presence of an extraocular (highly damped ?) circadian pacemaker. If the eyes were removed while animals were exposed to light cycles, "entrainment" persisted but there was a 180° phase shift in locomotor activity so that eyeless Bulla became diurnal.

We are not yet certain whether diurnal locomotor behavior in eyeless Bulla remains under the control of an endogenous pacemaker or is reflexively driven by the light cycles. However, placing eyeless Bulla into darkness results in 1 - 3 cycles of continued "diurnal" behavior. Thus we cautiously suggest that the switch to diurnal behavior following eye removal involves a change in the entrained phase angle of an extraocular pacemaker. Supported by NS15264.

18.7 DEMONSTRATION AND MANIPULATION OF BILATERALLY DISTRIBUTED CIRCADIAN PACEMAKERS CONTROLLING VISUAL SENSITIVITY IN CRAYFISH. B. Barrera-Mera and Gene D. Block, Depto. de Neurociencias C.I.F.C. U.N.A.M. Mexico, 20 D.F. and Dept. of Biology, University of Virginia, Charlottesville, Virginia 22901.

The eye of crayfish exhibits a clear circadian rhythm of retinal sensitivity as measured by the amplitude of the electroretinogram (ERG)[1]. Furthermore, the ERG rhythms of both eyes remain in phase in constant darkness, suggesting that the two eyes are controlled by tightly coupled pacemakers or by a single circadian pacemaker[2,3]. In spite of the fact that split brain preparations continue to exhibit bilateral circadian rhythms[3], the unambiguous existence of two separate pacemakers has not been reported.

In the current series of experiments the trito and deutocerebrum of the crayfish (Procambarus clarkii) were removed and the protocerebrum bisected. This operation neurally disconnected the two optic lobes from one another. Following recovery from surgery, brief pulses of light (1-3 S, 0.2 Cd/m^2) were delivered to each eye of an otherwise dark-adapted crayfish. Analysis of resultant ERG records revealed a clear circadian rhythm in both eyes which persisted for more than 3 weeks without significant damping. The circadian rhythms in the two eyes were typically in phase with one another but could be desynchronized by: (1) changing the test stimuli frequency to one eye which resulted in a change in the circadian period of the same eye, or (2) providing a long duration (60 minute) light pulse unilaterally. The change in phase angles between the two ocular rhythms in these bisected preparations demonstrates the existence of bilaterally distributed circadian pacemakers which are coupled in the intact crayfish.

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18.8 CIRCADIAN RHYTHM OF ERG B-WAVE IN FREE-MOVING ANOLIS. D. H. Fowlkes, C. J. Karwoski and L. M. Proenza. Vision Research Lab., Univ. of Ga., Athens, GA 30602.

The electroretinogram (ERG) was recorded from the free-moving lizard (Anolis Carolinensis). After a minimum of 3 wk entrainment to the solar light-dark cycle, silver wires (0.2 mm) were attached to the anesthetized (Alcaine) corneal surface of the immobilized lateral eye and under the scalp. Immediately following the implantation, usually performed at 1000 to 1100 h (E.S.T.), the animal was placed within a translucent hemisphere (18 cm dia.) situated on a horizontal white surface. The recording wires passed through a small hole in the top of the hemisphere allowing the animal free movement. The animal was then kept in constant darkness except that the hemisphere was illuminated once every 2 h with a 10 μsec flash (Grass Photostimulator PS2) which was about one log unit above threshold. ERG's were recorded through an AC preamplifier (Grass P15) with half amplitude filters set at 0.1 and 300 Hz.

During the first 24 h of recording (in constant darkness), b-wave amplitude during projected night was low (10-40 μV), began increasing shortly after projected sunrise, and was maximum (50-100 μV) 6-9 h after projected sunrise. Subsequent 24 h periods showed a similar pattern but with an increasing phase delay indicating a free-running rhythm with a period slightly longer than 24 h. The latency of the b-wave did not appear to vary with time of day. Since the latency and amplitude of the a-wave showed no circadian rhythm, the b-wave rhythm may originate from post-receptoral mechanisms.

18.9 ULTRADIAN RHYTHMS IN HAMSTER AND RAT EATING. L. P. Morin, Dept. of Psychology, Dartmouth College, Hanover, N.H. 03755.

Male hamsters and rats were housed under a variety of photoperiod conditions, with and without access to running wheels. An event recorder monitored the timing of eating from a modified tunnel feeder in which powder chow was continuously available. Power spectrum analysis of eating patterns indicates that timing of food intake is regulated, in large part, by endogenous ultradian rhythms in both species. Period length of these rhythms in hamsters varied from about 2.3 to 4.0 hr. In rats, the periods ranged from about 3.0 to 6.5 hr. In both species, two or more statistically significant ultradian rhythms were usually evident simultaneously. A circadian rhythm of eating was detectable in all rats, but only in some hamsters. Attempts to manipulate the periodicity of the hamster ultradian eating rhythms by phase shifting the light cycle, placing animals under a 2.5:2.5 hr light-dark cycle or by chronically exposing them to 24.6 or 23.3 hr days have been unsuccessful. The results raise questions pertinent to homeostasis and the regulation of food intake and to the generation of circadian rhythms.

18.10 DIURNAL RHYTHMS OF THE GUINEA PIG: RELATION OF EVOKED POTENTIALS AND BODY TEMPERATURE. Carl P. Browman, Anne H. Remmes* and Arthur D. Rosen. Departments of Psychiatry and Neurology, State University of New York at Stony Brook, Long Island 11794.

Time-of-day changes in visual evoked potential (VEP) waveform and body temperature were assessed in the guinea pig (Cavia porcellus). Six male animals age 99 days served as subjects. They were tested at 2-hr intervals from 8 am to 8 pm for two consecutive days, with additional single sessions at 8 am and 8 pm on separate days. The ECoG, recorded from the occipital cortex, was sampled at a 2 kHz rate for 250 msec. The first 10 responses within a session were excluded from data analysis. VEP waveforms were the average of 100 responses.

Five VEP components were reliably obtained: N15, P27, N51, P79, and N103. Latency variability across sessions for all subjects was rather constant and was similar to that across subjects. Variability generally increased from short-latency to long-latency components. Amplitude measures, in contrast, were highly subject specific. The degree of intersession amplitude variability was directly related to the particular animal. For the mean data, P27-N51 amplitude correlated with body temperature across time periods ($rho=.848$, $p < .05$). Correlation coefficients between body temperature and the other amplitude measures, as well as all latency measures, were nonsignificant.

A clear rhythm in body temperature is evident in our data. This is independent of most VEP waveform measures which generally did not show a systematic time-of-day change. Only P27-N51 amplitude correlates with body temperature. VEP latency variability is consistent across subjects and sessions, while there is considerable interindividual variability in amplitude measures. We concur with Kayser and Hildwein (1974) that diurnal rhythms of the guinea pig are for the most part difficult to demonstrate.

18.11 CENTRAL VISUAL PATHWAYS AND THE LIGHT-DARK DISTRIBUTION OF SLEEP IN 24-HR AND 1-HR LIGHT-DARK CYCLES. Cheryl L. Sisk and Friedrich K. Stephan. Dept. Psychology, Florida State Univ., Tallahassee, FL 32306.

In rats, about 30% of daily slow wave sleep (SWS) and rapid eye movement (REM) sleep occurs during dark hours in a 12 hr light-12 hr dark cycle (LD 12:12). The occurrence of REM sleep during darkness dramatically increases to 80-90% in rats under very short LD cycles, e.g., LD 0.5:0.5. The proportion of dark-occurring SWS is relatively unaffected by short LD cycles. This experiment investigated the roles of the primary, accessory, and hypothalamic retinal projections in the determination of this differential LD distribution of REM sleep within circadian- and ultradian-length LD cycles. Cortical EEG, neck EMG, and brain temperature were recorded in rats which received either 1) no lesion, 2) primary optic tract lesions anterior to lateral geniculate, 3) superior accessory optic terminal nuclei lesions, 4) inferior accessory optic terminal nucleus lesions, or 5) retino-hypothalamic tract/suprachiasmatic nucleus (RHT/SCN) lesions. For each rat, electrophysiological recordings were made in both a circadian (LD 12:12 or LD 10:14) and an ultradian (LD 0.5:0.5) LD cycle. Records were visually scored for the number of minutes spent in arousal, SWS, or REM sleep during light and dark for each recording day.

Primary and superior accessory optic tract lesions did not change the distribution of sleep and arousal on 24-hr LD cycles. Inferior accessory optic tract ablation increased the nocturnality of REM sleep but not SWS. RHT/SCN lesions abolished the circadian rhythm of sleep-wakefulness and its entrainment to the LD cycle. These results confirm and extend other studies which indicate that the RHT is responsible for the entrainment of circadian rhythms by light.

The dark-time enhancement of REM sleep observed in the intact rats on LD 0.5:0.5 was not abolished by any one of the four visual system lesions, although rats with primary optic tract lesions showed significantly more REM sleep in the light than other groups. Inferior accessory optic lesions accentuated the dark triggering of REM sleep so that virtually no REM sleep occurred during the short light periods. These results suggest that triggering of REM sleep by short dark periods is mediated by some hitherto undescribed retinal projection, or alternatively, can be mediated by several retinal projections.

The characteristic light-dark distributions of REM sleep in circadian and ultradian LD cycles may therefore be under different neural control by the central visual system.

18.12 SCN LESIONS ALTER THE FREE-RUNNING TEMPERATURE AND WAKE RHYTHMS OF RATS. C.I. Eastman, R. Mistlberger* and A. Rechtschaffen*. Univ. of Chicago Sleep Lab, Chicago, IL 60637

There are different multiple oscillator theories about the organization of mammalian circadian systems. In one (Pittendrigh and Daan, J. comp. Physiol. 106: 333-355, 1976), the circadian rhythm of activity is controlled by two oscillators which are normally coupled to sunrise and sunset respectively. In another theory (Aschoff and Wever, Fed. Proc. 35: 2326-2332, 1976), temperature is primarily controlled by one oscillator and activity by another. Moore-Ede et al. (Neurosci. Abstr. 6: 708, 1980) reported that in the squirrel monkey SCN lesions eliminated the rhythm of activity but not temperature; they suggested that the activity oscillator was in the SCN but that the temperature oscillator was elsewhere. The present experiments explored the role of the SCN in the generation of the circadian rhythms of temperature and wake in the rat.

Bilateral lesions of a variety of sizes were aimed at the SCN. Temperature was recorded from a thermistor on the brain surface. Wake and sleep were polygraphically scored by computer. All the data were stored in 30 sec epochs by a PDP-11 computer. So far, 10 SCN lesioned rats have been exposed to constant dim illumination (about 1.5 lux) and continuously recorded for up to four months. The circadian rhythms were assessed, in part, by computer produced "wheel-running style" graphs of both temperature and wake.

All SCN animals had abnormal rhythmic patterns compared to normal and sham lesioned controls. These abnormalities ranged from complete arrhythmicity in both temperature and wake to weak and labile free-running rhythms in both variables. In some cases, although the temperature rhythm was definitely abnormal, it seemed more regular and more robust than the wake rhythm. Some animals became more arrhythmic over months. One animal developed a bimodal pattern in both temperature and wake resembling "splitting", in which two components free-ran with the same frequency but about 180° apart. Histological analyses are in progress to evaluate the relationship between rhythm disruption and size and locus of lesion. The data will be discussed in terms of various multiple oscillator theories.

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18.13 FUNCTIONAL SYMMETRY OF THE SUPRACHIASMATIC NUCLEI. F. C. Davis* and R. A. Gorski. (SPON: L. Mallach). Lab. of Neuroendocrinology of the Brain Research Institute and Department of Anatomy, UCLA School of Medicine, Los Angeles, CA 90024.

Evidence for the control of circadian locomotor activity/rest rhythmicity by two oscillators has been observed in mammals, birds, and reptiles. A pacemaker system of coupled but functionally distinct circadian oscillators has been proposed for rodent wheel-running activity rhythms (Pittendrigh and Daan, J. Comp. Physiol. 106: 333, 1976). We tested the possibility that the bilateral suprachiasmatic nuclei (SCN) are functionally distinct circadian oscillators by measuring circadian rhythm properties in hamsters with right- or left-side unilateral damage to this region known to be critical for normal circadian rhythmicity. Wheel-running activity was recorded from sham-lesioned and lesioned male Golden hamsters during entrainment to a light/dark cycle, during a phase advance of the light/dark cycle and during constant light (350 lux). Comparisons among sham-lesioned, left-side lesioned, and right-side lesioned animals were made for the following parameters: 1) time of activity onset relative to lights off during entrainment; 2) rate of phase-shift following advance of the light/dark cycle; and 3) circadian period during constant light. The occurrence of "splitting" (two activity components with different circadian periods) was also noted. Since it is difficult to lesion the SCN unilaterally, the volume of intact SCN tissue was measured for the right and left sides of each animal. A lesion was considered unilateral if the volume of the SCN on that side was less than 35% of that of sham-lesioned rats, while the volume of the SCN on the other side was 50-100% of that of the controls. Moreover, SCN volume on the lesioned side had to be 50% or less of that on the intact side. These animals were also required to show entrainment. Right-side (N=6) and left-side (N=6) lesioned animals were not significantly different in any of the circadian rhythm properties measured. Furthermore, the lesioned animals did not fall into two obviously functionally distinct groups which would suggest functional assymmetry independent of right- or left-sidedness. The separate circadian oscillators evident in splitting do not appear to represent the two halves of the SCN; three unilaterally lesioned animals showed splitting. The circadian period of lesioned animals was shorter than that of the 4 shams (23.99 vs. 24.49, $P < .01$). Incorporating the data from all animals of the study (N=28), we found a positive correlation between size of the SCN and circadian period ($R=.723$). It is concluded that the right and left sides of the SCN do not represent functionally distinct circadian oscillators. Supported by NRSA 1 F32 HD05916 to F.C.D. and NIH Grant HD-01182 to R.A.G.

18.14 CONTRAVARYING CIRCADIAN RHYTHMS OF EPSP AND POPULATION SPIKE IN HIPPOCAMPAL DENTATE GYRUS AND THEIR RELATION TO STATE. L.J. Cauller*, Z. Boulos and G.V. Goddard. Psychology Dept., Dalhousie Univ., Halifax, Nova Scotia, Canada B3H 4J1.

Field potentials evoked at 15 min intervals by single biphasic electrical pulses to the perforant path were recorded over several days from the hilus of the dentate gyrus in chronically implanted freely-moving rats. In agreement with Barnes et al. (Science, 197:91, 1977) the slope of the field EPSP was greatest during the dark phase of daily light-dark (LD 12:12 or 14:10). The height of the population spike contravaried with the EPSP rhythm such that a given EPSP value was associated with a larger spike during the light than during the dark. This agrees with the observation by West and Deadwyler (Neurosci., 5:1597, 1980) that the granule cells are more excitable during the light. The contravarying circadian rhythms followed phase shifts of the LD cycle and persisted under constant light (LL) with free-running periods greater than 24 h for up to 14 days.

We recorded on videotape the behavior of rats kept in LL and collected cortical and hippocampal EEGs to determine the state of the rats at the time of stimulation. The mean EPSP slope was greater during SA (still alert) than during SWS (slow wave sleep) but their was no difference in the size of the population spike between these states. Since the greater EPSP input produced an equivalent spike output, we agree with the conclusion by Winson and Abzug (J. Neurophysiol., 41:716, 1978) that the granule cells are less excitable during SA than during SWS.

The potentials evoked during either SWS or SA had smaller EPSPs and larger population spikes at the time of the light phase of the prior LD cycle than at the time of the dark. Thus the circadian rhythms remained evident when the data were separated by state. The AA (awake active) state occurred almost entirely at times when the light was normally off and was associated with with large EPSPs and small spikes, whereas REM (paradoxical sleep) occurred almost entirely at times when the light was normally on and was associated with small EPSPs and large spikes. Too few examples were observed at the opposite circadian phase to demonstrate L-D differences within either of these states.

When EPSPs were evoked by stimulus intensities below spike threshold following arousal to SA, they had greater peak amplitude, shorter peak latency, faster rise time and narrower width at half amplitude in the dark phase than in the light.

18.15 CIRCADIAN VARIATION IN RAT BRAIN REGIONAL NEUROCHEMISTRY. S.G. Speciale. Dept. of Psychiatry, Univ. of Texas Health Science Center, Dallas, Texas 75235.

Circadian or 24-hour rhythms have been demonstrated for a wide variety of physiological parameters, including body temperature, motor activity, hormone secretion and several CNS neurotransmitters, including indole- and catecholamines. It was of interest therefore to measure some additional transmitter markers in brain regions of rats synchronized to a lighting schedule.

Male Sprague-Dawley rats were adapted to an automatic illumination cycle (0600-1800 hours light; 1800-0600 hours dark) for three weeks prior to use. Rectal temperature measurements verified entrainment (maximal during dark, minimal during light phase). Animals (6-8 per time point) were sacrificed by decapitation at 0600, 1000, 1500, 2000 and 2400 hours, the brains removed rapidly and dissected into hypothalamus (hpth), caudate nucleus (cn), midbrain (mb), brain stem (bs) and hippocampus (hip), then frozen on dry ice. Trunk blood was also collected for plasma hormone assay.

For the initial phase of these studies the activity of glutamic acid decarboxylase (GAD) and choline acetyltransferase (ChAT), synthetic enzymes and neuronal markers for GABA and acetylcholine, respectively, were measured in these tissues using the radiometric assays of Fonnum et al. (Biochem. J. 115:465, 1969; Brain Res. 20:259, 1970). GAD activity was highest in the hpth, intermediate in hip and cn, while mb and bs were one-tenth the hpth activity. ChAT activity was highest in cn, intermediate in hip, hpth and bs, while minimal in mb.

Enzyme activities in a number of regions varied significantly with the time of day (the majority at $P < 0.005$ level or greater). GAD in hip was elevated 63% at its maximum (2000 hours) over the minimum at 0600 hours. mb GAD was relatively constant through the day, except for a large increase at 2400 hours. bs GAD was similar to mb but the elevation at 2400 hours was not as marked. cn and hpth GAD were bimodal; peaks occur at 1500 and 2400 hours in cn, while in hpth there are peaks at 1000 and 2400 hours.

mb and bs ChAT were relatively constant across the 24 hours except for significant increases at 2400 hours. cn ChAT was also constant except for a reduced activity at 0600 hours, differing significantly from values at 1500 and 2000 hours. hpth ChAT was bimodal, with maxima at 1500 and 2400 hours and minima at 0600 and 2000 hours. hip ChAT was without significant variation through the 24 hours.

These results will be correlated with those for other transmitter systems in these regions and circulating hormone levels.

(Research supported by NIH grant EY033257).

18.16 DIURNAL AND PHOTOPERIOD-INDUCED VARIATION IN MUSCARINIC RECEPTOR CONCENTRATION IN SOME REGIONS OF THE RAT BRAIN. H.T. Tsui, S.H. Losee-Olson, F.W. Turek and W.L. Klein. Graduate Program in Neuroscience, Northwestern University, Evanston IL 60201

Cholinergic stimulation induces a 75 % loss of muscarinic receptors in cultured CNS cells(Siman,R.G. and Klein,W.L.,PNAS 76,4141-4145). Since Saito (Life Science 16,281-288) reported a circadian fluctuation of acetycholine levels in the telencephalon and pons plus medulla oblongata, but not in the mesencephalon plus diencephalon, we have tested for diurnal variation of muscarinic receptor concentrations in discrete regions of the rat brain. We also have tested the influence of photoperiod on hamster brain receptor levels and have investigated whether adding cholinomemetics to brain slices alters muscarinic receptor levels.

Rats maintained on LD 12:12 were killed at 4 hr intervals over a 24 hr period. The following areas were used: the suprachiasmatic nucleus, preoptic nucleus, the rest of the anterior hypothalamus, caudate putamen,amygdala, cortex and the brainstem. Of all areas examined,diurnal variations in receptor concentration were observed only in the caudate and the brainstem. The receptor level in caudate at midday was 33 % higher than the lowest of the other time points ($p<.01$,n =4). Receptor level in the brainstem peaked 2 h before lights on (32 % higher than the lowest, $p<.025$,n=4 to6) and stayed 23 % higher than the lowest concentration for the next 8 hrs. Receptor levels in various brain regions were also determined in hamsters maintained on LD 6:18 and 14:10 and killed at midpoint of the day. Hamsters kept on long days had significantly higher receptor levels in the cortex, and in the anterior and posterior hypothalamus than the short day hamsters (20-36 % difference, n=5, $p< 05$). No differences were seen in the caudate and the brainstem.

To determine if cholinergic activation of brain slices induces a loss of receptor, 300 um sections of brainstem were put on filter paper barely covered with oxygenated medium. The slices were incubated at 37°C in an oxygenated chamber within 15 min. of the animal's death. During a 6 hr incubation, with oxotremerine at 10^{-3} to 10^{-6}M, or without oxotremerine, receptor binding assays indicated no significant changes in receptor levels.

These results suggest that diurnal variation in muscarinic receptor concentration may exist in some regions of the brain where diurnal variation of acetycholine was previously observed and that muscarinic receptors in other regions may be affected by photoperiod. Presently we have no direct evidence that this variation results from changes in cholinergic stimulation. (supported by NIH grant NS-15299 to WLK and HD-12622 to FWT)

18.17 PRACTICE OF THE TRANSCENDENTAL MEDITATION (TM) AND TM-SIDHI PROGRAM MAY AFFECT THE CIRCADIAN RHYTHM OF URINARY 5-HYDROXYINDOLE EXCRETION. K.G. Walton, M. Lerom*, J. Salerno* and R.K. Wallace. Biochemistry Research Laboratory, Department of Biology, Maharishi International University, Fairfield, Iowa 52556.

A rise in the urinary excretion of 5-hydroxyindoleacetic acid (5-HIAA), the main metabolite of serotonin, during or immediately after an afternoon practice session of the TM technique has been reported[1]. Similar results were obtained with total urinary 5-hydroxyindoles (5-HI) in subjects who practiced the more advanced TM and TM-Sidhi program[2]. However, in the latter experiments little or no rise of 5-HI excretion was associated with a morning (0800 to 0900) session in averaged data from 11 subjects. New data to be presented come from experiments in which the total urine was collected in 8 consecutive 3-hour periods per day for 3 days or longer. All subjects were practicing the TM and TM-Sidhi program regularly. The 5-HI excretion rate (calculated as mg/h for each 3 h period) from a subject who was studied for 14 consecutive days exhibited 27 maxima. Twenty-six of these peak periods either included (18 peaks) or came immediately after (8 peaks) practice sessions of the TM and TM-Sidhi program. The magnitudes of the two peaks present each day were consistently unequal. The afternoon maxima were 3 to 10 times larger in 11 of the 14 days, whereas the reverse was true of the other 3 days. Thus, at least in this subject, whose pattern was unusually regular, there was a clear rise of 5-HI excretion associated with the morning as well as the afternoon session.

The significance of a correlation between high 5-HI excretion and practice sessions of the TM and TM-Sidhi program is not known. However, there is the possibility that such a program might serve as a zeitgeber for CNS or peripheral rhythms in serotonergic activity. The amplitudes of circadian rhythms in brain levels of serotonin and 5-HIAA are quite large in rodents, but there is no data which would indicate whether these brain rhythms correlate with urinary excretion of 5-HI. Previous studies in man have suggested a circadian rhythm in 5-HIAA excretion, although detailed studies within subjects should give a clearer picture. A change in peripheral serotonergic activity might be involved in the redistribution of blood flow seen with the TM technique[3]. On the other hand, among the physiological effects reportedly due to practice of the TM and TM-Sidhi techniques, increased phase coherence of the EEG[4] and altered hypothalamic-pituitary-adrenal function[5] might both be due to altered CNS serotonergic activity.

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18.18 TRANSCUTANEOUS NERVE STIMULATION (TNS) EVOKES FREQUENCY AND WAVEFORM DEPENDENT SUBJECTIVE SENSATIONS IN HUMANS. J.J. Katims* and L.K.Y. Ng. Division of Research, National Institute on Drug Abuse, Rockville, Maryland 20852

TNS treatment for relief of pain is now widely accepted in medical practice. Less well accepted are its applications transcranially for insomnia, anxiety, depression and drug withdrawal. As yet, little is known about its possible mechanisms of action.

Humoral changes associated with transcranial TNS in humans include alterations in catecholamines, thyroxine, endogenous opiate-like substances and gastric acid secretion. Recent evidence suggests that frequency of the electrical stimulus may be an important parameter in its therapeutic effect.

Twenty healthy volunteer subjects participated in a single blind investigation of the influence of various frequencies and waveforms of TNS on evoked subjective sensations. TNS was administered bilaterally anterior to the ear tragi at various frequencies (5-10,000 Hz) using either a sinusoid, biphasic square, or 0.2 msec biphasic rectangular pulse waveform. Current intensities were used which evoked a mild tingling sensation at the electrode sites approximately 0.25 mA peak to peak. With the sinusoid waveform stimulation the subjects reported 3 distinct frequency dependent sensations including: oscillations in visual field perception (without apparent movement of the retina, 5-19 Hz), a flickering illumination of the visual field (12-80 Hz), and a unique resonance sensation within the head (60-573 Hz). These same sensations also were reported with ipsilateral stimulation. Using a biphasic square waveform of stimulation the subjects reported all three sensations as occurring concomitantly (5-573 Hz) without resolution of frequency dependent channels. The 0.2 msec biphasic rectangular pulse waveform stimulation evoked no oscillatory, flickering or resonance sensations in 60% of subjects tested and the remainder reported only a slight cranial resonance sensation. The sensations described above do not resemble cochlear electrophonics or phosphene effects associated with TNS around the ears and eyes.

The findings from this study support the hypothesis that the neuronal systems which mediate subjective sensations possess frequency sensitive channels capable of waveform discrimination. This specificity may allow for the nonpharmacologic stimulation of discrete neuronal populations within the brain, a psychophysiological specificity which may have important diagnostic and therapeutic applications for the medical practitioner and neuroscientist.

19.1 ANALGESIC EFFECTS OF MORPHINE MICROINJECTIONS IN THE AMYGDALA OF THE RAT. C. G. Lineberry. Dept. Physiol., Sch. of Med. and Dent. Res. Center, Univ. N. Carolina, Chapel Hill, N.C. 27514.

Twenty-one rats were implanted for injection of drug solutions via 33 g cannulas placed in the amygdaloid nuclear complex. Analgesia was assessed by noting thresholds for reflex tail movement and vocalization elicited by electrical tail stimulation.

Ten rats were bilaterally injected with 0.5 µl solutions of saline, or 5, 10 or 20 µg morphine sulfate. Morphine produced dose-related increases in the vocalization threshold that averaged approximately 200% of control after 10 µg morphine.

Twenty sites in the amygdaloid nuclear complex were investigated in 11 additional rats using unilateral, 0.5 µl injections of 5 or 10 µg morphine, or saline. Ten of the twenty sites produced elevations in vocalization thresholds equal to or exceeding 150% of saline control values. Reflex tail movement thresholds were unaffected. The increase in mean vocalization threshold for active sites was 146±20% of saline control values after 5 µg, and 242±21% after 10 µg morphine. When 10 µg morphine was preceeded by s.c. naloxone (1 mg/kg), the mean vocalization threshold was 160±27% of saline control values (n = 9 sites). Viewed individually by morphine injection site, subcutaneous naloxone completely reversed the effects of morphine injections in 5 sites, partially reversed the effects in 3 sites, and was ineffective in one site. The effects of the 10 µg morphine microinjections were apparent within minutes after injection, reached peak values at 30 and 60 minutes, and declined by 50% by 180 minutes. These effects were comparable in magnitude and duration to those obtained with 4 to 8 mg/kg systemic morphine.

The results of these studies demonstrate dose-related, naloxone-reversible analgesic effects of unilateral or bilateral microinjections of morphine into the amygdaloid nuclear complex of the rat, in spite of numerous published reports that have failed to demonstrate such effects. In the only published report of analgesic effects produced by microinjection of morphine into the amygdala (Rogers, R.J., Pharmacol. Biochem. Behav., 6:385-390, 1977) only bilateral injections were effective in producing even a weak analgesic response as measured by the flinch-jump procedure. The analgesic effects of the morphine microinjections were confined to vocalization thresholds, indicating that the affective-emotional, rather than the sensory-reflexive dimension of pain is modulated by neural systems containing amygdaloid opiate receptors.

19.2 ENHANCEMENT OF MORPHINE ANALGESIA BY ENVIRONMENTAL CUES SIGNALLING PAIN. J. E. Sherman*, J. W. Lewis, R. E. DeWetter*, H. Strub* and J. C. Liebeskind (SPON: F. Krasne). Depts. of Psychology and Anesthesiology, UCLA, Los Angeles, CA 90024.

Central pain inhibitory mechanisms activated by direct electrical stimulation and by opiate drugs are now known to be activated also by noxious unconditioned (US) and conditioned (CS) stimuli. We have shown (Sherman et al., 1981) that opiate analgesia is enhanced after repeated noxious stimulation and suggested this to be partly attributable to conditioned fear. If so, this enhancement should only or best be seen in the same test environment (CS) in which noxious stimuli (US) were originally presented.

To test this hypothesis two groups of rats received 18 days of discrimination training in which one environment was paired with 45 sec of inescapable footshock (for shock sessions 1-7, .8 mA; sessions 8-9, 1.0mA) and the other was safe. For Group RM, shock was administered in a distinctive room, and handling without shock was conducted in the homecage environment. For Group HC, these environmental conditions were reversed. Exposure to the environments was alternated daily and was counterbalanced across groups. On the 19th day, all rats were transported to the distinctive room where they received morphine (5 mg/kg, s.c.). Pain sensitivity was assessed with the hot-plate test beginning 30 min after morphine.

RM rats displayed significantly greater morphine analgesia than HC rats ($p < .05$). Subsequent experiments revealed that this conditioning procedure itself caused significant analgesia that was unaffected by naltrexone (7 mg/kg, s.c.).

Thus the expectation of pain, i.e., conditioned fear, both diminishes responsiveness to painful stimulation and enhances opiate analgesia by activating nonopioid mechanisms of pain inhibition. (Supported by NIH grant #NS07628.)

19.3 CHANGES IN SENSITIVITY TO APOMORPHINE FOLLOWING REPEATED MORPHINE INJECTIONS ARE SPECIFICALLY RELATED TO THE PLACE WHERE MORPHINE IS EXPERIENCED. J. Stewart. Dept. of Psychology, Concordia University, Montreal, Canada, H3G 1M8.

An experiment was done to determine whether changes observed in the activity of the dopamine systems after repeated exposure to morphine are, at least in part, conditioned to environmental cues associated with morphine injections.

Activity was measured for 90 min immediately following a daily injection in two groups of rats, and sometime before a daily injection in two other groups of rats. In each condition, some animals received, intraperitoneally, 20 mg/kg morphine sulphate as the daily injection and others received saline. After at least two weeks of morphine administration, test days were run in which all animals were injected, intraperitoneally, with either saline or with 0.5 mg/kg apomorphine HCl. Following a morphine-free period of two to three weeks when all animals remained in their home cages, the same tests were repeated.

The results showed that animals that normally received morphine while in the activity boxes had significantly higher activity levels in response to apomorphine than did their saline control animals. Animals that received equivalent daily doses of morphine, but never associated with the activity boxes, responded to apomorphine as did their saline control animals. These effects were observed both during the period of daily morphine injections and following the morphine-free period. It appears that the changes that resulted in greater responsivitity to apomorphine were conditioned to, and thus were elicited by the environment that was associated with the morphine injections. The fact that these conditioned changes were elicitable several weeks after the last morphine injection are of considerable interest and parallel conditioned changes in body temperature previously reported by us (Eikelboom, R. & Stewart, J. Psychopharmacology, 61, 3, 1979, and 72, 147, 1981. (Supported by a grant from the Medical Research Council of Canada, MA 6678).

19.4 PINCH INDUCED CATALEPSY: A NATURAL DEFENSIVE RESPONSE INVOLVING BLOCKADE OF CENTRAL DOPAMINE SYSTEMS BY ENDOGENOUSLY RELEASED OPIOIDS. S. Amir and K. Ornstein*. Department of Isotope Research, The Weizmann Institute of Science, Rehovot, Israel.

Repeated pinching to the scruff of the neck in mice evokes a potent catalepsy, i.e. imposed postures, except inversion in a supine position, are retained for an exceedingly long time (Bouts of catalepsy lasting up to 20 min were observed). Moreover, concomitantly to this pinch-induced catalepsy (PIC) there is a profound analgesia (S. Amir et al., Life Sci. 28: 1189-1194, 1981); not only that the response latencies on a hot plate (53°C) and in tail-flick testing are delayed, but painful leg and tail-pinches potentiate catalepsy rather than inducing the normal pain responses. The coincidence of catalepsy and analgesia suggests that PIC is mediated by opioid-dopamine interactions.

Pretreatment with low doses of morphine (0.1-10.0 mg/kg) or with the dopamine antagonist haloperidol (0.1-1.0 mg/kg) markedly enhances PIC without inducing catalepsy in unpinched controls. In contrast, both the narcotic antagonist naloxone (0.1-1.0 mg/kg) and the dopamine agonist apomorphine (1.0-10 mg/kg) blocked the development of PIC. Naloxone did not reverse ongoing PIC (after the response had been established to a duration of >90 sec) but it readily abolished the analgesia. Apomorphine reversed PIC in all doses tested without affecting analgesia. These findings indicate that the development of PIC is mediated by endogenous opioids. Additionally, there is indication that the maintenance of PIC is mediated by blockade of dopaminergic transmission, which is independent of opioid activity subserving analgesia.

Recently, we reported that a catalepsy resembling PIC is elicited in mice attacked by a cat (K. Ornstein and S. Amir, JCPP, in press). This suggests that PIC may be a biologically meaningful defensive behavior, which is instrumental in reducing the frequency of attacks in predator/prey confrontations.

Only the concomitant occurrence of catalespy and analgesia enables an attacked mouse to withhold pain-induced struggling and escape attempts, which would reverse the advantages of catalepsy. This conicidence of analgesia and catalepsy is warranted by the above described opioid-dopamine interaction and can be split in its components by naloxone or apomorphine.

19.5 DOPAMINERGIC MEDIATION OF OPIATE REWARD. M.A. Bozarth and R.A. Wise. Center for Research on Drug Dependence, Department of Psychology, Concordia University, Montreal, P.Q. H3G 1M8 CANADA.

The rewarding properties of opiates appear to be mediated by a drug action in the ventral tegmental area (VTA). This notion has been suggested by studies showing that rats will self-administer morphine directly into this brain area, but not into other brain regions containing opiate receptors (Bozarth & Wise, Soc. Neurosci. Abst. 6:309, 1980). Since dopaminergic (DA) cells in the VTA have been implicated in reward from a variety of sources, it is of special interest to examine the involvement of DA in opiate reward.

The conditioned place preference (CPP) paradigm offers a useful method of assessing the affective properties of a drug. Rats that have experienced the rewarding effects of a drug associated with a specific environment will, when given the opportunity, return to the place where they experienced these effects (Rossi & Reid, Physiol. Psych. 4:269, 1976). In the present studies, CPP was measured using a shuttle box with a plywood floor on one side and a plywood floor covered with wire mesh on the other. The amount of time spent on each side of the box was automatically recorded. Rats were allowed access to the entire shuttle box for 15 min on 5 consecutive days. Next, they received 4 daily injections while being forced to remain on the non-preferred side for 30 min. Following the 4 days of conditioning, the rats were injected with vehicle and tested again for their place preference. Increases in the amount of time spent on the side of putative conditioning were interpreted as an indication of drug-induced reward. Rats subcutaneously injected with heroin (0.5 mg/kg) during the conditioning trials showed a preference for the side of the box where they experienced the drug effect. This CPP was blocked by pretreatment with either naloxone (3.0 mg/kg) or pimozide (0.5 mg/kg) suggesting that this is an opiate receptor mediated effect, and that it is dependent on a DA mechanism.

In another experiment, rats were unilaterally injected with morphine through chronically indwelling 22 gauge guide cannulae implanted in the VTA. A 2.5 µg dose of morphine sulfate was infused over 28 sec in 0.5 µl of Ringer's solution during the conditioning trials. Rats with cannula placements proximal to the DA cell bodies of the VTA showed a strong CPP while rats injected with morphine at sites caudal to this area did not. These results, combined with an earlier report showing CPP with VTA injections, but not with placements more dorsal (Phillips & LePiane, Pharmac. Biochem. Behav. 12:965, 1980), suggest that DA cells in the VTA are important in mediating the rewarding properties of opiates.

(Supported by grant DA 02285 from NIDA.)

19.6 A COMPARISON OF THE STIMULUS-EFFECT CURVES OF MORPHINE FOR ANALGESIA AND INHIBITION OF GASTROINTESTINAL TRANSIT IN RATS. R. B. Raffa*, F. Porreca*, R. B. Murray, A. Cowan and R. J. Tallarida*. Department of Pharmacology, Temple University School of Medicine, Philadelphia, PA 19140.

According to Stephenson's modification of classical drug-receptor theory (Br. J. Pharmacol. 11:379, 1956), the sequence of events following the administration of an active drug (leading to an effect) can be divided into separate drug-dependent and drug-independent portions. The drug-dependent portion is the reaction between drug and receptor leading to a drug-receptor complex and is characterized by an apparent dissociation constant (K_A). The complex provides a stimulus (S) which, in turn, induces the effect (E). The relation between the stimulus and fractional receptor occupancy is assumed to be linear, whereas the relation between the magnitude of the stimulus and the magnitude of the effect is an unknown function, presumed to be independent of the drug. In this study, we examined two effects of morphine, one mediated centrally (analgesia) and one with both central and peripheral components (inhibition of gastrointestinal transit). Male Sprague-Dawley rats (180-220 g) were given s.c saline or morphine sulfate (0.3 to 30 mg/kg) 30 min prior to testing in 57°C hot-water tail flick and the charcoal meal tests (Green, Br. J. Pharmacol. 14:26,1959). Other rats were predosed with s.c. buprenorphine hydrochloride (0.3 mg/kg) or vehicle 30 min prior to morphine (3 to 80 mg/kg). The latter rats were tested 60 min after buprenorphine, a time at which buprenorphine's own agonist actions are minimal and partial irreversible blockade of morphine is produced (Tallarida and Cowan, Fed. Proc. 39:759, 1980). Both of these conditions are required in the calculation of K_A for agonists using the procedure of Furchgott and Bursztyn (Ann. N.Y. Acad. Sci. 144:882, 1967). The K_A for morphine was 2.9×10^{-5} M for analgesia and 1.1×10^{-5} M for inhibition of gastrointestinal transit. The stimulus-effect curves were found to have different shapes. In a plot of per cent maximum effect against stimulus, the curve corresponding to inhibition of gastrointestinal transit is roughly hyperbolic and passes through the constrained point S=1 at 50% effect. This same shape was previously observed in another smooth muscle preparation, namely, rabbit aorta (Raffa et al., Arch. int. Pharmacodyn. 241:197, 1979). On the other hand, the curve corresponding to analgesia is steeper, intersecting the first curve at S=1. These differences in shape support the applicability of Stephenson's theory to in vivo opiate action by reflecting the general differences in the effector mechanisms underlying endpoints mediated through the CNS and smooth muscle.

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19.7 PHARMACOLOGIC CHARACTERISTICS OF MORPHINE (M) AND ETHYLKETOCYCLAZOCINE (EK) FOR ANALGESIA AND INHIBITION OF GASTROINTESTINAL TRANSIT (IGIT) IN RATS. F. Porreca*, R.B. Raffa*, R.J. Sheldon*, A. Cowan and R.J. Tallarida*. Dept. Pharmacol., Temple University School of Medicine, Philadelphia, PA 19140.

The interaction of an agonist with its pharmacologic receptor is characterized by a dissociation constant, K_A (1/affinity), and an A_{50}. The determination of K_A requires the use of an irreversible antagonist, a compound generally unavailable for opiates. Recently, Tallarida and Cowan have reported that under certain conditions, buprenorphine (B) seems to act as an irreversible antagonist of M for analgesia (Fed. Proc. 39:759, 1980). These observations have been extended to a second effect of M (IGIT) and a second compound (EK) for analgesia and IGIT.

Analgesia was measured 30 min after s.c. injection of M sulfate or EK methanesulfonate to groups of 8-12 male, S.D. rats (180-220 g) using tail compression and hot water (57° C) tail flick tests; IGIT was determined 20 min after a forced charcoal meal and 30 min following M or EK. When B was used as an antagonist, rats received B hydrochloride (0.3 mg/kg, s.c.) or vehicle 30 min prior to graded doses of s.c. M or EK. Testing for analgesia (tail flick) and IGIT took place 60 min after B, a time when B's agonist action is minimal in both endpoints. The dose-response curves of M and EK were shifted to the right with a decreased maximum after B allowing calculation of K_A's (method of Furchgott and Bursztyn, Ann. N.Y. Acad. Sci. 144:882, 1967). A_{50}'s (mg/kg) of M and EK were:

Compound	IGIT	Thermal	Pressure	Thermal/Pressure ratio
M	1.4	3.7	6.2	0.60
EK	2.7	0.4	0.6	0.67

Compound	IGIT(K_A)	Tail flick(K_A)
M	1.1×10^{-5} M	2.9×10^{-5} M
EK	7.5×10^{-5} M	0.28×10^{-5} M

K_A for EK (not previously reported) has been determined using two endpoints. It should be noted that the A_{50} does not equal K_A. The ratio of A_{50}'s of M and EK is approximately equal to 10 for both thermal and pressure tests as is the ratio of K_A's of M and EK for analgesia. The A_{50}'s do not support the contention that mu and kappa agonists may be differentiated on the sole basis of thermal/pressure ratios. Finally, since B is thought to act on the mu receptor, its ability to antagonize EK at this dose along with our previously reported identical naloxone pA_2 values against M and EK (8.1) indicate that M and EK may act at the same receptor to produce analgesia. (DA 02322 from NIDA)

19.8 THE EFFECTS OF INTRAVENOUS MORPHINE SELF-ADMINISTRATION UPON CONCURRENT FOOD AND WATER REINFORCED RESPONDING. N.E. Goeders, M.P. Sands,* D.R. Cherek, J.D. Lane, and J.E. Smith. Psychiatry Research Unit, Department of Psychiatry, Louisiana State University Medical Center, Shreveport, LA 71130.

This study was initiated to evaluate the effects of morphine self-administration on concurrent responding for food and water reinforcement. Male Fisher F-344 rats were trained to lever press for food and water presentation on concurrent FR10 schedules available on non-reversible option. Presentation of each reinforcer was contingent upon responding on the appropriate retractable lever. The first response on a lever resulted in the other levers being withdrawn, their stimulus lights extinguished, and a 120 sec limited hold initiated. After the completion of a fixed-ratio and presentation of a reinforcement or the limited hold elapsed, the lever was retracted and its stimulus light extinguished for a 30 sec time out. The levers were once again extended and the stimulus lights illuminated following the 30 sec time out. Once stable baselines of food and water responding were obtained, the rats were implanted with chronic jugular catheters and made physically dependent on morphine with hourly automatic infusions. All three levers were then re-extended, and the animals allowed to respond for either food, water or morphine reinforcement with 24 hr access. Morphine self-administration was maintained throughout both of the reversed 12 hr light, 12 hr dark daily cycles, and this in turn disrupted the animals' normal patterns of responding for food. Prior to morphine self-administration, patterns of responding for food and water tended to accompany the daily light and dark cycles, with over 75% of the food and water presentations occurring during the dark cycle. After morphine self-administration was initiated, periods of responding for greater than 20 food pellet presentations in rapid succession tended to decrease, while episodes of lever pressing for no more than 5 food pellets with less than a 5 min pause between presentations increased. Responding for food reinforcement became interspersed throughout each daily 24 hr period, with at least 40% of the food presentations occurring during the light (inactive) cycle. There was no significant difference from baseline values in the patterns of responding for water. Lesions produced by 6-hydroxydopamine and kainic acid in the nucleus accumbens are presently being studied to determine if food, water and morphine reinforcement processes are mediated through a common neuronal pathway. (Supported in part by USPHS Grants DA-01999 and MH-31835)

19.9 MORPHINE-INDUCED WATER CONSUMPTION. J. M. Stapleton, A. J. Castiglioni, F. Bermudez-Rattoni* and J. C. Liebeskind. Dept. Psychol., UCLA, Los Angeles, CA 90024.

Although opiates are thought to have primarily depressant effects, there is evidence that for certain measures, (locomotion, body temperature, and self-stimulation), their effects are biphasic in both dose and time. Soon after a moderately large dose of morphine, for example, depressant effects are seen, followed by delayed facilitation. Small, even subanalgesic doses can produce facilitation without prior depression. The opiate antagonist, naloxone, given alone, produces decreases in these measures. It is well established that naloxone also reduces feeding and drinking in rats, but there have been few reports of morphine-induced increases in appetitive behaviors. We have confirmed and extended Maickel et al.'s (Neuropharm., 1977) report of dose-dependent increases in fluid consumption induced by morphine (Stapleton, Castiglioni & Liebeskind, Proc. West. Pharm. Soc., in press). This presentation will summarize a series of experiments studying morphine-induced water consumption.

Morphine-induced water consumption does not show tolerance with repeated injections and can be reversed by naloxone. The time course of the effect is more similar to the time course for morphine-induced hypermotility than to the time course for analgesia or hyperthermia. Preliminary data suggest that the effect may be observed following microinjection into the lateral ventricle or lateral hypothalamus but not into frontal cortex.

It has been suggested that excitatory effects of morphine may be due to acute withdrawal effects, a rebound from the decreases seen soon after the injection. To test this hypothesis, rats were given morphine (10 mg/kg) or saline immediately before a 6 h test and later given naloxone (1 mg/kg) or saline 4 h after morphine. We reasoned that, if the morphine-induced increases in water consumption were due to withdrawal effects, they should not be blocked by naloxone given at this time, and might even be exacerbated. The results indicated that even given 4 h after morphine, naloxone blocked the facilitation of water consumption.

Current studies are seeking to uncover the mechanism involved in morphine-induced water consumption, including possible hormonal involvement. Morphine facilitation of water consumption and other excitatory effects of morphine, such as hyperthermia and hypermotility, may represent different behavioral manifestations of a common underlying neural process. These excitatory effects may be as important as the better known depressant effects in understanding the role of endogenous opioid systems in behavior. (Supported by NIH grant #NS07628.)

19.10 THE EFFECTS OF KAINIC ACID LESIONS OF THE NUCLEUS ACCUMBENS ON INTRAVENOUS MORPHINE SELF-ADMINISTRATION. J.E. Smith, C. Co*, C.M. Crenshaw*, T.S. Barr* and J.D. Lane. Psychiatry Research Unit, Department of Psychiatry, Louisiana State University Medical Center, Shreveport, LA 71130.

The mesolimbic dopaminergic system has been implicated in stimulant and opiate reinforcement processes. 6-hydroxydopamine lesions of the nucleus accumbens attentuate amphetamine (Lyness, et al, Pharmac. Biochem. Behav. 11:553, 1979) and cocaine (Roberts, et al, Pharmac. Biochem. Behav. 12:781, 1980) self-administration. Rats intracranially self-administer morphine into the nucleus accumbens (Olds, Neurosci. Abst. 5:535, 1979) and ventral tegmental area (Bozarth and Wise, Life Sci. 28:551, 1981). This experiment was initiated to determine the role of the nucleus accumben's neurons and brain stem feedback pathways in opiate reinforcement processes. Four pairs of adult male F-344 littermates were implanted with bilateral 23-gauge guide cannulae into the nucleus accumbens (A9.5,V-5.5,L ±1.2 - König and Klippel) and with chronic jugular catheters. Each pair of littermates was made physically dependent on morphine and then allowed to intravenously self-administer the drug (10 mg/kg, delivered over 5 sec with 24-hr access) on an FR10 schedule. After stable baselines of self-administration were obtained, each pair was lightly anesthetized with intravenous thiopental and one littermate bilaterally injected with 2 µg kainic acid in 1 µl into each nucleus accumbens while the other littermate received equivalent bilateral injections of the vehicle (saline). The animals were given two days to recover from the treatments, receiving automatic injections of morphine equivalent to their pre-lesion baseline intake and then again allowed to self-administer morphine. On the 14th day post-lesion, the rats were sacrificed by decapitation and the nucleus accumbens rapidly removed and homogenized. The content of dopamine (DA), norepinephrine (NE), serotonin (5-HT), aspartate (Asp), glutamate and gamma-aminobutyric acid (GABA) were concurrently measured and the high affinity uptake of choline (Ch), GABA, DA, and 5-HT determined. Kainic acid lesions resulted in significant increases in morphine intake [9.5 ± 3.0 to 21.8 ± 4.2 injections per 24 hours (values are mean ± S.D.)] while injections of vehicle had no effect (8.2 ± 2.1 to 11.6 ± 0.6). GABA and Glu content was decreased while DA increased. High affinity uptake of Ch was also decreased. The significant increase in morphine intake as a result of the lesion indicate that neurons involved in opiate reinforcement processes have been adversely affected, resulting in a compensatory increased intake to overcome this deficit. (Supported in part by USPHS Grant DA-01999-04).

19.11 ANALGESIA PRODUCED BY KINDLED SEIZURES IN RATS. D. W. Berman*, S. Caldecott-Hazard, Y. Shavit and J. C. Liebeskind (SPON: M. Demetrescu). Dept. of Psychology, UCLA, Los Angeles, CA 90024.

The release of opioids by fully kindled seizures has been suggested by naloxone's reversal and morphine's prolongation of post-ictal depression (Frenk et al., 1979). Additional evidence for opioid release comes from the additive effect of morphine or an enkephalin analogue on the inhibition produced by a kindled seizure on subsequent seizures (Caldecott-Hazard et al., 1980). A more traditional index of opioid release, post-ictal analgesia, has been studied during the development of kindled seizures (Frenk and Yitzhaky, 1981). However, fully generalized seizures were not tested for post-ictal analgesia. The present investigation studied post-ictal analgesia after fully generalized seizures and during the kindling process, using both tail-flick and hot-plate tests. The hot-plate test is thought to assess higher levels of pain integration than the tail-flick test.

Chronic bipolar stimulation and recording electrodes were implanted in the amygdala and rats were stimulated daily to kindle seizures. After each daily stimulus, either tail-flick (5 trials at 1 min intervals immediately following the seizure) or hind-paw lick latencies on the hot plate (1 trial, either immediately, at 2 min, 30 min, or 24 hr after the seizure) were measured. Stimulation and analgesia testing continued until all rats showed 3 fully generalized (stage 5) seizures. One week later, the rats were injected with naloxone (2 injections of 10 mg/kg at 15 min intervals), given a seizure, and immediately tested on the hot plate. Another group of kindled rats was made tolerant to morphine, then also given seizures and tested on the hot plate.

Tail-flick analgesia was not seen during the kindling process or following fully generalized seizures. Analgesia on the hot plate was found following fully generalized (stage 5) seizures but not during earlier stages of kindling. The analgesia was complete immediately after the seizures and partially present at 2 min post-ictally. Paw-lick latencies at 30 min and 24 hrs were not different from those of non-stimulated rats. Neither naloxone nor morphine tolerance affected this post-ictal hot-plate analgesia. Thus, these findings suggest that non-opioid mechanisms are involved in kindled post-ictal analgesia. The presence of analgesia in the hot-plate but not the tail-flick test suggests an important role for supraspinal structures in this effect. (Supported by NIH Grants #NS07628 and #NS06289.)

19.12 OPIATE MODIFICATION OF INTERMEDIATES STAGES OF AMYGDALOID-KINDLING. William S. Stone* and Robert F. Berman. Department of Psychology, Wayne State University, Detroit, Michigan 48202.

Recent data from our laboratory indicate that naloxone and naltrexone may be effective in reducing the severity of Stage 5 amygdaloid-kindled seizures (Stone, Eggleton & Berman, 1980). Although several lines of evidence suggest an opiate influence on amygdaloid-kindling, most earlier attempts to demonstrate effects have been marginal or unsuccessful. This is somewhat unexpected in view of the epileptogenic properties of morphine and the enkephalins, and the high concentration of opiate receptors, terminals and enkephalins in the amygdaloid region. The present experiment was an attempt to further examine conditions under which opiates might affect kindling by examining earlier stages of the kindling process.

Thirty-eight adult, male, Long-Evans rats were stereotaxically implanted, bilaterally, with bipolar electrodes into the medial amygdala. After recovery from surgery, each animal was electrically stimulated (100 Hz, 0.1 msec pulse, biphasic, symmetrical, rectangular pulses) through one of the implanted electrodes beginning at 10 microampres to determine the afterdischarge (AD) threshold. Animals were then assigned to one of 5 groups and kindled with 1 sec of stimulation daily at the AD threshold. Two groups of rats were injected daily with 10 mg/kg morphine sulfate from the start of kindling up to Stage 3 (MS, n=9), or from Stage 3 to Stage 5 (MS-3, n=9). Additional groups were injected daily with 10 mg/kg naloxone HCl from initiation of kindling up to Stage 3 (NAL, n=5), or daily from Stage 3 to Stage 5 (NAL-3, n=6). A control group received daily saline injections throughout kindling up to Stage 5 seizures (SAL, n=9). Morphine and naloxone injections were made 15 or 20 min prior to kindling, respectively, and all injections were made intraperitoneally.

Naloxone injected rats (NAL) required an average of 21.8 ± 7.6 days to reach Stage 3 seizures. This was significantly longer than that required by saline (SAL, 9.4± 2.5 days) or morphine (MS, 14.0 ± 5.6 days) injected rats. In contrast, morphine reduced the number of days required to kindle from Stage 3 to 5 (MS-3, 1.7 ± 0.8 days) compared to saline (SAL, 4.2 ± 3.4 days) or naloxone (NAL-3, 5.7 ± 2.7 days) injected rats.

These results indicate that opiates are more effective in modifying earlier stages of amygdaloid kindling than Stage 5. Compared to the results of Stone, et al (1980), morphine at Stage 3 is more potent than at Stage 5. Naloxone from day 1 similarly had a greater effect. The findings presented here may be indicative of different mechanisms involved in the acquisition versus the maintenance of kindled seizures.

19.13 BRAIN STEM SITE OF ACTION FOR REM-SLEEP SUPPRESSING EFFECTS OF MORPHINE. I. de Andrés*, J.R. Villablanca and Ch. E. Olmstead (SPON: W. Wyrwicka). Ment. Ret. Res. Ctr. Depts. Anat. & Psychiat. UCLA, Los Angeles, CA. 90024

In all species, including man, morphine produces a marked reduction of rapid eye movement sleep (REMs). We reported that in cats with bilateral removal of the cerebral hemispheres (diencephalic) REMs is partially suppressed and barbiturates produce a dramatic REMs rebound (Arch.Ital.Biol.110:383;1972). We thought, therefore, that this preparation would be a good model for assessing telencephalic versus brain stem loci for the effects of morphine on sleep. Following a two-stage removal of the hemispheres, 4 cats were implanted with electrodes in the pontine reticular formation, nuchal muscles and the orbits and permitted to recover for at least 15 days. On day 1, a 4 hr. baseline recording preceded morphine SO_4 (1.5 - 2.0 mg/kp, i.p.) and the animals were followed for 4 to 24 hr post-injection. In 2 cases 8 to 12 hr recordings were repeated on days 2,3 and 4. Wakefulness (W), NREMs and REMs were defined on the basis of polygraphic and behavioral criteria. The hourly percentage of W, REMs and NREMs were calculated and the values are given in median and ranges. For the predrug period the values were: W, 55% (32.5 - 68%); REMs, 5.3% (1.3 - 8%); NREMs, 40.1% (30-65.5%);N:8. Morphine totally suppressed REMs for at least 4 hr (range 4 to 8). REMs showed a slow recovery to pre-morphine levels over the 4 to 12 hr post-injection. On days 2 and 3 there was a marked REMs rebound to 24.4% (20.7 - 25.6%) which tended to decline to pre-morphine levels by day 4. For NREMs there was a large increase during the 1st (81.7%; 59-97%) and 2nd (54.2%; 0-100%) post-morphine hours which decreased into a dominant W during the 3rd (100% 11.5 - 100%), 4th (100%; 15.8 - 100%) and 5th (63.8%; 3.0 - 100%) hours. Thereafter both W and NREMs tended to pre-morphine levels with some depression of W paralelling the REMs rebound of the last 3 days. In conclusion, the morphine induced REMs suppression appears to be due to a direct action upon REMs mechanisms in the brain stem. These morphine effects upon sleep-waking follow a protracted time course which suggests that the 3 stage behavioral effect of morphine described for intact and a-caudate cats (Soc.Neurosci.Abstr.4:489;1978) is partially organized at brain stem level. Supported by USPHS Grants HD-05958 and HD-04612.

19.14 STEREOSPECIFIC EFFECTS OF NALOXONE ON RAT HYPOTHALAMIC AND SEPTAL NEURONS. F. Baldino, Jr. and A.L. Beckman. Dept. of Pharmacol., CMDNJ Rutgers Med. Sch., Piscataway, NJ 08854 and Alfred I. duPont Institute, Wilmington, DE 19899.

The purpose of this study was to investigate the possible direct actions of both (+)- and (-)-naloxone on individual neurons in the preoptic/anterior hypothalamus (POAH) and septal area (SA) of the rat brain.

Male Sprague-Dawley rats (250-300g) were anesthetized with urethane (1 g/kg i.p.) and placed in a stereotaxic instrument. Recording of single unit discharges was accomplished through one barrel (NaCl, 4 M) of a five-barrel glass micropipette. A second barrel, filled with 0.5 M NaCl, was used for automatic current balancing and tests for spike discharge current sensitivity. Test compounds were administered iontophoretically through the remaining barrels which alternately contained aqueous solutions of morphine sulfate (0.02 M, pH=4), (-)-naloxone HCl (0.1 M, pH=4), and (+)-naloxone HCl (0.1 M, pH=4).

Morphine and (-)-naloxone, the active isomer of naloxone, were applied to 31 neurons, eleven in the SA and twenty in the POAH. Similar results were observed in both regions. Morphine depressed the spontaneous activity in 19 of 31 neurons. (-)-naloxone at currents less than 10 nA did not influence the firing rate of these neurons. However, (-)-naloxone applied in excess of 10 nA reduced spontaneous activity in 28 of 29 neurons. This effect of (-)-naloxone was stereospecific; (+)-naloxone, the inactive isomer, did not alter the spontaneous rate in 12 of 14 cells when alternately applied with (-)-naloxone at the same current intensity. Application of (+)- and (-)-naloxone with currents above those necessary to elicit the direct inhibitory action produced a diminution of spike amplitude and an increase in the duration of the action potential.

The results of this study indicate that naloxone reduces spontaneous activity via two mechanisms. One involves a direct stereospecific action and a second produces a non-specific reduction in spike amplitide and a prolongation of spike duration which is reminiscent of that produced by local anesthetics. (Supported by NIDA Grant DA 02254 and the A.I. duPont Institute).

19.15 EFFECTS OF OPIATES ON SENSORY EVOKED POTENTIALS IN THE DENTATE GYRUS OF THE CHRONIC RAT DURING CONDITIONING. E.P. Christian*, M.O. West, J.H. Robinson* and S.A. Deadwyler* (SPON: T.J. Miller) Dept. of Physiol. & Pharmacol., Bowman Gray School of Medicine, Winston-Salem, NC 27103.

A recent report demonstrated the existence in the rat of a dual component averaged evoked potential (AEP) to auditory (tone) stimuli which was confined to the outer molecular (OM) layer of the dentate gyrus. The early, N1 component was mediated by the perforant path projection to the dentate gyrus from entorhinal cortex, while the later, N2 component was controlled by projections from medial septum (Deadwyler et al., Science 211:1181-1183, 1981). Rats (n=6) were prepared for chronic recording by implantation of a removable microelectrode drive positioned above the dorsal hippocampus and dentate gyrus, and an ipsilaterally placed entorhinal stimulating electrode. Two of the animals were additionally implanted with intracerebral ventricular (ICV) cannulae. Animals were trained to criterion in 1) a single tone discrimination task or 2) a differential discrimination task. After extensive training in the single tone paradigm, the N1 component of the OM AEP (50 trial average) was attenuated or absent, while N2 amplitude remained constant. Following injections of morphine sulfate (7-10 mg/kg, i.p.), or the synthetic opioid peptide FK-33824 (Sandoz) (3 mg/kg, i.p.), significant increases were observed in N1 amplitude. N2 was either unchanged or was decreased in some cases. Direct ICV injections of morphine (20 µg) or FK-33824 (36 ng) produced even more pronounced increases in N1 amplitude without affecting behavior. Input-output (IO) curves of the extracellular perforant path elicited synaptic potential also changed after opiate administration in that curves were shifted to the left and the maximal potential was increased. Duration of the effect paralleled the time course for changes seen in the OM AEP. Naloxone (2 mg/kg, i.p.) injections alone had little effect on the OM AEP, but created a significant shift to the right and a lower maximal potential in IO curves. Naloxone administration *15 min prior to morphine* in most cases reduced the morphine effect on OM AEPs and IO curves. These results provide evidence that opiate agonists are capable of producing increased sensory activation of perforant path synapses.

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19.16 REGIONAL BRAIN GLUCOSE UTILIZATION DURING NALOXONE-INDUCED MORPHINE WITHDRAWAL. P. DiStefano*, G.F. Wooten, R.C. Collins and E.M. Johnson* (SPON: S.G. Eliasson). Dept. Neurol. and Pharmacol., Wash. Univ. Sch. Med., St. Louis, MO 63110.

We have studied regional cerebral glucose utilization (GU) in rats during acute morphine withdrawal by ^{14}C-2 deoxyglucose autoradiography. One pellet containing 75 mg of morphine base was implanted S.C. on day 1. On day 4 two pellets were implanted S.C. On day 7 the pellets were removed and morphine withdrawal was induced by injection of naloxone 0.5mg/kg S.C. Fifteen minutes after injection of naloxone, when rats had developed tachypnea, hyperactivity, wet dog shakes and diarrhea, ^{14}C-2 deoxyglucose was administered. Controls included naive rats, morphine pretreated rats that received no naloxone on day 7, and naive rats treated with naloxone 0.5mg/kg S.C.

During naloxone-induced morphine withdrawal the most prominent increases in GU (relative to morphine dependent controls) occurred in central nucleus of amygdala (51%), lateral mammillary nucleus (40%), lateral habenular nucleus (39%), medial mammillary nucleus (35%), and medial septal nucleus (35%) (all $P < 0.01$). Significant increases also occurred in bed nucleus of stria terminalis (23%), interpeduncular nucleus (28%), anterior medial thalamus (30%), anterior ventral thalamus (30%), parataenial nucleus (25%), and lateral septal nucleus (26%). A 35% reduction in GU occurred in entorhinal cortex ($P < 0.05$). No changes were found in any other neocortical areas, hippocampal formation, dentate gyrus, basal ganglia, or posterior and ventral thalamic groups. GU in morphine dependent rats was decreased in frontal cortex, striatum, anterior ventral thalamus, and medial habenular nucleus; while GU was uniformly increased (23-54%) in hippocampus, dentate gyrus and preoptic hypothalamic areas. Naloxone 0.5mg/kg S.C. administered to naive rats produced no changes in regional GU.

Morphine withdrawal is associated with a marked increase in GU in the central nucleus of the amygdala and in several other limbic structures that may be activated in series by amygdalofugal pathways: these structures include bed nucleus of stria terminalis, lateral habenular nucleus, interpeduncular nucleus, mammillary nuclei, and anterior thalamic groups. Several of these structures, particularly the central nucleus of the amygdala, contain large concentrations of endogenous opiates and opiate receptors.

19.17 MODIFICATION OF MORPHINE ACTION BY THE PITUITARY.
B.G. Kasson* and R. George* (SPON: S. Eiduson). Dept. of Pharmacology, UCLA School of Medicine, Los Angeles, CA 90024.

Although a vast literature exists on the pharmacologic effects of opiates, there are few data on the modulation of opiate action by pituitary hormones. An approach taken in the past to begin investigation of this problem has been the examination of the effects of hypophysectomy on antinociceptive responses to morphine or various forms of electroshock. The results from early studies were inconclusive and contradictory. In 1977, J.W. Holaday et al. (P.N.A.S. USA 74:4628-4632, 1977), described a sensitization to opiate effects by hypophysectomy which was reversible by ACTH administration. We have conducted a more thorough examination of the effects of hypophysectomy upon morphine responses and have found some interesting differences not previously reported.

Male Sprague Dawley rats (160-180 gr.) were hypophysectomized by the parapharyngeal technique and compared with normal intact males for antinociceptive and thermoregulatory responses. It was found that, for antinociceptive responses, the two groups did not differ with respect to ED_{50} values (2.6 mg/kg hypophysectomized, 2.1 mg/kg intact) but hypophysectomy (Hx) dramatically altered the slope of the dose response curve. Hx animals, as shown by Holaday, were considerably more sensitive to morphine than intact animals, but only at high doses (greater than the ED_{50}), whereas, unlike that reported previously, with low doses of morphine (less than the ED_{50}) Hx animals were considerably less sensitive. Treatment of Hx animals with ACTH, at a dose which restored normal adrenal weight, not only shifted the dose response curve back to the same slope as intact animals but also significantly increased the ED_{50} (4.0 mg/kg). Temperature response curves were also altered by hypophysectomy. Hx produced a more hyperthermic response at any given dose of morphine. ACTH administration decreased the hyperthermic response and also made these animals less sensitive to the hypothermic effects of morphine. Further experiments will determine which other pituitary hormones play a role in the modulation of morphine actions. (This work supported by USPHS DA-01006).

20.1 HORSERADISH PEROXIDASE IDENTIFICATION OF THE MOTONEURONS TO THE TENSOR TYMPANI MUSCLE IN RHESUS AND OWL MONKEYS. Norman L. Strominger, Steven M. Silver*, Timothy C. Truscott, and Jerome C. Goldstein*. Department of Anatomy and Division of Otolaryngology; Albany Medical College of Union University, Albany, New York 12208.

The tensor tympani muscle was exposed in a series of monkeys using a transmastoid approach to the middle ear. An operating microscope was used to enhance visualization. Horseradish peroxidase (20%-15%, Sigma Type VI) was injected into the tensor tympani through a micropipette under air pressure. Dried flakes of the enzyme also were tattooed into the muscle belly in some cases. Animals were perfused transcardially two to three days postoperatively with 0.9% saline followed by 8% gluteraldehyde. Blocks of brainstem including the pons and midbrain were cut at 50μ on a freezing microtome in the transverse or horizontal plane. Material was reacted with tetramethyl benzidine and stained with neutral red and/or thionin.

Three cases with unilateral injections demonstrated the completely ipsilateral location of the motoneuron pool. Four other monkeys were given bilateral injections. Labeled perikarya formed a compact cluster in the ventral or parvocellular division of the motor trigeminal nucleus. The labeled cells were much smaller than large unlabeled motoneurons of the more massive dorsal or magnocellular division. In a couple of instances, enzyme leaked onto the chorda tympani nerve and labeling occurred in the superior salivatory nucleus. Cells so labeled were spatially some distance from the motoneurons to the tensor tympani. Neurons in the mesencephalic trigeminal nucleus did not contain label.

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20.2 TOPOGRAPHIC ORGANIZATION OF MOTOR NEURONS INNERVATING PINNA MUSCLES IN THE CAT. J.M. Zook, H.A. Patterson and J.C. Middlebrooks. Coleman Lab. and Depts. of Physiology and Anatomy, Univ. Calif. San Francisco, San Francisco, Calif.,94143.

Many mammals rely upon the directional sensitivity of mobile pinnae to aid in sound localization and discrimination. Although control of pinnae movement is intimately related to the auditory system, little is known of this specific sensory-motor interaction. In the cat movement of the pinnae is controlled by at least seventeen distinct muscles. Most pinna muscles are innervated by the auriculotemporal and posterior auricular branches of the seventh cranial nerve, the facial nerve. Previous studies have shown that these branches of the facial nerve originate from motor neurons of the dorsomedial division of the motor nucleus of the facial nerve. We have systematically examined the innervation of the individual muscles effecting pinna movement by the method of retrograde transport of horseradish peroxidase (HRP). The results indicate that there is a topographic organization within the dorsomedial division of the facial nerve nucleus of the motor neurons innervating individual pinna muscles.

For these experiments individual pinna muscles were exposed under general anesthetic. With careful blunt dissection a muscle group with intact sheath was partially isolated from surrounding tissue. A 30% solution of HRP in saline was injected into the muscle. In all cases clusters of HRP-labeled cells were found in the dorsomedial division of the facial nerve nucleus. These clusters of labeled cells formed columns, or sheets, running the entire rostral-caudal extent of the nucleus. When viewed in the transverse plane labeled cells in each case were located within a different sector of the dorsomedial division. Little overlap was found in the position of these cell columns from muscle to muscle. For example, considering the transverse plane, labeled neurons were consistently found only in the medial part of the dorsomedial division after injections in the levator auris longus, labeled neurons were found only in the ventral part after injections in the depressor conchae and labeled neurons were found only in the lateral part after injections in the adductor auris superior.

Further investigations have been directed towards establishing the degree of overlap in the innervation of pinna muscles by these motorneuron columns using double retrograde label techniques. In light of this columnar organization the main question is the relationship between these columns and the nuclei from which pinnae movements are controled. To address this question we are investigating the specific organization of descending projections to the facial nerve nucleus and the relationship between this organization and co-ordinated pinna movement evoked by microstimulation at the sources of projections to the dorsomedial division of the motor nucleus of the facial nerve.

20.3 QUANTITATIVE STUDY OF BRAINSTEM MOVEMENT AND METHODS WHICH MAY BE USED TO REDUCE IT: DEVELOPMENT OF A NON-PULSATILE CARDIOPULMONARY PERFUSION SYSTEM FOR THE CAT. R.H. Britt and G.T. Rossi. Div. Neurosurgery, R155, Stanford Sch. Med., Stanford, CA 94305 and Palo Alto Veterans Hospital, Palo Alto, CA 94304.

For investigators attempting intracellular recording in the brainstem auditory pathway, brain pulsations caused by the cyclical arterial and venous pressure fluctuations from cardiac and respiratory cycles make penetration and remaining within neurons of 30-50μ difficult. The authors have quantitatively measured brainstem pulsations; measured the effects of standard methods used to reduce pulsations (pneumothoraces, elevation of the head, cerebrospinal fluid (CSF) drainage, and reduction of mean arterial pressure (MAP)); and have developed a new non-pulsatile cardiopulmonary bypass system which eliminates movement of the brain.

In 10 pentobarbital anesthesized cats a wide decompressive posterior fossa craniotomy was performed and the cerebellum aspirated. A reflective foil target was placed on the floor of the fourth ventricle on which a fiber-optic displacement transducer was focused. This system measured the planar, one-dimensional motion normal to the cat's dorsal medulla. Two types of displacement were recorded: 1) relatively low amplitude (110-266μ) and short duration (330-400 msec) excursions corresponding to the pressure wave peak of each systole and 2) a slower (10-12/min), high amplitude, plateau-like displacement (300-950μ) lasting for a time (2.4-5.1 sec) corresponding to each inspiration. Bilateral pneumothoraces reduced movement by 50%. Elevation of the head further reduced movement to a mean 68% below the original excursions. Lowering MAP from 113 to 35 mmHg, reduced cardiac induced movement by 40%.

To eliminate brainstem movement completely, a non-pulsatile cardiopulmonary bypass system was developed utilizing a Bio-Medicus centrifugal flow pump and a SciMed membrane oxygenator resulting in steady-state blood flow (MAP 50-70 mmHg). Brainstem auditory evoked responses remained normal for periods of 2 to 8 hours on bypass. Of 15 cats placed on bypass, problems typically developed after 2-3 hours including: 1) a bleeding disorder (probably hyperfibrinolysis) and 2) metabolic acidosis (correctable by adding bicarbonate). Because of limited donor cat blood, the feasibility of using blood substitutes (20% Fluosol-DA, Alpha-Therapeutic Corp.) is being investigated. The ability of this system to eliminate all brain pulsations has potential application in all neurophysiological studies requiring intracellular recording techniques. This system will be evaluated in a series of intracellular recording studies in cat cochlear nucleus.

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20.4 GIANT RING-SHAPED TERMINALS IN THE COCHLEAR NUCLEUS OF CAT. Doyle R. Jones* (SPON: D.K. Morest). Dept. Anatomy, Univ. Ct. Health Center, Farmington, CT 06032.

Axonal terminals with neurofibrillar rings have been described in many parts of the nervous system. Cold acclimation of poikilotherms increases the number of these rings, which in the "cochlear gray" of lizards (Boycott et al,Proc. Roy.Soc.,'61) are described as particularly large (up to 4 μm in diameter) and formed by thick bundles of neurofilaments. Cold-induced neurofibrillar rings may reflect a compensatory process which maintains "physiological properties, such as intracellular transport and synaptic efficacy" during periods of cold-induced stress (Potter,J. Neurocytol.,'73). The present study describes a specific population of exceptionally large neurofibrillary rings in the anteroventral cochlear nucleus(AVCN) of the cat.

The morphology and locations of the giant ring-shaped endings were studied in AVCN of 9 adult cats with the Protargol and rapid Golgi methods. The origin of these endings was established by labeling with HRP following cochlear injections and by examination of cochlear nuclei in labyrinthectomized cats. The giant ring-shaped endings reside in the dorsal half of the anterior division of AVCN and like the end-bulbs of Held are terminals of cochlear nerve fibers. They arise from the largest axons in AVCN, disappear following labyrinthectomy, are labeled with HRP after cochlear injections, and are specifically related to bushy cells. These endings range up to 20 μm in diameter and are formed by thick bundles of neurofilaments which emerge from the axon trunk. The ring-shaped end-bulbs range in complexity from those which are relatively smooth with few appendages to those with many appendages. Some rings form in an appendage of a large end-bulb and occasionally two rings form in an end-bulb.

The key to the functional significance of these specialized nerve terminals may lie in their spatial distribution within AVCN, which is predominantly confined to a region of bushy cells receiving middle to high frequency input. Bourk (Dissertation, MIT,'76)reported that some of the PPO and PP1 units (presumably corresponding to bushy cells) with high characteristic frequencies(CF) have higher thresholds than those with lower CF. It is possible that the high CF, high threshold units correspond to bushy cells receiving ring-shaped end-bulbs. Perhaps these neurofibrillar rings reflect a lower level of turnover of neurofilament proteins in these endings.

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20.5 CYTOARCHITECTURAL ORGANIZATION OF THE DORSAL COCHLEAR NUCLEUS. J. K. Moore. Dept. of Anat. Sci., SUNY at Stony Brook, Stony Brook, NY 11794.

The cytoarchitecture of the DCN has been studied in the hedgehog, several species of rodent, cat, monkey, and man, using drawings and photographs of Nissl and Golgi material to define cell types and cell mapping of the entire DCN to determine the distribution and density of the various cell types in different species. Regarding cell types and distribution; (1) most large neurons in all species are fusiform cells, with elongate to triangular somas in the size range of 15μ X 35μ, though somewhat larger in the cat. These cells may be either oriented radial to the nuclear surface or oblique to longitudinal in the deeper region of the DCN. Somas of deep and radial cells do not differ in appearance in Nissl material, but their dendritic arborization varies in that radial cells develop an apical dendritic branching that is highly arborized and covered with dendritic spines. Basal dendrites of radial cells and all dendrites of deep cells are more coarsely branching and have sparse appendages. The presence of spines on apical dendrites is presumably related to formation of synapses with granule cell axon terminals as described by Mugnaini et al., 1980. In monkey and man, all fusiform cells have a pattern similar to deep fusiform cells of nonprimate species: (2) true giant cells with large nuclei and somas up to 50-80μ are rare in all species, though most common in the cat: (3) most smaller cells are round (12μX12μ) or oval (12μX20μ), with nuclei smaller than fusiform cells and sparse chromatin. Their appearance in Nissl material is so uniform as to allow them to be regarded as a single cell type, but differential dendritic patterns occur in cells located in the superficial layers or deep region of the nucleus. These patterns have been described as the stellate, elongate, and cartwheel types. In primates cells of this type have dendritic patterns equivalent to cells of the deeper region in nonprimate species: (4) two categories of very small cell, the small fusiform cell and cochlear granule cell, are common in the superficial layers of the nucleus in nonprimate species, but are seen as scattered cells in the central DCN in both nonprimates and primates, including man.

20.6 THE NEURONAL ORGANIZATION OF THE MOUSE DORSAL COCHLEAR NUCLEUS. F.H. Willard* and D.K. Ryugo. Dept. Anatomy and Neurobiology, Univ. Vermont Med. School, Burlington, VT 05401; Dept. Anatomy, Harvard Med. School, Boston, MA 02115 and Eaton-Peabody Laboratory, Mass. Eye & Ear Infirmary, Boston, MA 02114.

In the mouse dorsal cochlear nucleus(DCN), sheets of large cells (x-sectional area of cell body >250 μm^2) project in a topographic fashion to restricted laminae in the central nucleus of the inferior colliculus(Ryugo et.al., Brain Res.,210:342-349, 1981). Moreover, all members of this distinctive class of large cells project to the inferior colliculus and are positioned in layers II(75%), III(15%), and IV(10%). Although they are distributed in different layers, the cells nonetheless appear morphologically similar in Nissl and HRP preparations; consequently, we have undertaken a more detailed analysis of these cells using Golgi techniques.

Two separate categories of large cells may be recognized on the basis of differences in dendritic morphology. Principal cells (a.k.a. pyramidal cells, fusiform cells, bipolar cells) constitute approximately 85% of the large cell population and are only found in layers II and III. Each cell has 3-5 primary dendrites whose branches radiate along a plane oriented perpendicular to the interface between layer II and III, and form disc-shaped dendritic fields. The disc-shaped principal cells form a series of laminae whose orientation corresponds to the cochleotopic course of auditory nerve fibers. This fibro-dendritic arrangement, so characteristic of other auditory nuclei, presumably underlies tonotopy in DCN.

The other group of large cells are confined to layer IV and the deepest part of layer III. These neurons have very long and relatively unbranched dendrites which extend in all directions. Therefore, they lack a planar shape and potentially may be contacted by primary fibers originating from long segments of the cochlear partition. Such cells correspond to what have previously been called giant cells.

Both principal cells and giant cells comprise the thin neuron sheets which project topographically to the inferior colliculus; their cell bodies and collicular projections maintain a cochleotopic order. Yet differences in their dendritic morphology and relationship to primary afferents suggest that each must subserve different aspects of auditory function.

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20.7 INTRACELLULAR RECORDING AND STAINING OF FUSIFORM CELLS IN CAT DORSAL COCHLEAR NUCLEUS. W.S. Rhode*, D. Oertel and P.H. Smith. Dept. of Neurophysiology, Univ. of Wisconsin Med. Sch., Madison, WI 53706.

Fusiform cells in the dorsal cochlear nucleus (DCN) have been studied electrophysiologically and identified anatomically by the intracellular injection of horseradish peroxidase (HRP). In sodium pentobarbital-anaesthetized cats extra- and intracellular recordings were made from 15 fusiform cells which were reconstructed in camera lucida drawings. Before impalement, extracellular responses of these cells to tone bursts at their characteristic frequency (CF) display the "pauser" or "buildup" poststimulus time histograms (PST) described by Pfeiffer (Exp. Brain Res. 1:220, 1966) and often a "chopper" pattern is superimposed on the PST. After impalement tone bursts at CF frequently elicited a "chopper" pattern from the same cell. "On-off" response patterns were sometimes recorded to tones at frequencies away from CF.

In the absence of sound stimuli the intracellular potentials of fusiform cells were noisy and the cells occasionally discharged. In response to tone stimuli at CF, fusiform cells were depolarized by large, noisy excitatory postsynaptic potentials (psps) and then hyperpolarized following the tone burst. During the hyperpolarization the noisiness of the membrane potential temporarily decreased before returning to its resting level. We have observed no inhibitory psps even when the cells were polarized with current.

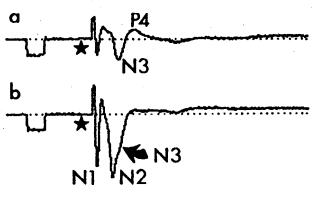
Fusiform cells are characterized by two tufts of dendrites, apical dendrites which extend dorsally to the surface of the nucleus and basal dendrites which extend vertically (Kane, J. Comp. Neurol. 155:301, 1974). Reconstruction of injected cells has demonstrated that typically 4 to 7 apical dendrites branch and may end in as many as 50 terminal processes. Apical dendrites are densely covered by spines. Basal dendrites, in contrast, lack spines. They branch less and are longer, reaching the deep DCN. Occasionally a basal dendrite has an apical branch which is covered with spines. Axons usually arise from a basal dendrite, but can arise from the cell body and run into the dorsal acoustic stria. Axon collaterals sometimes terminate in the region of the cell soma and dorsomedial to the soma in the higher frequency region of the DCN.

20.8 TEMPORAL PROPERTIES OF SYNAPTIC ACTIVITY IN THE DORSAL COCHLEAR NUCLEUS. Paul B. Manis* and William E. Brownell, Depts. of Neuroscience and Surgery (ENT), University of Florida College of Medicine, Gainesville, FL 32610.

Potentials evoked by repetitive electrical stimulation of the eighth nerve were examined in the dorsal cochlear nucleus (DCN) of decerebrate unanesthetized and barbituate anesthetized cats. Shock of the eighth nerve elicits a complex response, which for convenience may be divided into four successive components: N1 N2, N3, and P4. The illustration presents potentials recorded at two depths. Trace "a" was recorded from layer 2, while trace "b" was recorded 300 μm deeper. The calibration pulse is -1 mV by 1 msec, and the time of stimulus delivery is indicated by the star. The stimulus artifact has been suppressed.

The ability of these potentials to follow high rates of eighth nerve stimulation was examined by averaging the response of the last 5 of 30 stimuli at a given frequency. The N1 followed stimulus frequencies up to 350 Hz with little decrement, but was reduced at 500 Hz. The N2 component of the field potential followed stimuli up to 250 Hz quite well, exhibiting in some cases an increased magnitude between 100 and 250 Hz. The N2 decreased in magnitude more rapidly than the N1 at higher frequencies, being severely depressed at 500 Hz. In general, N3 behaved the same as N2. The P4 component was abolished for stimulus frequencies above 75 Hz. Similar results were obtained in a paired-shock paradigm. The N2 and N3 showed a slight facilitation for intervals between 7 and 30 msec. The P4 component was depressed by more than half over the same intervals and recovered to baseline for intervals longer than 50 msec.

The response components can be differentiated on the basis of their ability to follow high-frequency afferent volleys. The latency and current source-sink distribution of the N2 suggests that it is a monosynaptic response, although this needs to be verified by independent means. If the N2 is synaptically mediated, then this synapse is unusual compared to other central nervous system synapses, which usually show response decrement for frequencies above about 100 Hz. Rapid neurotransmitter release and degradation would be consistent with the high afferent discharge rates seen in the auditory nerve.



Supported by USPHS grants MH10320, MH07855 and NS12209.

20.9 SOME RECURRENT PROJECTIONS OF THE SUPERIOR OLIVE TO ANTEROVENTRAL AND DORSAL COCHLEAR NUCLEI IN CAT. Glenn R. Farley and W. Bruce Warr, Human Communication Laboratories, The Boys Town Institute, Omaha, Nebraska 68131.

The purpose of this study was to examine the differences in the organization of recurrent projections to the anteroventral (AVCN) and dorsal cochlear nuclei (DCN) using retrograde and anterograde tract-tracing methods. Injections of 30-50 nl of 30% HRP were made into either the AVCN or DCN, and, following survival times of about 24 hours, labeled cells were found bilaterally throughout the longitudinal extents of the same general periolivary cell groups, regardless of injection location. In most cases, labeling was greater ipsilaterally. Labeling was most profuse in the complex of cells embracing the lateral superior olivary nucleus (LSO) and in the ventral nucleus of the trapezoid body (VNTB), including a superficial cell group lying directly ventral to the medial nucleus of the trapezoid body. Injections into AVCN resulted in a greater preponderance of labeling in rostrally located cells in the ipsilateral lateral periolivary regions than in those located caudally, whereas DCN injections produced a gradient of labeling in the opposite direction. In regard to the possible collateral projections of olivocochlear neurons, injections in AVCN labeled neurons bilaterally in the VNTB and in the dorsomedial periolivary nucleus which, when examined in material counterstained for AChE, were found to be intensely positive for this enzyme. In contrast, injections in the DCN failed to produce this pattern of labeling, although cells in these nuclei were clearly labeled with HRP. A similar examination of the small AChE-positive neurons surrounding LSO produced no evidence of combined markings with HRP and AChE in any of our experiments.

The particularly heavy projection from the lateral periolivary cell croup to AVCN and DCN was further examined by injecting tritiated Leucine into the lateral nucleus of the trapezoid body at the level of the S-shaped portion of the LSO. Autoradiographs demonstrated heavy projections ipsilaterally to (1) the polymorphic and fusiform layers of the DCN, (2) the spherical cell area, and (3) to a lesser extent, to other portions of cochlear nucleus, excluding the granule cell layer. Also of interest was an apparent projection to the lateral dendrites of the ipsilateral medial superior olivary nucleus.

Our findings suggest that the descending influences upon AVCN and DCN, while by no means identical, exhibit a surprising degree of commonality in terms of their nuclear origins.

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20.10 QUANTITATIVE EVALUATION OF CENTRIFUGAL CHOLINERGIC PATHWAYS TO THE RAT COCHLEAR NUCLEUS. D.A. Godfrey[1], J.L. Park[1]*, J.R. Rabel[1]* J.D. Dunn[2], J.T. Smith[1]* and C.D. Ross[1], Depts. Physiol.[1] and Anat.[2], Oral Roberts Univ., Tulsa, OK. 74171.

The contribution of centrifugal pathways to cholinergic synapses in the rat cochlear nucleus was studied by an approach involving lesions and quantitative histochemical mapping procedures. Surgical lesions were aimed just medial to the right cochlear nucleus to cut virtually all its central connections, leaving the left nucleus as control, and animals were sacrificed one week later. Distributions of cholinergic neural elements were estimated by measurement of choline acetyltransferase (ChAT) activities for tissue samples microdissected from freeze-dried 20µm-thick sections. Acetylcholinesterase (AChE) activities were measured, as a secondary marker, for samples from other nearby sections. For four rats in which the lesion missed the region medial to the cochlear nucleus, regional ChAT and AChE activities averaged across the group were not significantly different between left and right cochlear nuclei. Data for ChAT activities, in µmoles/kg dry wt/min (38°C), for the three rats with the most accurately placed lesions, are summarized below. (Average regional data given as lesion side/control side (%); AVCN, DCN, PVCN: anteroventral, dorsal, posteroventral cochlear nucleus; differences significant: +at $p < .01$, *at $p < .001$)

Region	Rat A	Rat B	Rat C
DCN molecular layer	30/167(18%)*	8/282(3%)*	23/231(10%)*
DCN fusiform soma layer	80/351(23%)*	30/476(6%)*	71/576(12%)*
DCN deep layer	46/248(19%)*	17/276(6%)*	72/399(18%)*
PVCN	43/236(18%)*	13/286(5%)*	44/358(12%)*
AVCN rostral part	56/553(10%)*	20/431(5%)*	130/393(33%)*
AVCN granular regions	138/416(33%)*	64/244(26%)+	100/183(55%)+

The lesion in rat A encroached least onto the medial part of the DCN, while the lesion in rat B encroached most. The higher lesion/control % in the DCN and PVCN of rat A might relate to a sparing of the caudalmost part of the trapezoid body by the lesion in this rat, while the higher AVCN and granular region % in rat C may relate to a sparing of the rostralmost part of the trapezoid body in this rat. These data for ChAT are consistent with the possibility that almost all cholinergic elements in the bulk of the rat cochlear nucleus represent fibers and terminals of cholinergic centrifugal pathways, while as much as a third of the ChAT activity in the granular regions may relate to cholinergic interneurons. Lesion-side AChE activities were usually at least 50% of control-side activities for any given region, suggesting that much of this enzyme activity may be related to cholinoceptive structures in the rat cochlear nucleus.
(Supported by Oral Roberts University intramural research funds)

20.11 EFFECTS OF MODERATELY-INTENSE, PURE-TONE STIMULI ON AUDITORY SENSITIVITY AND COCHLEAR NUCLEUS SINGLE UNITS IN THE RABBIT. G.K. Martin*, B.L. Lonsbury-Martin and M.F. Frampton*. Dept. Otolaryngol., Univ. Washington Sch. Med., Seattle, WA 98195.

Auditory thresholds to 14 discrete test frequencies representative of the major portion of the rabbit hearing range were obtained using a classically-conditioned nictitating membrane response (Martin et al., Hear. Res., 2:65-78, 1980). Following determination of monaural thresholds, the effects of moderately-intense, continuous stimuli on pure-tone detection capability were evaluated in terms of amount and duration of threshold shifts using a standard protocol. Rabbits were first assessed at the test frequency using an adaptive testing procedure to determine preexposure threshold. Next, a 90-dB tone systematically related to the frequency of test tone bursts was presented for 3 mins. Immediately upon its termination, test stimuli were reinstated to track the magnitude and duration of the temporary elevation in threshold. An inverted U-shaped function best described the amount of threshold shift with the maximum effect of 15-20 dB occurring for test stimuli ranging from 4-11.3 kHz, while considerably smaller elevations were observed for test frequencies on either side of the sensitive mid-frequency region. Recovery times were linear in log time and paralleled the amount of hearing loss with greater threshold shifts requiring longer recovery periods. However, when initial threshold shifts approached 20 dB, recovery times frequently exceeded the routine 30-min recovery interval. Both trained and untrained animals were prepared for acute recording from single cochlear nucleus neurons in order to relate neural threshold shifts and recovery times to those measured in the earlier behavioral experiments under identical sound-exposure conditions. Two classes of pathological single unit responses were identified. For the majority of neurons, a decrement in driven discharge rate was noted for all stimulus levels tested. In contrast, a number of units demonstrated decreased poststimulatory activity for only threshold-related test stimuli, while for higher level tone bursts, discharge rate increased above control values. Short-lasting alterations were also observed for spontaneous discharge rates which could be either facilitated or decreased. In similar behavioral and neural studies in nonhuman primates, initial neural threshold shifts and corresponding recovery time courses were much greater than those predicted by their behaviorally measured counterparts. In contrast, preliminary findings in our rabbit preparation suggest a much closer correspondence between neural and behavioral results. A comparison of results between rabbit and monkey models for sound exposure will be discussed.
(Supported by The Deafness Research Foundation.)

20.12 MECHANISMS OF AUDITORY INFORMATION PROCESSING FOR PITCH PERCEPTION. G.E. Loeb, M.W. White*, and M.M. Merzenich. Coleman Memorial Lab., UCSF, San Francisco, CA 94143, and Lab. of Neural Control, NINCDS, NIH, Bethesda, MD 20205.

Recent attempts to restore hearing in deaf patients by selective electrical stimulation of the auditory nerve have focused attention on the problem of how sound is normally processed. The percepts reported by such patients suggest that the nervous system uses the subtle timing information inherent in the phase-locking of afferent activity to sound frequencies in the band 500-5000 Hz. These are the frequencies for which electrical stimuli cause pitch and loudness sensations not relatable to the predictions of place or periodicity pitch detection mechanisms.

We have proposed a neural mechanism for the extraction of pitch by spatial cross-correlation of the traveling wave on the basilar membrane. In our model, phase-locked neural signals from pairs of loci in each cochlea are non-linearly combined at the level of the medial superior olive (MSO). This converts the task of frequency detection from analysis of temporal-spatial cochlear patterns to spatial pattern recognition of the MSO neural ensemble. We here examine several properties which arise from computer simulations of this process and compare them with electrophysiological data which we and others have obtained in the cat anteroventral cochlear nucleus (AVCN) and MSO.

In particular, the model suggests that the loci whose signals are combined should be separated by about 0.4 wavelengths of the frequency to be detected. The output of the MSO, where the signals converge, should be highly sensitive to changes in the arrival time of these action potentials. A four-way excitatory/excitatory convergence is proposed (two loci from corresponding locations in each cochlea) which would account for certain complex psychophysical phenomena such as the pitch of dichotic band-delayed white noise and the pitch of the dichotic missing fundamental. Both are examples of phenomena consistent with a central representation of sound spectra which is binaural yet early enough in the synaptic chain for phase-locking to be preserved.

This model would account for the psychophysical reports of auditory prosthesis patients if their electrical stimulation resulted in neural activity with spatial localization and phase-locking similar to that produced by acoustic stimuli. We have demonstrated such activity in AVCN cells in cats during similar electrical stimulation.

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20.13 AUDITORY INPUT TO CELLS IN THE DEEP LAYERS OF THE CAT SUPERIOR COLLICULUS. P.B. Schechter, Judith A. Hirsch* and Tom C.T. Yin. Dept. of Neurophysiology, Univ. of Wisconsin Medical School, Madison, Wis. 53706.

Previous studies of the superior colliculus (SC) of mammals have shown that some cells in the deep layers are polymodal, receiving visual, somatic and/or auditory inputs. Although the receptive field properties of the visual and somatic inputs have been mapped thoroughly, little is known about the auditory inputs to SC. We have studied the acoustic properties of these cells, with special emphasis on their binaural characteristics.

We have used two different preparations: barbiturate anesthetized cats stimulated with pure tone stimuli delivered dichotically and paralyzed, nitrous-oxide anesthetized cats with click and tonal stimuli delivered in a free sound field. In the paralyzed animals, the visual receptive field properties of the cells in the superficial and deep layers were also studied.

Cells which responded to the acoustic stimuli were not found in many of the penetrations that were made into the deep layers of the SC. In some cases such neurons were located in narrow laminae several hundred micra thick. Most cells were excited by input from the contralateral ear and inhibited by input from the ipsilateral ear. Thus, most cells were binaural and could be driven by pure tone stimuli. We also studied response areas, tuning curves, and interaural intensity and time difference functions. By using high frequency sinusoidal tones that were gated rapidly with a trapezoidal envelope, we found that even cells with high characteristic frequencies were sensitive to interaural time differences. This was consistent with results obtained from the free field preparation: some of the cells had spatially restricted auditory fields along the azimuth. One surprising finding was that many of the cells had response latencies of 6.5 to 10 msec. This is much shorter than measurements made in the inferior colliculus (IC) under identical conditions. This auditory input must be mediated via fast conducting pathways that do not traverse the IC and may be responsible for the component of the early brain stem evoked response that has been thought to originate from the IC.

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20.14 A FLUORESCENT DOUBLE-LABELING STUDY OF ASCENDING AUDITORY PROJECTIONS TO INFERIOR COLLICULUS OF CAT. K. K. Glendenning, M. S. Bull*, and R. B. Masterton. Dept. of Psychology, Florida State University, Tallahassee, FL 32306

Retrograde labeling and degeneration studies have demonstrated that several auditory brainstem nuclei project to both the ipsilateral and contralateral inferior colliculi (IC). These studies have not shown whether the origin of these projections is in two separate subpopulations each projecting either ipsilaterally or contralaterally, or in a single population of neurons each sending axon collaterals to both colliculi. Retrograde double-labeling experiments with fluorescent compounds suggest that all three types of projection exist.

Cats were subjected to large bilateral injections (>1.0 ul) of 2.5% Fast Blue in one IC and 1.0% Nuclear Yellow in the other IC. A systematic variation of the survival times showed the optimal survival time for Fast Blue and Nuclear Yellow in this system to be about 72 and 10 hours, respectively.

Some labeled neurons were found in each of the divisions of the cochlear nuclei. The majority of these neurons were labeled only by the contralateral marker. A few neurons were labeled with the ipsilateral marker and only very few were labeled with both markers. In the superior olivary complex, the medial superior olive was fully labeled and the medial nucleus of the trapezoid body was sparsely labeled, each almost exclusively by the ipsilateral marker. In the lateral superior olive, however, at least three populations of neurons were intermixed. The largest population was labeled only by the contralateral marker, a somewhat smaller population was labeled only by the ipsilateral marker, and a small population was labeled by both markers.

These results indicate that collateral projections to both inferior colliculi arise from the cochlear nucleus and the superior olivary complex but this type of projection is not prevalent.

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20.15 EFFECTS OF NEONATAL UNILATERAL COCHLEAR ABLATIONS: AN ANATOMICAL INVESTIGATION OF ASCENDING PROJECTIONS TO THE INFERIOR COLLICULUS. K.S. Wrege* and L.M. Kitzes* (SPON: T. O'Connor). Depts. Psychobiol. and Anat., Univ. of Calif., Irvine, CA 92717.

Unilateral ablations of the left cochlea were made in 2 day old gerbils. At 4-6 months of age, the central nucleus of the inferior colliculus (ICCN) was examined physiologically in both neonatally lesioned and normal animals. In lesioned animals there was no evidence of auditory input from the ablated cochlea. Sigma IV HRP (30%, .15 µl) was then pressure injected into either the left or right ICCN. The animals were transcardially perfused 48 hrs later. The tissue was then processed according to the technique of Mesulam (J. Hist. Cyt., 1976) and counter-stained with neutral red. Data were collected from only those subjects in which 1) the reaction product was apparent throughout the injected IC, 2) there was no evidence of HRP diffusion beyond the limits of the IC, and 3) the medial superior olive contralateral to the injection was devoid of labelled cells. Labelled cells were counted in the fusiform layer of dorsal cochlear nucleus (DCN), and in postero-ventral (PVCN) and antero-ventral (AVCN) cochlear nucleus.

It is apparent in Nissl stained material that neonatal cochlear ablations result in severe transneuronal degeneration of both PVCN and AVCN on the lesioned side. In contrast, degeneration within the fusiform layer of DCN is minimal.

As demonstrated by the number of HRP labelled cells, normal animals exhibit ipsilateral projections to the IC from all 3 divisions of the cochlear nuclear complex. The ratio of ipsilateral to contralateral projections is 22% for DCN, 5% for PVCN and 1% for AVCN. In neonatally lesioned animals, the ipsilateral projections from PVCN and AVCN on the normal side are significantly greater than in normal animals ($p < .01$). In contrast, the ipsilateral projection from DCN does not differ significantly from normals. A differential effect was also apparent in the contralateral projections from the lesioned side. PVCN and AVCN exhibit greater reductions in their contralateral projections (90%) than does DCN (50% reduction). The ipsilateral projection from all 3 divisions on the lesioned side are significantly reduced (90%) compared to normal animals. The contralateral projections from the normal side are also significantly reduced, though to a lesser extent (40%).

These data show that neonatal unilateral cochlear ablations affect the morphology and connectivity of both ventral and dorsal cochlear nucleus. However, DCN appears less affected than either PVCN or AVCN, suggesting an important influence of descending input to DCN.

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20.16 PATTERNS OF LOCAL CEREBRAL GLUCOSE UTILIZATION IN THE INFERIOR COLLICULUS OF THE RAT. C. Huang, Dept. of Physiology, Univ. of So. Ala., Mobile, AL 36688, J. W. Dickson, J. Fex Laboratory of Neuro-otolaryngology, NINCDS and L. Sokoloff Laboratory of Cerebral Metabolism, NIMH, Bethesda, MD 20205.

The uptake of [^{14}C] 2-Deoxy-glucose (2DG) in the brain has been used extensively in the study of cerebral metabolic and functional activation. Although it is a potential tool for exploring the functional organization in the auditory system, a detailed and quantitative report has not appeared. This report deals with the effects of auditory stimulation in the inferior colliculus (IC) of the awake rat.

The general experimental protocol, including preliminary surgery and animal restraining, plasma sampling and analysis of glucose and 2DG, tissue processing and autoradiography, film reading and computation of local cerebral glucose utilization (LCGU) have been described (Sokoloff et al., 1977). Nineteen albino rats were divided into three groups. The first group of animals received binaural pure-tone stimulation at 2,4,8, and 16 kHz in a sound-proof room. Animals in the second group had one cochlea removed 24 hours before stimulation. The third group received no tonal stimulation and was placed in the sound-proof room or a laboratory with ambient noise.

In the stimulated animals, at least one of the IC showed one or two characteristic bands indicative of elevated metabolic activity. Densitometric readings for some of the bands showed a half-width of 100µm, approaching the practical limit of the resolution of the [^{14}C]2DG method. Frequently, a second band, approximately 500 µm in width also appeared. Values of LCGU for IC regions within the band often reached twice that of regions outside the band, which was 108 ± 25µ mole/100gm/min.

No such band was observed in animals that received no stimulation. However, LCGU for the IC from rats placed in the sound-proof room (135±7µ mole/100gm/min) did not differ significantly from that of rats receiving ambient noise (134±25µ mole/100gm/min). In the IC contralateral to the absent cochlea, LCGU was much lower, 55 ± 6 mole/100gm/min.

The results were consistent with previous evidence that the IC in the rat is primarily innervated through the contralateral cochlea, that the metabolic activation during sound stimulation within the IC can reach extremely high values and is not homogenous, suggesting a functional subparcelling. The three-dimensional representation of metabolic activation under pure-tone stimulation, noise stimulation, and no stimulation will be presented.

(supported by College of Medicine, Univ. of South Alabama).

Sokoloff, L. et al., J. Neurochem. 28 897-916 (1977).

20.17 EFFECTS OF NEONATAL UNILATERAL COCHLEAR ABLATIONS: A PHYSIOLOGICAL STUDY OF THE INFERIOR COLLICULUS. L.M. Kitzes* and K.S. Wrege* (SPON: J. Sassin) Depts. Anat. and Psychobiol., Univ. of Calif., Irvine, CA 92717.

Unilateral cochlear ablations were made in 2 day old gerbils. When the animals were 4-8 months old, multiple and single unit responses were studied in the inferior colliculus (IC) ipsilateral to the normal ear. These ipsilateral responses were compared to data obtained in normal animals. Pt-Ir electrodes were advanced through the IC in 100 μm steps and the response threshold for ipsilateral stimulation was determined. In order to assess the quality of the normal ear in the experimental animals and to compare ipsilateral and contralateral thresholds, at least one penetration was made into the IC contralateral to the normal ear. In each animal, the neonatal cochlear ablations were confirmed by the absence of activity evoked by stimulation of the ablated ear. Electrode tracks were reconstructed in all animals. In a series of experimental animals used for anatomical anlysis, the IC was explored with glass micropipette electrodes advanced in 250 μm steps.

In normal animals ipsilateral stimulation evoked excitatory responses in only 30% of the examined loci. In the remaining 70% ipsilateral stimulation evoked either inhibitory responses, or no response. A designation of no response was made if stimulation evoked no response at a stimulus level more than 40 dB above the contralateral threshold. The 40 dB cut off value was required because of possible contamination of threshold determinations by acoustic cross over. In many instances where ipsilateral stimulation evoked no excitatory activity, inhibitory interactions were apparent at stimulus levels within 10 to 15 dB of the contralateral theshold. In 42% of the loci in which both ears were effective, ipsilateral excitatory thresholds were between 15 and 40 dB greater than contralateral thresholds.

The proportion of ipsilateral excitatory responses in the IC greatly increased in animals subjected to neonatal cochlear ablation. In contrast to normals, ipsilateral stimulation evoked excitatory activity in 80% of the examined loci. The thresholds of ipsilateral stimulation were well within the range of contralateral thresholds. At suprathreshold stimulus levels the multiple unit responses were reliable and robust.

Our anatomical data indicate that neonatal cochlear ablations result in altered afferent projections to the IC. These physiological data suggest that the amount of ipsilateral excitation is dependent upon the integrity of the contralateral pathways.

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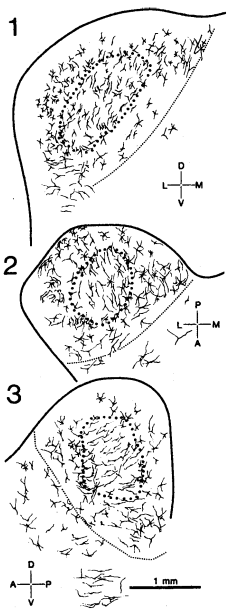
20.18 STRUCTURE OF LAMINAE IN THE CENTRAL NUCLEUS OF THE INFERIOR COLLICULUS OF THE BAT (ANTROZOUS PALLIDUS). Jeffery A. Winer. Department of Physiology-Anatomy, University of California, Berkeley, California 94720.



Studies of the architecture of the central nucleus of the mammalian inferior colliculus have demonstrated the orientation of some principal cell dendrites and the afferent axonal plexus into parallel, sheet-like aggregates termed laminae. These laminae could subserve physiological isofrequency contours imposed by cochlear nucleus axons on the central nucleus. To define the size and shape of individual laminae, serial maps of Golgi-Cox stained neurons have been reconstructed in the transverse, horizontal and parasagittal planes. These maps reveal that bat central nucleus laminae form gently curved and overlapping sheets stacked upon one another. The laminae are oriented as follows: in the transverse plane, from dorsomedial to ventrolateral at about a 75° angle (Fig. 1); in the horizontal plane, from anteromedial to posterolateral (Fig. 2); and, in the parasagittal plane, from anterodorsal to posteroventral, as inverted parabolae (Fig. 3). The laminae are most conspicuous in Golgi impregnations in the horizontal plane, and least apparent in Nissl-stained material. The pattern of nuclear organization recapitulates, with some important exceptions, the structure of individual nerve cells, and is present, with some variation, throughout the central nucleus. Four varieties of neurons are common to the central nucleus. The most common, (1) the disc-shaped cells, have a polarized soma from which dendrites emerge to form the long, flattened axis of the lamina. On the basis of the size of their perikaryon and dendritic field, both large and small disc-shaped cells occur. (2) Neurons with radiate, spherical dendritic arbors are also present. (3) Translaminar neurons have large dendritic fields orthogonal to the main axis of the laminae. (4) Both spiny and sparsely spined stellate cells occur. The dendrites of these classes of cells impose a distinctive pattern on the intrinsic and translaminar circuitry of the central nucleus.

21.1 BIOCHEMICAL AND HISTOCHEMICAL OBSERVATIONS ON THE FIFTH CRANIAL NERVE AND ITS RELATION TO MENINGES AND ITS BLOOD VESSELS. L. Liu-Chen, M. Mayberg*,M.A. Moskowitz. Dept. of Neurosurgery, Dept. of Neurology, Mass. General Hospital, Boston;Dept of Nutrition and Food Science, M.I.T., Cambridge, Ma 02139.

Histochemical and biochemical experiments using horseradish peroxidase (HRP) and radioimmunoassay were used to identify the existence of a neuronal pathway arising in the Vth cranial nerve and terminating in the meninges around the middle cerebral artery(MCA). In all 7 cats, cell bodies containing HRP were identified in the Vth ganglia after the enzyme was placed on the MCA. Neurons were found strictly ipsilaterally and were located among the cell bodies which project to the forehead. In other experiments the chemistry of the Vth nerve was examined in cat and rat. Measurable levels of substance P (SP) were detected in these species and ranged from 173.6 to 223.0 fmole/mg protein. In addition, levels of SP were easily measured in meningeal tissues (37.8-48.2 fmole/mg protein).

To determine the extent to which meningeal fibers containing SP arise from the Vth ganglia, the following experiment was performed. Male Sprague - Dawley rats (250-300 gms) were subjected to electrolytic lesioning of the right trigeminal ganglia using stereotaxic apparatus. The extent of the lesion was determined by the sensory deficits in response to pin prick over each division of the Vth nerve and the histological sections of this ganglia. Rats were sacrificed between 10 and 14 days after the lesion. SP content in the meninges was measured by radioimmunoassay. SP levels in meninges was significantly decreased on the lesioned side compared with control hemisphere. Animals subjected to electrolytic lesion but without evidence of sensory deficit failed to show a consistent reduction in SP content. Similar results were obtained in several preliminary experiments in cats following unilateral trigeminal ganglionectomy. These results suggest that trigeminal neurons which are SP-containing project to the meninges. The existence of such a pathway may be important in the transmission of pain or other sensory stimuli from meninges and its associated blood vessels.

21.2 ULTRASTRUCTURAL LOCALIZATION OF NEUROTENSIN-LIKE IMMUNOREACTIVITY IN THE DORSAL HORN OF THE RAT SPINAL CORD. V. Seybold and B. Maley. Dept. of Anatomy, Univ. of Minnesota Medical School, Minneapolis, MN 55455

The neurotensin-like immunoreactivity (NLI) observed within substantia gelatinosa at the light microscopic level is interpreted to be contained in axons and terminals of intrinsic neurotensin-containing neurons. After colchicine treatment, it has been possible to identify such cells in laminae II and III of the lumbosacral spinal cord of the rat (Soc. Neurosci. Abs. 6:428,1980). To confirm the localization of NLI in terminals and vari-cosities and to begin to appreciate the synaptology of neurotensin-containing neurons in the spinal cord, NLI profiles were studied at the ultrastructural level.

Normal and colchicine-treated (30 µg colchicine, intrathecally at the lumbosacral enlargement) rats were perfused intravascularly with phosphate buffered 4% paraformaldehyde and 0.1% glutaraldehyde. The lumbosacral spinal cords were excised, and 100 µm transverse sections were cut with a vibratome and immediately processed for immunohistochemistry using the PAP procedure of Sternberger. The rabbit anti-neurotensin serum was used at a dilution of 1/1000, and all antisera used in the procedure were diluted in 0.3% Triton X-100 and 1% normal sheep serum in phosphate buffered saline. Absorption controls revealed that the primary antiserum was specific for neurotensin.

The distribution of NLI processess and cell bodies observed at the ultrastructural level parallels the distribution observed in earlier studies. In normal animals, NLI was localized in varicosities and terminals within substantia gelatinosa. The terminals ranged in size from 0.5-2 µm and contained small, round, clear vesicles (40 nm) and several large granular vesicles (LGV, 100 nm). The DAB reaction product was generally observed to be adjacent to the cytoplasmic side of membrane-bound structures and over the LGV. Labeled terminals formed asymmetrical synapses onto unlabeled dendrites and spines. Axons containing NLI were unmyelinated and about 0.2 µm in diameter. NLI labeled cells, which were observed only in colchicine-treated rats, were contacted by unlabeled boutons (0.5-1 µm) containing round or pleomorphic vesicles.

These ultrastructural studies lend further evidence for a local modulatory role for neurotensin-containing cells in the dorsal horn.

Supported by grants from the Pharmaceutical Manufacturers Association Foundation and the Minnesota Medical Foundation.

21.3 THE LOCALIZATION OF BRAIN STEM ENKEPHALINERGIC AND SUBSTANCE-P NEURONS WHICH PROJECT TO THE RODENT NUCLEUS RAPHE MAGNUS. S.M. Prichard and A.J. Beitz. Dept. Anat., School of Med., Univ. of S. Carolina, Columbia, SC 29208.

The raphe magnus is considered an important component of the descending pain control system. The present investigation was undertaken to study the sites of origin of enkephalin (ENK) and substance-P (SP) projections to the nucleus raphe magnus (RM) in an attempt to elucidate some of the peptidergic systems which influence this region. Ten adult Sprague-Dawley rats were stereotaxically injected with HRP into the RM and 1-3 days later received an intraventricular injection of colchicine. The colchicine treated rats were sacrificed by intracardiac perfusion with 3.8% paraformaldehyde followed by PBS containing 25% sucrose. Brain sections were reacted with $CoCl_2$ and DAB to yield a black HRP reaction product in neurons projecting to the RM. Following HRP histochemistry alternate sections were incubated in antisera against ENK or SP and processed for PAP immunohistochemistry which yields a brown immunoreactive product in cells containing ENK or SP. Double-labeled neurons which contain either ENK or SP and project to the RM thus displayed both brown and black reaction product. Immunohistochemical controls were carried out by pretreating the antisera with homologous and heterologous antigens. The greatest number of both double-labeled ENK neurons and double-labeled SP neurons occurred in the nucleus (n) reticularis paragigantocellularis, the n. solitarius, and the n. cuneiformis. Double-labeled ENK cells were also observed in the inferior vestibular nucleus, the n. reticularis ventralis, the n. gigantocellularis, the n. pontis oralis and the superior colliculus. Additional substance-P immunoreactive neurons which project to the RM were found in the inferior vestibular nucleus and in an area located between the lateral reticular nucleus and the trigeminal nucleus caudalis. These results indicate that the majority of SP and ENK projections to the RM arise from the same brain stem nuclear groups. The functional significance of these dual inputs requires further investigation. (Supported by NSF BNS 7906486. We thank Dr. Robert Elde for the anti-ENK and anti-SP serum).

21.4 IS GABA THE NEUROTRANSMITTER OF BARNACLE PHOTORECEPTORS? L. C. Timpe*, K. King*and A. E. Stuart. Dept. of Physiol., U.N.C. Chapel Hill, C. H. North Carolina; Dept. of Neurobiology, Harvard Med. School, Boston, Mass.

We have examined the proposal that photoreceptors of the barnacle use gamma-aminobutyric acid (GABA) as their neurotransmitter (Millecchia and Gwilliam, Science 177:438-441,1972; Koike and Tsuda, J. Physiol. 305:125-138, 1980).

Recording intracellularly from the cell directly postsynaptic to the photoreceptors, the inverting or I-cell, we have studied the effects of bath-applied GABA and picrotoxin. The synapse between photoreceptor and I-cell inverts the response to light from a depolarization in the photoreceptor to a hyperpolarization in the I-cell, so we expect the natural transmitter to hyper polarize the I-cell. At concentrations less than 10^{-5}M GABA has no effect on the I-cell. At 10^{-5}M GABA slightly hyperpolarizes the I-cell; the response to a light in the presence of 10^{-5}M GABA is the same as in normal saline. Picrotoxin (10^{-4}M) affects neither the light response nor the resting membrane potential of the I-cell.

The proposal that GABA is the transmitter of photoreceptors is based partly on the observation that picrotoxin blocks the "off-response" of the visual system, a burst of impulses which can be recorded extracellularly from the circumesophageal nerve (Millecchia and Gwilliam, 1972). These spikes are generated in the A-cell, directly postsynaptic to the I-cell, when the I-cell depolarizes in response to the dimming of light. Recording intracellularly from A-cells we found that 10^{-4}M picrotoxin abolishes the large synaptic depolarization at the offset of light; this depolarization normally generates the spikes of the off-response. Our observation is consistent with the observation of Millecchia and Gwilliam. GABA is not the transmitter released from I-cells onto A-cells, however, because bath-applied GABA does not depolarize the A-cell.

The axonal GABA concentration of a known GABAergic cell, the inhibitory motoneuron of the lobster, is about 100mM (E. A. Kravitz and D. D. Potter, J. Neurochem. 12:323-328). Axons of non-GABAergic excitatory fibers from the same preparation contain 1mM GABA. Using high performance liquid chromatography we measured the GABA level of the barnacle's median ocellar nerve, which contains four large photoreceptor axons as well as many smaller fibers. If the total amount of GABA we measured were distributed uniformly in only the photoreceptor axons, its concentration would be less than 2mM.

Because GABA and picrotoxin have little effect on transmission from photoreceptor to I-cell, and because the photoreceptor contains only a small amount of GABA, we conclude that the barnacle photoreceptor is unlikely to use GABA as its neurotransmitter.

21.5 LIGHT STIMULATED RELEASE OF SUBSTANCE P FROM THE ISOLATED PERFUSED RABBIT RETINA. C. Schenker* and S.E. Leeman. Dept. of Physiology, Harvard Medical School, Boston, MA 02115 and Dept. of Physiology, U.Mass.MC, Worcester, MA 01605.

The vertebrate retina contains several subpopulations of peptidergic amacrine cells and immunohistochemical evidence suggests that each different peptidergic amacrine cell also corresponds to a different morphological subtype (for review see Stell etal, TINS, Dec 1980). It is therefore likely that each of these different cells subserves a different as yet unknown function and each may be activated by a different pattern of environmental light/dark input. Assuming that a neuron releases its transmitter upon activation one can use transmitter release to monitor the activity of chemically identified neurons. In this sense we have used the isolated perfused rabbit retina to monitor the activity of substance P containing amacrine cells under different conditions of stimulation. Perfusates were collected at 2min intervals and assayed for immunoreactive substance P (I-SP) by radioimmunoassay. In the dark a small and variable baseline efflux can be detected. Upon stimulation with flashing light (50msec pulses, 3Hz) the I-SP levels rise significantly above baseline during the period of stimulation. The rate of I-SP release varied with light intensity. At the highest intensity of light used (500$\mu W/cm^2$) the mean increment over baseline ranged from 10-20 fmol/2min. With light stimuli of lower intensity smaller increments in the release of I-SP over baseline were obtained. The release of I-SP (increment over baseline) also varied in duration with the duration of the stimulating period. This interesting feature of the pattern of release of I-SP is particularly apparent at high photopic stimulation. Under these conditions and for periods of stimulation of 3-12 min the total response time is ca. 2.5x the period of stimulation.

At this point one can only speculate about the role played by the I-SP containing amacrine cells in the retina. The persistence of the release of I-SP after cessation of a high photopic stimulus could indicate that the I-SP containing amacrine cells participate in the formation of an after-image.

Supported in part by grant AM 16510 to SEL and NIH training grant T32 GM0725 (CS).

21.6 BEHAVIOR ELICITED IN RATS AFTER INTRATHECAL ADMINISTRATION OF PEPTIDES. G.L. Wilcox, J.L.K. Hylden and V.S. Seybold. Depts. of Pharmacology and Anatomy, Univ. of Minnesota Medical School, Minneapolis, MN 55455

Biochemical and immunohistochemical evidence indicates that substance P (SP) and somatostatin (SOM) are contained in small diameter primary afferent neurons. Excitatory or inhibitory effects observed in response to these substances in neurophysiological studies supports their role as neurotransmitters in primary afferent neurons. Given a putative role in somatosensation one would expect exogenous application of these substances to the spinal cord would elicit behavior consistent with perception of a somatosensory stimulus. This observation of behaviors in mice in response to intrathecal SP and SOM (Hylden and Wilcox, Brain Res. In Press) led us to study the effects of these peptides in rats.

Rats (300 g, male, Holtzman) were implanted with indwelling 8.5 cm PE-10 intrathecal catheters a minimum of 7 days prior to the study. Animals exhibiting impaired motor function were excluded from the study. Rats were conditioned for 30 min a day for 3 days to sound insulated operant chambers equipped with interior lights and a one-way mirror. Peptides (in a vehicle of 0.01M acetic acid in saline), vehicle or saline (5-10 µl, 10 µl/min) were infused with an infusion pump connected with the catheter via PE-10 tubing. The rats were observed blind in the closed chamber for 3 min before and 5-15 min after infusion. Each bite to the abdomen or hind quarter and each episode of hind limb scratching was counted as 1 behavioral event.

Intrathecal SP (20 ng-10 µg) elicited dose related biting and scratching, with a maximum of 29 ± 5 responses/5 min occurring at the highest dose. The duration of the response was 5 min, and the peak effect occurred at 2 min after the start of the infusion. Intrathecal SOM (1-20 µg) caused dose related hind limb scratch-ing only, with a maximum of 40 ± 8 responses/10 min. The duration of the response was at least 10 min, and the peak effect occurred 5-6 min after the start of the infusion. Infusion of vasoactive intestinal polypeptide (VIP, 10 µg) or neurotensin (10 µg) was without effect.

These results are the first demonstration of behavioral effects of SP and SOM at the spinal level in rats. The different composition of behaviors elicited by each of the peptides suggests that each mediates different aspects of somatosensation. The differences in their time courses are consistent with the ob-served differences in their distribution within the laminae of the dorsal horn. Supported by USPHS grants DA01933, T32GM07397, and RR05385 and by the PMAF.

22.1 NEURAL DISCHARGE PATTERNS AND MECHANORECEPTOR TRANSDUCTION. D. J. Barker, Dept. of Physiology, Texas College of Osteopathic Medicine/North Texas State Univ., Fort Worth, TX 76107.

The work reported here stems from an attempt to infer transfer functions of mechanoreceptors in raccoon glabrous skin from quantitative studies of stimulus-response relationships utilizing a variety of mechanical stimuli. A mechanical model of the skin and underlying receptors was derived from a subset of the data. The model consisted of a skin simulation and a simulated neuron, and was constructed under the assumption that the temporal pattern of firing of first-order neurons reflected the shape of the generator potential which in turn was related to the force actually applied to the receptor core. For any given stimulus input, the model would output the neural response pattern. The nature of the transduction process could be inferred from the model and predicted outcomes tested experimentally. The data presented here concern an experimental test of the model. The data from which the model was constructed was collected in 9 acute recording experiments under pentobarbital anesthesia. Responses of single neurons innervating the glabrous skin of the nose were recorded from the trigeminal ganglion. Stimuli were delivered with a feedback controlled axial displacement generator. Single unit data was digitized and analyzed by computer. Responses of 23 neurons to constant velocity trapezoidal stimuli were used to construct the simulated mechanoreceptors on an analog computer. Based on the temporal pattern of firing, two distinct mechanoreceptor response types were discernable: the first type (N=13), called IR, included both slowly and rapidly adapting units and showed an increasing rate of discharge to a constant velocity ramp. The second type (N=10), called DR, showed the opposite firing pattern, a decreasing rate of discharge, to the same stimulus. The model suggested that the effective stimulus for the IR neurons was a filtered first derivative of the applied stimulus, or velocity, while the effective stimulus for the DR neurons was a filtered second derivative, or acceleration. The IR and DR neurons also responded differently to sinusoidal stimulation with respect to the point in the sine wave cycle where the second spike appeared. This difference was predicted by the model. The model was then used to predict the response of DR neurons to a constant acceleration (parabolic waveform). The model suggested that DR neurons would respond with an IR pattern in response to constant acceleration. This finding was confirmed in two subsequent experiments. The results suggest that the model may be useful in describing the transduction process of glabrous skin mechanoreceptors in raccoon rhinarium. Further experiments in other species will be needed to determine the generality and utility of this mechanoreceptor model. Supported by USPHS Grants 5326 and 06225.

22.2 EFFERENT INPUT MODULATES THE PHYSIOLOGY OF THE LIMULUS LATERAL EYE. R. Batra*, L. Kass*, and R. B. Barlow, Jr. Institute for Sensory Research, Syracuse University, Syracuse, NY 13210.

Circadian efferent activity increases the sensitivity of the Limulus lateral eye at night. At least two receptor mechanisms mediate the increased sensitivity: structural changes that enhance quantum catch (absorbed photons/flash) and physiological changes that increase gain (optic nerve impulses/absorbed photon). We report here several quantitative studies of the circadian changes in the physiological properties of single ommatidia in the dark adapted Limulus eye. In each study light stimuli were delivered to single ommatidia via a 70-μm light pipe that optically isolated the receptor and thus minimized the effects of lateral inhibition. The nighttime state of the retina was simulated by shocking the optic nerve.

Frequency transfer characteristics (FTCs) of optic nerve responses were measured for various response rates. The FTCs at high impulse rates exhibit a circadian rhythm. Those at night for high rates resemble those during the day for low rates.

Temporal distribution of optic nerve impulses also exhibits a circadian rhythm. For a dark adapted eye during the day the optic nerve discharge is irregular at low response rates and becomes progressively more regular at high rates. At night an irregular distribution of nerve impulses also characterizes high discharge rates. Interval histograms of the impulse discharge in the absence of illumination and at low light intensities reveal a peak at short intervals (less than 100 ms) which is pronounced at night, but reduced or absent during the day. This peak may reflect a period of elevated excitation in the neighborhood of a nerve impulse.

Coding of light intensity depends both on level of illumination and time of day. Single photon absorptions may be more likely to generate multiple impulses at night than during the day--a possible mechanism for increasing photoreceptor gain.

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22.3 SYSTEM IDENTIFICATION APPLIED TO THE VENTRAL PHOTORECEPTOR OF *LIMULUS*. N. A. Farahbakhsh* and E. R. Lewis. Electronics Research Laboratory, University of California, Berkeley, CA 94720.

An irreversible thermodynamic network approach proved to be particularly convenient and enlightening in the characterization of linear responses and steady-state light adaptation in photoreceptors, and led directly to hypotheses regarding the mechanisms underlying those phenomena (Fuortes, M.G.F and Hodgkin, A. L., J. Physiol., 172:239, 1964; Baylor, D.A., Hodgkin, A.L. and Lamb, T.D., J. Physiol., 242:759, 1974). The network approach capitalized on the fact that when linear small-signal behavior and nonlinear large-signal behavior (such as light adaptation) are combined in a single device, the linear behavior can be characterized in terms of the pole-zero distribution of the small-signal impulse response and the nonlinear behavior can be characterized in terms of the shifting of that distribution in response to large signals. Recently-developed, digital-computer algorithms make possible the identification of pole-zero distributions implied by small-signal impulse response data in a manner much more precise and refined than that available for the earlier photoreceptor studies. We are applying one such algorithm (Provencher, S.W., Biophys. J., 16:27, 1976) to the small-signal impulse response of the ventral photoreceptor of *Limulus polyphemus*, looking not only at the steady-state pole-zero distributions corresponding to various degrees of adaptation, but also at the dynamics of the distribution shifts. Since small-signal impulse-response pole-zero distributions generally are directly interpretable in terms of networks of irreversible thermodynamic processes, the shifting of those distributions should lead directly to insights regarding the mechanisms underlying phototransduction and light adaptation.

22.4 DROSOPHILA MUTATIONS DISPLAYING SEMIDOMINANT EFFECTS ON RHODOPSIN CONCENTRATIONS. N.J. Scavarda*, J.E. O'Tousa* and W.L. Pak. Dept. of Biol. Sci., Purdue Univ., W. Lafayette, In. 47906.

A series of EMS induced single-gene mutations that reduce the rhodopsin concentration in the compound eye of Drosophila have been isolated.These fall into five complementation groups (nina A, B, ..E). The prolonged depolarizing afterpotential (PDA), present in photoreceptors of wild type when a substantial amount of rhodopsin is photoconverted to metarhodopsin, is either absent or reduced in size in these mutants. The third chromosome mutation, ninaE, is of special interest because (1) its effect is specific to one particular class of photoreceptors and (2) at least one allele, ninaE^{P332} exhibits semidominant effects with respect to both the ERG phenotype and rhodopsin concentration. Heterozygous ninaE^{P332} flies less than 2 days old display an ERG phenotype indistinguishable from homozygous mutant flies. As the heterozygotes age from 3 to 14 days, their ERG phenotype becomes progressively more wild type. In vivo microspectrophotometric measurements of homozygous ninaE^{P332} individuals indicate rhodopsin levels of 0-1% of wild type levels. Heterozygous individuals up to 2 days of age contain approximately 20% of wild type levels, while 3-14 day old heterozygotes have approximately 55% of wild type levels. Optical studies indicate that ninaE^{P332} has normal rhabdomeres in all classes of photoreceptors. Complementation analysis and preliminary mapping results suggest, however, that ninaE^{P332} may be allelic to the previously described ora^{JK84} (outer rhabdomeres absent: Koenig and Merriam, 1975; Harris et al., 1976), which lacks the rhabdomeres in R1-6 photoreceptors. O'Farrell's two dimensional gel electrophoresis was used to examine eye protein patterns in ninaE mutant and wild type flies. Flies were exposed to various regimens of light and dark adaption prior to immersion in liquid nitrogen, acetone-dry ice dehydration and dissection of the ommatidial layer. Preliminary findings indicate that protein patterns from wild type, homozygous and heterozygous mutant flies are affected differently by different adaption regimens.

22.5 VISUAL INFORMATION GENERATES CONCURRENT DIFFERENT CODE TYPES. C. Torda.Res.Dpt.N.Y.C.PA.Training,N.Y.,N.Y.,10028(currently at Stanford University,P.O.Box 4866,Stanford,Calif.,94305).

Photic stimuli activate specific tunable photoreceptors in the retina.Intracellular recordings of bioelectric processes of the illuminated rods of Bufo marinus were performed during changing concentrations of Ca of the intermembrane space of the outer rod segment in presence of changing concentrations of cyclic GMP.The conclusion was reached that the illumination dependent membrane hyperpolarization is a Ca-dependent process. The illumination intensity dependent rapid changes of hyperpolarization is made possible by near-threshold maintenance of membrane potential by cGMP. Several mechanisms involved in regulation of the cyclic GMP concentration have been identified. The Ca-dependent hyperpolarization seems to be the first encoding of photic stimuli.Through automatically ongoing prevalently parallel processing at various loci between retina and CNS centers the photic energy is transformed to spatial frequency codes. The ascending retinal 2-D codes are transformed in the lateral genicular to 3-D interference patterns.- Part of the visual stimuli ascending through the superior colliculuc activate saccade programming neurons contained in the intermediate and deeper layers of SC.The saccade programming neurons transmit this code into the firing rate of phasic units located in the rostral zone of the paramedian pontine reticular formation. By integration of these phasic pulses the periabducens neurons generate the tonic components of the saccade. The rapid saccades contain and transmit a partial code of the visual stimulus that originated them. Since this code ascends in a 1 or 2-D form it is able to function as a fast allocator of visual memory traces deposited in the diffuse memory system. Interference with memory retrieval of severe modifications of rapid saccades have been studied through behavior and bioelectric potential measurements during the early phases of conditioning of the nictitating membrane reflex of the rabbit to an unusual visual stimulus.The results led to the above conclusions.--One of the pertinent steps of impulse processing by the CNS is the evaluation of their novelty value. This is accomplished by match-mismatch processing at various loci incl. the hippocampus. Bioelectric measurements of the activities of CA3,CA1 hippocampal pyramidal neurons and dentate fascia granule cells during various experimental conditions, incl. transsection of the perforate pathways and exposure to new and old stimuli suggest presence of significant differences in CA3 bioelectric potentials during new and old stimuli, and presence of two types of CA1 pyramidals. The CA1(1) type codes the impulses into a form destined for incorporation into higher memory systems.The CA1(2) type(stimulus modality specific cells) program a decision for selection of the executory mechanisms able to create a stimulus adequate response.Encoding from retina to CNS depends on Ca gating.

22.6 ADAPTATION IN HAIR CELLS: IN VITRO INTRACELLULAR RESPONSES AND IN VIVO MICROPHONIC POTENTIALS FROM A VESTIBULAR ORGAN. R. A. Eatock and A. J. Hudspeth. Division of Biology, California Institute of Technology, Pasadena, CA 91125.

Hair cells of the bullfrog sacculus adapt in vitro to prolonged stimuli, showing a progressive decline in the extracellularly recorded microphonic potential and in the intracellular receptor potential (Eatock, R. A., Corey, D. P., and Hudspeth, A. J., Soc. Neurosci. Abstr. 5: 19, 1979). The intracellular studies have been extended so that adaptation of the receptor potential and receptor current of single cells can be compared. We also report in vivo adaptation of the microphonic potential.

For intracellular recording the sacculus is dissected from the animal and placed in a saline-filled chamber. Single hair cells are stimulated by displacement of a probe inserted between the stereocilia and the kinocilium of the hair bundle; either the receptor potential or receptor current is then measured, the latter by a two-electrode voltage clamp. In response to a prolonged deflection of the hair bundle (step stimulus), the receptor current rises to a peak, then declines with a time course that is consistent with the rate of adaptation of the in vitro microphonic response. Adaptation occurs to stimulus steps toward or away from the kinocilium, of saturating amplitude or within the operating range of 2^{o} hair bundle bend. The decline in response results from a change in the cell's displacement-response curve, which relates displacement of the tip of the hair bundle to the membrane potential or current. During adaptation the curve shifts along the displacement axis without a significant loss in sensitivity. Although the form of the receptor potential during a step is complicated by voltage-sensitive conductances, the shift in the cell's displacement-response curve follows the same time course whether the response is measured as receptor potential or current. Adaptation has at least two phases with different rates, the early phase being considerably faster than the later. By 100 ms after the onset of a step, the response approaches a plateau level of 0-60% peak value.

The natural stimulus for the frog's sacculus is thought to be linear acceleration. For in vivo studies of adaptation, the animal is mounted on an electrodynamic shaker which delivers steps of constant acceleration. The microphonic potential recorded from the sacculus reveals adaptation to such stimuli at a rate similar to that of the in vitro preparation. Because the mechanical properties of the sacculus have not been described, the relative contributions of mechanical and receptor relaxations to the in vivo adaptation remain to be determined.

(Supported by NIH grants NS-13154 and GM-07616)

22.7 THE BASIS FOR RESPONSE COMPRESSION & SHIFT OF THE OPERATING CURVE DURING ROD LIGHT ADAPTATION. K. N. Leibovic, Biophysics, SUNY/B Buffalo, NY 14214

It is well known that rod photoreceptors, functioning over some six orders of magnitude of light intensity, operate only over some three orders of magnitude, at most; and during light adaptation the operating range shifts to higher light intensities, while at the same time the response amplitude is reduced.

The basis for these phenomena was studied with the aid of intracellular recording in rods of Bufo Marinus. The retina was isolated from dark adapted animals and the responses to 100 mS flashes were recorded in the presence of different light backgrounds and at different levels of bleaching.

We have confirmed that the response is reduced in amplitude and speeded up in time in the presence of a background. We have, furthermore, obtained evidence that the response amplitude is reduced, but that the response kinetics remain invariant after bleaching up to 90% or more of the pigment. Based on our model of the internal transmitter we have carefully analyzed the response waveform in the light of our results. On the assumption that the internal transmitter levels, controlled by the light flux, modulate the outer segment membrane conductance, we have shown that bleaching reduces the effect of light on the amount of transmitter either released or sequestered.

We conclude that bleaching

(1) reduces response amplitude

(2) raises the threshold and shifts the operating range through the mechanism which reduces the effect of light on active transmitter.

This work was done in Dr. Dowling's laboratory at Harvard during the author's leave from SUNY/B.

22.8

WITHDRAWN

22.9 CHEMICAL SYNAPTIC TRANSMISSION BETWEEN TYPE I HAIR CELLS AND THE CALYX OF VESTIBULAR PRIMARY AFFERENTS.
D.A. Schessel* and S.M. Highstein. Dept. Neuroscience, Albert Einstein College of Medicine, Bronx, NY 10461.

The mode of synaptic transmission between the Type I hair cell and the caliform terminal of vestibular primary afferents was investigated in the lizard Calotes versicolar. Afferents at the base of the horizontal semicircular canal were impaled within 1-6 microns of the synapse with horseradish peroxidase (HRP) or KCl loaded glass microelectrodes. Resting potentials were typically-60mV and spontaneous action potentials (APs) occurred at either irregular or regular intervals (500-5ms). A calyx termination in the horizontal semicircular canal crista was demonstrated for single irregular afferents injected intracellularly with HRP. In irregular afferents a high activity level of sub-threshold synaptic events (0.5-5mV) typical of the chemical EPSPs seen in Type II hair cell-afferent synapses in other systems was demonstrated. APs in irregular afferents arose from these summated EPSPs. Local application of $CoCl_2$ (15-20uM) reversibly blocked these EPSPs. Application of tetrodotoxin (TTX) 1uM blocked APs leaving sub-threshold synaptic activity unchanged. In control or TTX experiments the frequency of EPSPs and resting potential were modulated by hot and cold caloric probes. Resting potential changed by $\pm$ 8mV. With cold stimulation the smallest EPSPs were in the range of 0.5mV. At "normal" or calorically increased release rates EPSP rising and falling phases were roughly symmetrical. At calorically reduced release rates EPSP decay times were prolonged. Further, bridge hyperpolarization augmented, and depolarization decreased EPSP amplitude. These findings are consistent with chemical transmission between Type I hair cells and the calyx of vestibular primary afferents. Release rate dependent EPSP decay times suggest that the calyx adapts its time constant to sample discrete (not summated) events at high levels of synaptic activity.

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23.1 ULTRASTRUCTURE OF AGING AT NEUROMUSCULAR JUNCTIONS OF FAST AND SLOW MAMMALIAN MUSCLES. M. A. Fahim* and N. Robbins. Dept of Anatomy, Case Wes. Res. Univ., Sch. of Med., Cleveland, Ohio 44106.

An ultrastructural morphometric comparison was made between the motor endplates of aged (29 mo.) and young mature (7 mo.) male CBF-1 mice, in the extensor digitorium longus (EDL; fast twitch) and soleus (Sol; slow twitch) muscles. In old mice, nerve terminals of both EDL and Sol exhibited pronounced loss of synaptic vesicles and increases in numbers of microtubules, neurofilaments and smooth endoplasmic reticulum compared with young mice. These changes were particularly severe in the Sol where postsynaptic folds devoid of nerve terminals of Schwann cells were occasionally observed. Also, axoplasmic fragmentation with envelopment of axon terminals by Schwann cells was observed in several aged terminals, as was the extension of Schwann cells into synaptic clefts, resembling the early stages of denervation. Mitochondrial area (as a % of nerve terminal area) was lower in all aged nerve terminals than in young terminals. Abnormal mitochondria, when present in axon terminals, were associated with similar abnormalities in the more proximal axon. Synaptic clefts were widened in old animals and the junctional folds of aged terminals were more branched with frequent vacuole-like vesicles in the subsarcolemmal zone. Lipofuchsin deposition was prominent within the muscle fibers of aged Sol and EDL muscles but other muscle structures were not qualitatively different from young mice. Thus, the major ultrastructural changes associated with age in this study were presynaptic, although minor postsynaptic changes were also evident. These findings contrast with corresponding physiological data (Robbins & Kelly, abst. this vol.) indicating no deficit in short-term evoked transmitter release at mouse neuromuscular junctions. Therefore, profound morphologic changes observed with aging are either not rate-limiting or are compensated by other factors.
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23.2 EVOKED TRANSMITTER RELEASE IN YOUNG AND OLD MOUSE NEUROMUSCULAR JUNCTIONS. N.Robbins and S.S. Kelly* (SPON:J.P. Conomy). Dept. Anat., Case Western Res. Schl. Med., Cleveland, OH 44106.

Profound ultrastructural changes occur at aging neuromuscular junctions (e.g. Gutmann et al,J. Physiol. 219:331,1971; Fujisawa, Exp. Gerontol. 11:43,,1976; Fahim and Robbins, this vol.). In order to determine whether such changes imply functional deficit, and whether muscles with different activity patterns show differential age changes in synaptic function, neuromuscular transmission was examined in 3 muscle groups of young (11-13 mo) and old (34-35 mo) CBF-1 male mice. The muscles were: extensor digitorum longus (EDL), a phasic muscle; soleus (SOL), a tonic muscle, and the diaphragm (DIAPH), a rhythmically active muscle.

Action potential voltage threshold and resting membrane potential were unchanged with age in all 3 muscle groups. Endplate potentials (EPPs) were measured in curarized (2.6 uM) preparations stimulated at 2 and 20 Hz. In old EDL and SOL, initial EPP amplitudes were increased 122% and 93%, respectively, even though miniature-EPP (MEPP) amplitudes in uncurarized preparations were unchanged. During trains of 50 EPPs, plateau EPP amplitude was also increased by about 100% (EDL) or 67% (SOL). The ratio, $(\text{mean EPP amplitude})^2/(\text{EPP variance})$, showed the same proportional age-related increase as EPP amplitudes. Thus, the relative safety factor of transmission was markedly increased, most probably through an increase in average quantal content.

By contrast, under the same conditions, the DIAPH showed no change in EPP amplitude or safety factor with age, even though MEPP amplitude was increased 54%. Thus, DIAPH ages differently from limb muscles, and the two limb muscles with different activity patterns, show similar age changes in evoked release. Further, none of the aging muscles examined showed deficits in synaptic output during short trains. In old EDL and SOL junctions, where number of synaptic vesicles is reduced (Fahim and Robbins, op. cit.), compensatory mechanisms such as increased rate of vesicle recycling may serve to maintain transmitter output.
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23.3 RAPID REORGANIZATION OF SYNAPSES ON MATURE PURKINJE CELLS. S. Chen* and D.E. Hillman (SPON: John Pearson). Dept. Physiol. and Biophys., New York Univ. Med. Ctr., 550 First Avenue, New York, N.Y. 10016.

Purkinje cells in adult rats responded to partial deafferentation of parallel fibers by alteration in spine size and synaptic profile contact lengths (Hillman & Chen, Soc. Neurosci. Abst 6:822, 1980). Observation of lesioned parallel fiber beams for early changes revealed reinnervation of Purkinje cell spines before degenerating boutons were removed. In addition, new synapses developed and new spines formed. Since reactive synaptogenesis could not completely compensate for the massive loss of afferents, synaptic contact size increased inversely to the deficit in afferents.

Adult rats were anesthetized and the cranium opened over the cerebellar vermis. Parallel fiber beams were sectioned twice at a 1 mm interval by inserting a microknife through the dura and transverse penetration of the folial crest. Animals were allowed to survive for 2, 4, 6, 9, 14, 21, 24 and 48 hours and were then perfused with phosphate-buffer paraformaldehyde and glutaraldehyde. Sagittal slices of the folia were postfixed with a osmium-ferrocyanide mixture and block stained with 2% uranyl acetate.

Ultrastructural examination of thin sections revealed that degeneration of parallel fiber shafts and boutons began by 4 hours. At 6 hours the shafts were fragmented and phagocytized by glia; while at 9 hours, dense boutons showed stripping from the spines. Other spines remained shrouded by ghosts of parallel fiber plasmalemmae that were sandwiched between the glia and the spine. At this time, the frequency of dual parallel fiber synapses on spines increased significantly over control preparations indicating marked synaptogenesis on original spines. Within 10 hours one parallel fiber bouton was normal and the other showed degeneration. Each normal bouton formed a new synaptic site that was adjacent to the original contact. Later (24 hours) numerous spines contacted two normal boutons. New parallel fiber connections also formed with spiny dendritic shafts and new spines emerged. These primitive contacts developed on dendritic shafts not shrouded by glia. Numerous coated vesicles were observed in dendrites near the reactive zone. As the synaptic site differentiated, synaptic vesicles accumulated at the synaptic site. Other changes included complex spine formation and small side projections of parallel fibers. Supported by USPHS Grants HD10934 and NS13742.

23.4 BILATERAL CIRCUITRY REMODELING IN THE DENTATE GYRUS OF YOUNG ADULT VERSUS AGED RATS AFTER A UNILATERAL ENTORHINAL LESION, S.F. Hoff, S.W. Scheff, C.W. Cotman. Dept. Psychobiology, University of California at Irvine, Irvine, CA 92717.

Partial deafferentation of the hippocampal formation after a unilateral entorhinal lesion brings about the reinnervation of the denervated zones by the remaining afferent imputs. We have studied the reinnervation in denervated zones and also the concurrent response in zones not directed denervated by the lesion in aged vs young adult animals. Unilateral electrolytic lesions of the entorhinal cortex were placed in 90 day old and 2 year old male Sprague-Dawley rats. These animals were allowed to survive for 2,4,10,60,120 or 180+ days post-lesion and were then sacrificed and prepared for electron microscopy. Over the time course synaptic profiles were quantified on electron microscopic montages of the dentate molecular layer, which was divided into inner (innervated by the commissural/associational system), middle and outer (innervated by the entorhinal cortex) zones. In all, 71,700 normal and degenerating synaptic profiles were counted over 130,000 square microns of tissue.

Within the denervated outer two-thirds of the ipsilateral molecular layer, approximately 85-90% of all synapses are lost after an entorhinal lesion. Synaptic replacement begins very quickly in young adult rats, but has a slower onset in aged rats. Both age groups attain their pre-lesion synaptic density by the end of the time course. The delayed onset of reinnervation in the aged rats appears to correspond to the slower removal of degeneration by glial cells (astrocytes). This response may involve the elevated glucocorticoid levels observed in aged rats, which cause astrocytic hypertrophy and stabilize lysosomal membranes, thus impeding the clearance of degeneration debris. It is interesting to note that within the ipsilateral inner molecular layer (non-denervated) both age groups demonstrate a non-degenerative loss and reacquisition of 20% of their synapses. Within the contralateral molecular layer, however, the young adult rats demonstrate a 37% loss of synapses after an entorhinal lesion, with the majority replaced by the end of the time course; aged rats do not show a significant change in synaptic density over the same time course. Our data show that aged rats have a diminished capacity to respond to a lesion as demonstrated by a slower rate of reinnervation in denervated zones and a lack of response in the contralateral molecular layer, possibly resulting from an age-related stabilization of neuronal circuitry and subsequent decrease in sensitivity to impaired circuitry activity. Supported by NIA grant AG00538 and NIMH grant MH19691.

23.5 MODIFICATIONS OF GRANULE CELL DENDRITES DENERVATED BY ENTORHINAL LESION. A. Caceres* and O. Steward (SPON: E. Rubel). Dept. of Neurosurgery, Univ. of Virginia, Sch. of Med., Charlottesville VA 22908.

Following lesions of the entorhinal cortex (EC), spine density on granule cells of the dentate gyrus falls 30% below control levels, and then increases to normal values with the reinnervation of the dendrites (Parnavelas et al., Nature:248,71, 1974). Although this and other aspects of the response of the dentate granule cell to partial deafferentation have been extensively studied, there are still no data about the effect of EC lesions on the organization of dendritic fields. This study presents some qualitative observations about such effects.

Adult male rats were sacrificed at 2,4,8,10,14,30 and 60 days after unilateral lesions of the EC. Tissue including the mid-septotemporal portions of the hippocampus was immersed in fixative overnight, impregnated by the rapid Golgi method and embedded in celloidin. Sections were cut at 180 μm and camera lucida drawings were made of granule cells at a final magnification of 580x. A minimum of 15 granule cells was analyzed for each survival time; granule cells of the contralateral hippocampus and from non-lesioned animals served as controls.

Dendritic fields of normal granule cells are highly polarized, with a characteristic inverted cone appearance. To obtain this shape dendrites arch rapidly after leaving the granule cell layer and with successive branching extend laterally as they ascend through the molecular layer; the majority of them end in close proximity to the outer boundaries of the molecular layer. Following EC lesions, dendritic fields of granule cells are narrower and dendrites display an abnormal morphology. The dendrites appear normal in the inner molecular layer; however, when they reach the denervated zone they suddenly change direction and follow a course that tends to be parallel to the granule cell layer. Moreover, the dendrites only occasionally reach the more distal portions of the molecular layer. In addition, there is a progressive dendritic distortion more evident in the outer molecular layer; this includes an apparent loss in dendritic mass, varicosities, and sudden reductions in dendritic diameter. These abnormalities are most evident between 8 and 14 days postlesion. By 30 and 60 days postlesion distortions are no longer evident although some cells still display abnormal dendritic fields.

The early postoperative changes may be related to the physical disruption of synaptic contacts, and/or the elimination of the presynaptic input either in terms of bioelectrical and/or trophic activity. The normal appearance of dendrites at long postlesion intervals may reflect dendritic remodeling with reinnervation.

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23.6 THE EFFECTS OF COMPLEX OR ISOLATED ENVIRONMENTS ON CORTICAL DENDRITES OF MIDDLE-AGED RATS. Edward J. Green*, Barbara E. Schlumpf*, and William T. Greenough. Dept. of Psychol. & Neur. & Behav. Biol. Program, Univ. IL, Urbana-Champaign 61820.

Previous reports have described alterations in dendritic branching subsequent to housing rats in complex or isolated environments at weaning or as young adults. Studies involving older organisms have demonstrated the continuing neural responsiveness of the brain to experience, as reflected in gross morphological and biochemical measures. Recent evidence suggests that some neurons retain the capacity to grow and form new connections well into adulthood, and possibly, senescence. In the present experiment, female hooded rats (retired breeders) were housed two per cage until 15 months of age. They were then placed in either a complex toy-filled group environment (EC) with other rats of similar age or housed singly in standard laboratory cages (IC). After 45 days of differential housing their brains were removed and stained with the Golgi-Cox method. Each brain was evaluated independently for completeness of stain. Coronal sections (150μm) through visual cortex were taken, and 15 Layer IV stellate and Layer III pyramidal neurons from each animal were traced at 500x using a semiautomatic computer-microscope system. All data collection was done on coded slides which did not reveal the treatment of individual animals. The number of intersections of dendrites with concentric spheres spaced at 20μm intervals were used to estimate dendritic density. Preliminary statistical analyses reveal that stellate and pyramidal neurons sampled from EC rats each exhibited more total dendritic material than neurons from IC rats ($p<.02$ for both populations; two tailed Mann Whitney U test). Complete sphere analyses, as well as the results of a dendritic branching analysis will be presented. These results are the first demonstration of alterations in dendritic structure induced by differential environments in middle-aged rats.

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23.7 EFFECTS OF HANDEDNESS TRAINING ON DENDRITIC BRANCHING OF NEURONS IN FORELIMB AREA OF RAT MOTOR CORTEX. John R. Larson* and William T. Greenough. Depts. Psychol. & Anat. Sci. & Neur. & Behav. Biol. Program., Univ. IL, Urbana-Champaign 61820.

Prior work has shown altered dendritic patterns in adult rat occipital cortex following extensive maze training (Greenough et al., Behav. Neur. Biol., 26:287, 1979; Chang & Greenough, Soc. Neurosc. Abst., 1490, 1978). The handedness reversal paradigm allows investigation of neurons in a region known from lesion and electrophysiological studies to be involved in performance of reaching (Peterson & Devine, J. Comp. Physiol. Psychol., 56:752, 1963; Dolbakyan, et al., Neuroscience, 2:73, 1977). Unilateral handedness training also provides the advantage of a within subject control, since hemispheres opposite trained and non-trained paws can be compared.

Male rats (70-80 days of age) were tested for initial paw preference in reaching into a 1 cm dia. tube for food, then subjected to one of three training procedures: training on the initially non-preferred paw (reversal), training on the preferred paw (practice), or training both paws (alternation) in 33 sessions over 15 days. An additional group of control rats was tested for preference but not trained. Posttraining tests indicated reversal training effectively altered paw preference while other training strengthened original preference. Golgi-Cox stained Layer V pyramidal neurons (the major efferent cells) of the forelimb region of motor cortex (located by electrical stimulation) in each hemisphere were traced with a computer-based tracking system. Dendritic fields were quantified by counting intersections of dendrites with concentric spheres centered at the cell body.

In the Reversal group, distal (greater than 250μm) regions of apical dendrites of neurons in the hemisphere projecting to the trained paw had more extensive dendrites than those in the hemisphere projecting to the nontrained paw. In the Practice group similar but smaller effects were observed. Control and Alternation rats had no consistent interhemispheric differences relative to paw preference in apical dendritic branching, although there was a tendency for Alternation rats to have more extensive basal dendritic branching in neurons in the hemisphere projecting to the preferred paw. Tests of handedness on tasks other than reaching for food indicated that handedness in the rat is not a very general trait and that training effects on handedness were task-specific.

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23.8 CORRELATIVE HISTOCHEMICAL AND QUANTITATIVE IMMUNOCHEMICAL STUDIES OF SUBSTANCE P RECOVERY AFTER DORSAL RHIZOTOMY. A. Tessler, B.T. Himes,* R. Artymyshyn,* M. Murray, M.E. Goldberger. Departments of Neurology and Anatomy, The Medical College of Pennsylvania, Philadelphia, PA 19129.

Using the unlabeled antibody (PAP) technique, we have previously shown that deafferentation of the cat spinal cord by complete unilateral lumbosacral dorsal rhizotomy produces a reduction in substance P (SP) immunoreactivity in the deafferented dorsal horn at 10 days, which is followed by a partial return of SP in the dorsal horn. In order to establish the amount of the return we have used RIA to determine the amount of SP in the spinal cord of cats 10 and 30 days after unilateral lumbosacral dorsal rhizotomy. At 10 days the amount of SP immunoreactivity in the deafferented dorsal horn is reduced to 78% SP/mg protein of that found in the intact dorsal horn (N=5); at 30 days the reduction is 59% (N=6). Thus the 30 day dorsal horn contains 19% more than 10 day dorsal horn ($p<0.05$). Ventral horn immunoreactivity is not reduced at either time as determined by histochemical or immunochemical methods. Thus the results obtained by the two methods are roughly parallel, and we have used the immunohistochemical method in an attempt to locate the source of recovery of SP after deafferentation. This return is unaffected by lesions which destroy: 1) contralateral dorsal root afferents; 2) ventral root afferents; 3) ascending or descending fibers; 4) commissural fibers. These observations suggest that the restoration of SP reaction product depends on SP-containing interneurons located within lumbar spinal cord. In order to test the hypothesis that interneurons are responsible for the return, we have injected kainic acid, a neurotoxic agent that preferentially destroys nerve cell bodies, into the intact and chronically deafferented sides of the spinal cord and examined the SP reaction product. On the chronically deafferented side, where all neurons appear to have been destroyed, SP reaction product is virtually abolished from the dorsal horn at 10d post-injection. When all neurons are absent on the afferented side, SP immunoreactivity is only slightly reduced. These findings are consistent with the view that SP-containing interneurons contribute a small amount of the total SP present in the dorsal horn normally and that this amount increases in the dorsal horn chronically deafferented by dorsal rhizotomy.

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23.9 TRANSSYNAPTIC REGULATION OF TYROSINE HYDROXYLASE AND DOPAMINE SYNTHESIS IN THE OLFACTORY BULB. T. Kawano*, H. Baker, F. L. Margolis and T. H. Joh, Roche Inst. of Mol. Biol., Nutley, N. J. 07110 and Lab. Neurobiology, Cornell Univ. Med. Coll. New York, N. Y. 10021

Lesions of the olfactory epithelium produce profound decreases in the content of the afferent neuron components, olfactory marker protein and carnosine, as well as a decrease in the size of the olfactory bulb (OB). To investigate the transsynaptic consequences of disruption of OB afferent input we examined both dopamine (DA) and norepinephrine (NE) metabolism. OB NE is restricted to terminals of centrifugal origin while DA is found in intrinsic neurons and their processes. Following permanent destruction of the olfactory epithelium by intranasal irrigation with 0.17M $ZnSO_4$ in mouse or surgical lesion in rat, DA and DOPAC but not NE (expressed as pmoles/gm tissue (mean ± SEM) in table below) levels fall dramatically. Parallel decreases in DA content and immunocytochemically demonstrable TH staining occur. With Triton X-100 lesions of the mouse olfactory epithelium only a temporary OB deafferentation is produced since stem cell replacement of receptor cells can occur. As with $ZnSO_4$ lesions DA and DOPAC but not NE levels initially decrease reaching lowest levels at 2-3 weeks and returning to prelesion levels between 7 and 8 weeks. Staining for TH initially decreases and then returns suggesting that these intrinsic neurons did not degenerate.

Treatment	NE	DA	DOPAC	TH Staining
None	1755 ± 90	1141 ± 161	421 ± 37	3+
$ZnSO_4$ 21d	2388 ± 222	381 ± 85	96 ± 16	±
Triton 21d	1674 ± 171	485 ± 51	139 ± 16	1+
Triton 49d	1971 ± 239	1227 ± 248	515 ± 114	3+
None (Rat)	1976 ± 72	330 ± 5	218 ± 28	3+
Lesion (Rat 28d)	2558 ± 294	101 ± 33	55 ± 22	±

The decreases in DA but not NE levels as well as the loss of TH staining in the periglomerular neurons indicate that the majority of OB TH is contained within these intrinsic DA neurons and not in NE systems. Support for the continued presence of these neurons in deafferented animals was indicated by the demonstration of DA accumulation in OB following L-DOPA administration to reserpinized control and lesioned animals even though TH staining in these animals was minimal. These studies provide evidence for reversible transsynaptic regulation of dopamine metabolism possibly through regulation of the expression of the TH gene in periglomerular DA neurons. (Supported in part by grants - NIMH-MH33190 and NHLBI-HL18974).

23.10 COMPARISON OF THREE TIME COURSES OF DEGENERATION AND REINNERVATION AFTER VARYING DEGREES OF DEAFFERENTATION IN THE LATERAL SEPTAL NUCLEUS OF THE ADULT RAT. P.M. Field* and G. Raisman*. (SPON. F. Ebner). Laboratory of Neurobiology, National Institute for Medical Research, Mill Hill, London NW7 1AA, England.

The hippocampus projects through the fimbria to the septum of the same side and also to a lesser extent to a discrete dorsolateral quadrant of the septum of the opposite side. The numbers of degenerating and non-degenerating synapses were counted, using the electron microscope, in this dorso-lateral part of the septum after contralateral, ipsilateral and simultaneous bilateral lesions of the fimbria at various survival times from one day to over a year.

The numbers of degenerating terminals in the septum reached a maximum at 2 to 3 days after unilateral section of the fimbria and at this time the sum of the degeneration on the ipsilateral side plus the degeneration on the contralateral side was equal to that caused by a bilateral lesion. At longer survivals there was a marked difference in the rate at which synaptic changes occurred after the bilateral lesion as compared with the unilateral lesion. From 4-7 days after a bilateral lesion the number of degenerating synapses in the septum continued to increase whereas during this period after the unilateral lesion it began to fall. At its maximum value, the amount of degeneration caused by the bilateral lesion greatly exceeded the sum of the maximum amounts of degeneration after the two individual lesions.

We propose that among the population of terminals the degree of asynchrony between the rate of degeneration and its removal (by astroglial phagocytosis) is such that at no time after a single lesion can all the degenerating terminals be seen. A model is proposed which would estimate the total amounts of degeneration due to the unilateral lesions.

The removal of degeneration after the bilateral lesion is delayed by several weeks as compared with the unilateral lesion. This may be due to the time required to activate more astrocytes or to the fact that the origin of the reinnervating terminals after bilateral lesions is different from that after unilateral lesions.

23.11 A REEVALUATION OF INTRASPINAL SPROUTING OF PRIMARY AFFERENTS. B.E. Rodin*, S. Sampogna* and L. Kruger. Depts. of Anatomy and Anesthesiology and Ahmanson Lab. of BRI, UCLA, L.A., CA 90024.

Intact dorsal root fibers have been reported to form anomalous projections within the spinal cord after surrounding dorsal roots have been severed. These findings are difficult to interpret because of technical problems in previous studies with metallic impregnation of degenerating and normal fibers and cord distortion following rhizotomy. The present study constitutes an attempt to reexamine denervation-induced sprouting in spinal cord by labeling the normal axonal trajectory of sciatic afferents with horseradish peroxidase (HRP) at various intervals following partial denervation.

The central projection of the L4, L5 component of sciatic nerve was examined in rats that had been denervated five days (acute), two wks., one, two or three mos. previously. Unilateral dorsal root ganglionectomies were performed when animals were 60-70 days of age; the L4 or L5 ganglion was left intact, and 2-6 surrounding ganglia were excised. Five days before animals were sacrificed, the sciatic nerve on the denervated side was exposed, severed and desheathed at its proximal cut-end. HRP crystals were applied to the nerve and six days later, the spared ganglion and spinal cord processed for visualization of HRP.

The central projection of sciatic afferents was the same following acute and chronic denervation. There was no evidence that the rostro-caudal trajectory of sciatic afferents had expanded during the various post-denervation intervals, and the location and density of reaction product within the spinal segments that normally receive these afferents did not substantially differ in acute and chronic preparations. In a few animals that survived at least eight wks. following ganglionectomy, the lateral projection in laminae I, II of low lumbar segments was denser and more widespread (5-7u) than in acute preparations. This was not a consistent finding even among animals in the same experimental condition, and probably reflects lateral displacement of afferents in dorsal horn with the dorsal column shrinkage that accompanies long-term survival after ganglionectomy. This was evidenced by the fact that, in these exceptional cases, the medial projection in lamina II was sparser than usual.

These results with rats do not support previous findings with cats that the central projection of primary somatosensory afferents is altered considerably following chronic partial denervation. Although these conflicting results might be attributable to species differences in plasticity, it is possible that previous evidence for sprouting in spinal cord is an artifact of the methods used to demonstrate it.

23.12 Development of long-term potentiation in the in vitro goldfish optic tectum. D. Lewis, T. Teyler, and V. Shashoua. Neurobiology Program, Northeastern Ohio Universities College of Medicine, Rootstown, Ohio 44272, and Dept. of Biological Chemistry, Harvard Medical School, Belmont, MA 02178.

We have previously reported methods for maintaining in vitro explants of goldfish optic tecta in an incubation medium which approximates goldfish CSF and a 99/1% O_2/CO_2 atmosphere (Teyler, Lewis, Shashoua, and Brogna. Neuroscience Abstracts 6 (1980) 294). Stable responses can be maintained for up to 8h. Stimulation of the severed optic nerve stump gives rise to a short-latency population fiber volley and a longer latency negative, monosynaptic response centering in the stratum opticum. Stimulation with tetanic frequencies from 0.2 - 50 Hz with an equal number of stimuli gives an inverted U function of monosynaptic response amplitude vs. frequency. Tetanus frequencies of 1 Hz for 100 sec and 5 Hz for 20 sec resulted in a significant increase in the amplitude of the monosynaptic response above control values of 1/60 sec stimulation. An increased response of approximately 30% is seen at 30 min and 1h posttetanus. This potentiation lasts up to 3h. Tetanus frequencies below and above 1 and 5 Hz showed no significant potentiation. Higher frequencies of 33 and 50 Hz, frequently used to produce LTP in the mammalian hippocampus, produced response depression in the optic tectum. Hippocampal LTP is seen within 10 min while the potentiation of the goldfish tectum takes longer to develop, 30 min at the earliest. Since the goldfish is a poikilotherm its metabolism is slower than the homeothermic mammals; therefore, LTP may take longer to develop. These results demonstrate that the phenomenon of long-term potentiation may not be unique to the mammalian hippocampus.

23.13 CALCIUM INDUCED LONG-TERM POTENTIATION IN THE HIPPOCAMPUS. R.W. Turner*, K.G. Baimbridge* & J.J. Miller (SPON: J.A. Pearson). Department of Physiology, University of British Columbia, Vancouver, Canada V6T 1W5.

The effects of a transient increase in extracellular calcium concentration on the Schaeffer-commissural evoked excitatory post-synaptic potential (EPSP) and population spike responses of CA1 pyramidal neurones was investigated using the rat in vitro hippocampal slice preparation. Brief exposure of slices (5-10 min) to twice the normal concentration of calcium (2mM) induced a marked potentiation of both the EPSP and population spike that could persist for at least 3 hours. The total intracellular calcium content of individual slices, measured by atomic absorption spectrophotometry, was significantly increased for at least 1 hour following return to the control medium. No long-term changes were observed in either the presynaptic fiber volley or antidromically evoked CA1 population spike, indicating that this potentiation could not be attributed to an increase in the number of fibers activated or a generalized increase in cellular excitability. The response of CA1 pyramidal neurones to the iontophoretic application of L-glutamate in the apical dendritic zone was also unaffected after exposure to the high calcium perfusate, indicating a lack of alteration in excitability restricted to this region of the synaptic input.

These data indicate that brief exposure of in vitro hippocampal slices to a high extracellular calcium concentration results in a long-term increase in synaptic efficacy which is similar in many respects to long term potentiation induced by tetanic stimulation of hippocampal excitatory afferents. The results further suggest that the mechanisms underlying calcium induced long-term potentiation may reside in presynaptic terminals and involve an enhanced transmitter release.

23.14 RESIDUAL CEREBELLOTHALAMIC TERMINAL FIELDS FOLLOWING HEMISPHERECTOMY IN THE CAT. Ch. E. Olmstead, J.P. McAllister* II, J.R. Villablanca and F. Gomez* MRRC and Depts. of Psychiatry and Anatomy, UCLA School of Medicine, Los Angeles, CA 90024.

As part of a study of recovery of function in animals with uni- and bilateral removals of the cerebral hemispheres we are examining possible sites for interactive remodeling in subcortical areas where projections of the deep cerebellar nuclei overlap with both corticofugal and corticopetal neurons. The areas selected were the ventrolateral complex of the thalamus (VL), red nucleus, pontine nuclear groups, the cuneiform nucleus and inferior olivary complex. We report here preliminary results from injections of ^{3}H amino acids into the nucleus interpositus, both anterior (INA) and posterior (INP), and the subsequent mapping of projections to the VL. Intact (N=7) and hemispherectomized (HEMI:N=8) adult cats received unilateral injections of ^{3}H leucine-proline (0.2µl; specific activity of 42 and 10 Ci/mmole, respectively) into either the INA or INP on the right side. The HEMIs had undergone ablation of the left hemisphere at least 6 months earlier. This included all of the neocortex along with the hippocampus and striatum. Five days after the injections the animals were perfused intracardially. Serial frozen sections were cut at 50µ throughout the entire brain and processed for routine autoradiography. In intact cats amino acids injected into an area restricted to the antero-medial 1/3 of INP were transported to the rostral lateral portions of the contralateral VL. Five HEMIs receiving injections into that same portion of INP exhibited terminal fields in lateral portions of the contralateral VL. These terminals were patchy and appeared qualitatively identical to those seen in intact animals. Cytoarchitecturally, the regions containing these terminals were conspicuously gliotic and devoid of thalamocortical relay neurons. Parts of what appeared to be neuronal cytoplasm were occasionally observed, but neuronal nuclei were rarely seen. Two of these 5 animals showed additional terminal labeling in partially gliotic regions of the nucleus ventralis medialis and in 3 HEMIs sparse terminal fields were also observed in both normal and gliotic regions of the parafasicularis, central lateral, anterior pretectal and posterior nuclei. We are now trying to ascertain whether the projections from INP, but not INA, may extend their normal terminal fields in the magnocellular portion of the red nucleus to innervate more of its parvocellular division. These results indicate that the INP projections to the VL persist despite degeneration of the thalamocortical neurons, suggest that the site of the terminals is not directly upon such neurons, and might reflect attempts at reorganization in response to cortical ablation.

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23.15 A MODEL OF ALCOHOL-INDUCED BRAIN ATROPHY IN THE RAT. R. L. Wierimaa*, F. A. Holloway, J. A. Devenport*, and L. D. Devenport (SPON: D. M. Toth). Dept. of Psychiatry and Behavioral Science, Univ. of Okla. Health Sciences Center, Oklahoma City, OK 73190.

Cerebral atrophy (ventricular expansion, sulcal widening, and decreased density) is frequently associated with chronic excessive ethanol ingestion (e.g., Golden et al., Science 211:508, 1981). To begin unraveling the events intervening between alcohol and brain, we have developed an animal model. In so doing we have tested the possibility that adrenal steroids mediate the atrophic effects of chronic alcohol exposure.

Basing our methods on previous research (Devenport, Behav. Neur. Biol. 27:218, 1979; Devenport et al., Neurosci. Abstr. 6:382, 1981), rats were either adrenalectomized or sham-operated at 44 days of age. Half were maintained in separate cages in an alcohol inhalation chamber (modeled after Rogers et al., Behav. Neur. Biol. 27:466, 1979) where the atmosphere was adjusted to achieve continuous blood alcohol concentrations (BACs) of .2 mg percent (w/v). The rest were housed similarly, but their atmosphere was without ethanol. These conditions were in force across ages 46-65 days, after which blood samples were taken and, following anesthetic overdose, brains were removed.

Sham-operated rats required a considerably greater atmospheric concentration of ethanol (23.2 - 24.9 mg/l) than did adrenalectomized animals (16 - 19.3 mg/l) to reach the same BAC. Alcohol exposure reduced brain weights and volumes relative to controls. Body weights were unchanged. In keeping with our previous findings, adrenalectomy increased brain size above that of sham-operates. Unexpectedly, however, alcohol exerted a stronger effect in adrenalectomized rats than in sham-operates, suggesting that adrenocorticoids may play a protective role against the action of ethanol on gross brain structure.

This work was supported in part by USPHS grant R01AA04962 to LDD.

23.16 TIME AND EXPERIENCE ALTER BEHAVIOR AND HIPPOCAMPAL NEUROCHEMISTRY AFTER DAMAGE TO THE CA3 SUBFIELD. G.E. Handelmann,* D.S. Olton, T.L. O'Donohue,* C.J. Cummins, M.C. Beinfeld,* and D.M. Jacobowitz. Dept. of Psychol., The Johns Hopkins Univ., Balto., MD; Lab. of Clinical Science, NIMH, and Lab. Neurochemistry, NINCDS, Bethesda, MD 20205.

Bilateral microinjections of kainic acid into the hippocampal subfield in rats caused a destruction of the CA3 pyramidal cells and an impairment in the ability to perform a spatial maze task, with eventual recovery of behavioral function. Previous experiments suggested that the recovery of function may be mediated by alterations in the distribution or function of the various hippocampal pathways. In this experiment, alterations in hippocampal pathways were investigated by measuring neurotransmitters or their synthetic enzymes in the hippocampus and related structures at various times after loss of the CA3 pyramidal cells. In addition, the extent to which these changes in neurotransmitter levels were influenced by experience on the spatial maze task was investigated. Neurochemical assays for choline acetyltransferase (ChAT), glutamic acid decarboxylase (GAD), glutamate, norepinephrine (NE), and cholecystokinin (CCK) were performed on ten dissected regions of the hippocampal system.

The analysis indicated that a number of alterations in hippocampal neurotransmitter systems occurred either immediately after the surgery or at longer survival times, and that some of these changes were influenced by test experience. ChAT and GAD activities were unchanged immediately after surgery but declined in many regions over time in untested rats. Test experience prevented the decline. Glutamate levels decreased in the CA3 region and its projection areas, and remained low. NE levels were lowered in some regions immediately after surgery, but they increased with time to above control levels. Test experience increased the levels even more. CCK levels were lowered in some regions immediately after surgery. Test experience increased CCK in some regions, including the CA1 subfield and the entorhinal cortex.

These findings suggest that test experience prevents a decline in septohippocampal activity after loss of the CA3 cells, and influences a number of neurochemical systems which may modulate hippocampal activity. These mechanisms may be responsible for the behavioral recovery of function.

23.17 INDUCED COLLATERAL SPROUTING OF HIPPOCAMPAL 5-HT FIBERS;A QUANTITATIONAL HRP STUDY IN THE RAT. F.C.Zhou* and E.C.Azmitia ; Department of Anatomy, Mount Sinai,CUNY,New York,N.Y. 10029.(SPON/Shriver)

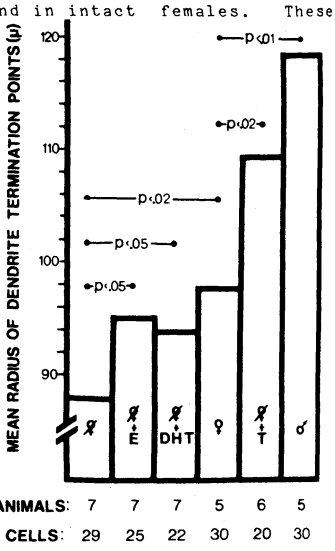
Serotonergic fibers from the median raphe nucleus (MR) use two pathways to innervate the hippocampus (HIPP)-a cingulum bundle(CB) and fornix-fimbria(FF) route (Azmitia and Segal,J.Comp.Neurol.179:641,1978). Removal of the CB 5-HT fibers results in structural and functional changes which recover after several weeks.This was attributed to FF 5-HT fibers undergoing collateral sprouting (Azmitia Nature 274;374, 1978). We here present evidence for reorganization of the raphe cells projecting to the hipp after 5,7-DHT destruction of CB-5HT fibers.

30 female rats were placed into four groups; uninjected control,sham injected,5,7-DHT (5 ugm in 400nl/min) microinjection into CB, and 5,7-DHT into CB followed by injection into FF (6.25ug in 500nl) 2 days before injection of HRP (Sigma VI,100nl of 10% solution) into dorsal hipp.These rats were perfused with 2.5% glutaraldehyde at various post-operated times (3,21,42 days).The HRP was visualized by TMB Method.

The organization of the raphe cells efferent to the dorsal hipp using the CB was different from those using the FF in that neurons located in the interfascicular part of the dorsal raphe nucleus (IF-DRN) used the CB route.The total number of neurons projecting to the dorsal hipp decreased by 58% and 95% 3 days following CB and CB-FF 5,7-DHT injections respectively.There was a significant increase in HRP labeled neurons in the MR 21 and 42 days compared to the 3 day group after CB injections (a 2% decrease and 38% increase compared to sham injected),but no change after CB-FF 5,7-DHT.The labeled cells were also seen to increase in size in the long survival CB group.No cells were localized in the IF-DRN in this CB group. These results indicate that CB-5HT fibers do not regenerate into the D-HIPP, but FF-5HT fibers increase their terminal territory by collateral sprouting in response to homotypic dennervation.Supported by NSF-grant BNS-79-06474.

23.18 GONADAL STEROID ADMINISTRATION CAUSES NEURONAL GROWTH IN A SONG CENTER OF ADULT FEMALE CANARIES. Timothy J. DeVoogd and Fernando Nottebohm. The Rockefeller University, New York, N.Y. 10021

Female canaries were ovariectomized at two weeks of age. At one year, they received Silastic implants containing estradiol (E), dihydrotestosterone (DHT), testosterone (T), or nothing (N). During the ensuing four weeks, the T-treated birds began to sing. After the four week period, all birds were sacrificed and their brains were stained by a rapid Golgi technique. The dendritic trees of cells in nucleus robustus archistriatalis, a telencephalic song control nucleus, were quantified using a computer-microscope system. The length, number of branches and dispersion of branches away from the cell body of dendrites from the brains of E- and DHT-treated birds were significantly increased over those of the N birds, yielding a pattern similar to that found in intact females. These dendritic parameters in the T-treated birds were significantly greater than in the E, DHT and intact female birds, approximating normal male dimensions. The figure to the right illustrates these changes for the mean distance from dendrite termination points to the cell soma.



Thus, gonadal steroids can cause neuronal growth in adult animals, and this growth is associated with acquisition of a species typical behavior.

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23.19 LESION-INDUCED CHANGES IN PDH ACTIVITY AND CALCIUM TRANSPORT IN HIPPOCAMPAL MITOCHONDRIA. Kessler, M.*, Baudry, M., Fuchs, J., Arst, D.*, and Lynch, G. Department of Psychobiology, University of California, Irvine, California 92717

Lesions of the perforant path have been widely used as a tool to study the phenomenon of sprouting and reactive synaptogenesis in the hippocampal formation. However little is known of the sequence of biochemical events which initiates and regulates sprouting. It has been shown that another form of hippocampal plasticity is accompanied by a transitory change in the phosphorylation of the α-subunit of pyruvate dehydrogenase (PDH). The demonstration of a link between this enzyme and the regulation of mitochondrial calcium transport has prompted the suggestion that hippocampal afferent fibers may exert a strong influence on calcium regulation in their targets. In light of this, we decided to investigate the effects of denervation on PDH activity and mitochondrial calcium transport in hippocampus before and during the period of sprouting.

Following unilateral entorhinal cortex lesions, the pyruvate-supported calcium transport is significantly decreased as soon as 1 day post-lesion whereas the succinate- and ATP-supported transport are not affected until about 3 days post-lesion. The pyruvate-supported calcium transport is maximally depressed at 5 days postlesion; it shows a 45% decrease as compared to the unoperated contralateral side, and remains significantly lower as long as 6 months post-lesion. There is also a progressive, although less marked, decrease in the succinate-supported calcium transport. The ATP-supported transport remains largely unchanged. The changes in pyruvate-supported calcium transport are highly correlated with the reductions in PDH activity.

These data support the idea that pre-synaptic elements influence PDH activity and calcium regulation in the hippocampus; the mechanisms by which this is accomplished are not known but it seems reasonable to assume that the phosphorylation and/or dephosphorylation of the alpha regulatory subunit of PDH is involved. The relationship of these denervation-induced disturbances to sprouting is unclear but a loss of calcium-buffering ability could serve to mobilize growth responses in both axons and dendrites. Finally, since both high frequency synaptic stimulation and deafferentation appear to affect PDH, it is possible that long-term potentiation and sprouting both employ, at some stage in their development, similar cellular processes.

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23.20 GROWTH, DIFFERENTIATION, AND CONNECTIVITY OF EMBRYONIC NEURAL TISSUE TRANSPLANTED INTO THE CEREBELLUM OF THE RAT. B. H. HALLAS. New York College of Osteopathic Medicine, Old Westbury, NY 11568.

Mammalian embryonic neural tissue was transplanted into the cerebellum or forebrain of various age host rats to study the development, growth, differentiation and connectivity of the transplants. Neocortical tissue obtained from either 15-day or 18-day-old embryos was transplanted into the cerebellum or forebrain of 30-day-old host animals and the host animals were sacrificed in a developmental sequence, 1,2,3,4,5,8,15,30, and 90 days after transplantation. In this study, differential patterns of growth were compared between transplants placed in the cerebellum and those in the forebrain and between the two ages of neocortical tissue.

In the second phase of this study, embryonic neocortical tissue obtained from 15-day or 18-day-old embryos was transplanted into either the cerebellum or forebrain of 5-, 10-, 15-, 20-, 25-, 30-, 35-, or 180-day-old rats. All transplants were found to have survived and differentiated with the largest transplants observed in the 5-day-old hosts. In older host animals, the size of the 15-day transplants were found to be progressively smaller while all 18-day embryonic transplants grew to approximately the same size. In all age host animals, the transplants exhibited fully differentiated neurons identical to their in vivo counterparts and in no host animal was there evidence of a neuroglial scar separating transplant from the host brain.

In a third group of 15-day or adult hosts, 15-day or 18-day-old embryonic neocortical tissue was transplanted into the right cerebellar hemisphere. Ninety days after transplantation, either a 20% solution of Horseradish peroxidase was injected into the transplants or electrolytic lesions were made in the contralateral spinal cord, inferior olivary nuclei, or pontine nuclei and the resulting tissue processed for degenerating fibers by the Fink-Heimer stain. In additional animals, lesions were made in the transplants directly and the host brain processed for Fink-Heimer staining for the study of the efferents of the transplants. Through utilization of these two techniques, it was demonstrated that the neocortical transplants had established connections with the host brain, thus providing evidence that the embryonic neocortical transplants had become an integral part of the host's brain.

23.21 RECOVERY OF ALTERNATION BEHAVIOR AFTER ENTORHINAL CORTEX LESIONS. Thomas M. Reeves, Douglas C. Smith and Robert N. Holdefer. Dept. of Psychology, Southern Illinois Univ., Carbondale, IL 62901.

Previous investigators have found evidence that recovery of alternation performance in a T-maze, following unilateral entorhinal cortex (EC) lesions, is correlated with the time course of collateral sprouting of fibers from the intact entorhinal area (Loesche and Steward, 1977). A subsequent attempt to confirm the correlation of recovery of alternation behavior and sprouting did not find an impairment after unilateral EC lesions (Ramirez, 1979).

Since the report of Loesche and Steward (1977) is the only demonstration of a behavioral recovery that is correlated with collateral sprouting in the adult mammalian brain, the discrepancy between their data and those of Ramirez (1979) is a critical issue which we have attempted to resolve.

Fifty-six male Sprague-Dawley rats learned to alternate in a T-maze for food reward after being reduced to 80% ad lib weight. Eighteen rats with unilateral EC lesions were tested after a 3-day recovery period. Their performance on postoperative days 3-8 was significantly below their own preoperative levels (t-values ranged from 2.70,$p<.01$ to 6.20,$p<.0005$). By postoperative day 9, performance was at pre-lesion levels. Rats permitted a 10-day recovery period did not differ from their preoperative level by the second day of post-lesion testing. Thus, recovery was not facilitated by experience in the maze during the post-lesion interval, day 3-8. This was consistent with the data of Loesche and Steward (1977), as were the following findings: 1) animals with bilateral EC lesions showed permanent deficits (56-days) even with daily postoperative training, 2) secondary EC lesions, in rats which sustained a previous unilateral EC lesion, caused permanent deficits, and 3) transections of the commissure thought to carry the sprouting fibers (dorsal psalterium) resulted in permanent alternation deficits.

These behavioral data are consistent with a collateral sprouting interpretation. Preliminary data regarding the electrophysiological correlates of the time course of sprouting and return of alternation performance within individual unilateral EC-lesioned animals will be presented.

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23.22 TRANSIENT AND LONG-TERM POTENTIATION IN THE NEOCORTEX OF THE RAT. D.A. Wilson* and R.J. Racine. Dept. of Psychology, McMaster Univ., Hamilton, Ont., Canada L8S 4K1.

Post-tetanic potentiation (PTP) and long-term potentiation (LTP) were examined in the neocortex following high-frequency stimulation of the corpus callosum in both anesthetized and unanesthetized adult rats.

Biphasic stimulation (0.1 msec each pulse, 50-1000 uA intensity) of callosal fibers in one hemisphere produced biphasic (positive-negative) or monophasic (negative) post-synaptic responses recorded near the surface of the contralateral hemisphere. These waveforms reportedly reflect the bimodal nature of callosal terminations in the neocortex (Grafstein, B., J. Neurophysiol., 22;504,1959), with surface-positivity corresponding to activation of terminations in deeper layers, and surface-negativity corresponding to activation of terminations in superficial layers.

Test pulses producing sub-maximal responses (40-50% of maximum on I/O curve) were presented at 0.1 Hz, preceding and following high-frequency (400 Hz, 100 msec) trains applied to the callosum. The surface-negativity showed marked PTP and LTP following tetani. The positivity was generally unaffected. The short-term effects were similar to those reported by Clare, Landau, and Bishop (EEG and Clin. Neurophysiol.,13:21,1961) in the cat callosal-neocortical system. A more complete analysis of PTP revealed a decay process best described as an exponential function with time constants similar to those reported in the hippocampus (McNaughton, B., Brain Res.,199:1,1980) and frog n.m.j. (Magleby, K. & Zengel, J., J. Physiol.,257:449,1976).

High intensity tetani produced LTP, lasting longer than one hour at levels up to 100%. The phenomena of LTP threshold and saturation were found to be similar to those reported for the perforant path-granule cell synapse (McNaughton, B., Douglas, R., & Goddard, G., Brain Res.,157:2771978).

Further, preliminary investigation into the ontogeny of LTP in the callosal-neocortical system of the developing rat suggests that LTP cannot be consistently produced prior to the third post-natal week; a period of synapse proliferation and myelin formation.

These findings suggest that LTP of post-synaptic responses is not specific to the hippocampal formation, but rather may be a more generalized phenomenon of forebrain pathways.

23.23 FUNCTIONAL PLASTICITY IN OPTIC CENTERS OF ADULT RAT - A 2DG STUDY. A.W. Toga, D.A. Stein* and R.C. Collins Wash. Univ. Sch. Med., St. Louis, MO 63110

Unilateral enucleation during infancy results in changes in the number and pattern of ipsilateral retinofugal projections in several rodents. Recent evidence suggests that functional changes can also be induced in the visual system of the adult (Chalupa and Henderson, Brain Res., 192, 1980). We have examined this issue further by measuring the functional metabolic response of the visual system of adult pigmented rats at different times following monocular enucleation.

Glucose utilization was quantitatively measured using the ^{14}C-2 deoxyglucose autoradiographic method (2DG). All subjects were fasted for 24 hours prior to the experiment and prepared and tested in ambient laboratory illumination. Femoral arterial and venous catheters were inserted and one eye enucleated under halothane anesthesia. Animals in the immediate post enucleation group (N=5) were given 2DG 4 hours later when animals were alert and mobile. The second group (N=5) had their catheters inserted and were given 2DG 30 days following monocular enucleation.

For all subjects the metabolic rates of optic centers on the side contralateral to the intact eye were higher than on the side contralateral to the enucleated eye . Visual structures contralateral to the intact eye did not significantly increase their metabolic rates. Glucose utilization in all deafferented visual structures, however, was greater when measured 30 days following monocular enucleation compared to rates measured on the same day. The largest increases were observed in the superior colliculus, particularly the lateral aspects (.90 versus .60 µmoles/gm/min.) and the lateral geniculate nuclei (.94 versus .73 µmoles/gm/min.). Nucleus lateralis posterior and pretectum also had greater metabolic rates 30 days after enucleation compared to immediatley post enucleation. Metabolic increases were also observed in visual cortex, but these changes occurred on both sides of brain - 1.00 µmoles/gm/min. to 1.12 on the deafferented side and 1.08 to 1.22 µmoles/gm/min. on the stimulated side. Comparing the asymmetry (stimulated/enucleated) in metabolic rates of each structure shows that immediately following eye removal there are large differences between the right and left sides (up to 47% in caudal aspects of the S.C.). Whereas after 30 days these right left differences become attenuated (26% difference in caudal S.C.).

These metabolic data further indicate that functional anatomic changes ("plasticity") occur in the adult visual system following monocular enucleation.

23.24 SLOW WAVE FIELD POTENTIALS IN THE RAT BRAIN DURING THE ONSET OF PUBERTY. J.F. Masken and R.J. Morgan, Dept. of Physiology and Biophysics, Colo. State Univ., Ft. Collins, CO 80523.

Cross-correlation analysis was successfully applied in this study to evaluate changes in the relationships between the AMY, POA, and ARC with respect to slow wave field potentials (signals) recorded simultaneously from these regions of the brain. Recordings were made during the onset of puberty and in the course of the estrous cycle. The correlation of slow wave activity between the AMY and POA was, in general, higher than that between the AMY and ARC and POA and ARC. The correlation between slow wave field potentials between the AMY and ARC was the lowest. This was the case in both the prepubertal and post-pubertal state. The correlation for all three combinations, i.e. AMY-POA, AMY-ARC, and POA-ARC, was lower in the prepubertal state than in the post-pubertal state. The change in the level of correlation appeared gradually and before vaginal opening in the case of AMY-ARC, and POA-ARC, but the correlation for AMY-POA increased rather abruptly and right at the first estrous. Changes in delay times (the length of tme it takes a signal in one area to appear in another, e.g. from AMY to POA), direction of signal traffic (in which area did the signal originate), and total activity of the three areas were also apparent from the analysis. There were also changes, particularly in the direction of signal flow, that could be of significance with respect to daily fluctuation in Gn-RH and LH release.

(Supported in part by NIH Grant HD09363-03.)

24.1 SYNAPTIC EFFICACY AT SINGLY- AND DUALLY-INNERVATED NEUROMUSCULAR JUNCTIONS IN THE CUTANEOUS PECTORIS MUSCLE OF THE FROG. Y.-M. Yao* & J. N. Weakly. Dept. of Physiology, Univ. North Carolina School of Medicine, Chapel Hill, N. C. 27514.

A portion of the end-plates in some amphibian skeletal muscle fibers receives the terminations of two or more motor axons. Mallart & co-workers (1,2), using the pectoral muscle of Xenopus laevis, reported that in cases where two different axons form synaptic contacts at the same end-plate, the efficacy (e.p.p. amplitude) of each component synapse is less than that of singly-innervated junctions. The reduced synaptic efficacy was attributed to reduced transmitter release from the individual nerve terminals in dually-innervated junctions. Moreover, the mean amount of transmitter released by both axons together and, hence, the sum of e.p.p. amplitudes at compound junctions was significantly smaller than at singly-innervated end-plates. It was suggested that the reduction in individual and aggregate synaptic efficacy could be explained by postulating mutual inhibitory influences operating between closely spaced nerve endings.

To test the generality of this hypothesis, we examined the e.p.p. amplitudes of 452 singly- and 85 dually-innervated end-plates in 10 lightly-curarized cutaneous pectoris muscles of R. pipiens. Resting membrane potential and input resistance were not significantly different in singly- and dually-innervated fibers.

In agreement with the work in Xenopus, the e.p.p. produced by each nerve ending alone in the dually-innervated junctions was significantly smaller ($p < 0.05$ by two-tailed t-test, df=8) than the average e.p.p. at singly-innervated junctions. However, in the present experiments, the sum of the two e.p.p.s at dually-innervated sites was not significantly less ($p > 0.05$ by two-tailed t-test, df=8) than the average e.p.p. amplitude at singly-innervated junctions. The total e.p.p. amplitude at dual junctions was 0.89 ± 0.44 (mean ± S.D.) of the average single junction e.p.p. The finding that the sum of the e.p.p. amplitudes from dual end-plates is equal to the e.p.p. amplitude at singly-innervated synapses is consistent with the idea that two (or perhaps more) axons may compete without mutual inhibitory influences for a limited synaptic space on each cutaneous pectoris muscle fiber.

References:
1. J. Physiol. (Lond.)(1979). 289, 203-218.
2. Ontogenesis and functional mechanisms of peripheral synapses, Taxi, J., ed. (Elsevier), pp. 213-218.

This work was supported by USPHS grant NS 15136.

24.2 CORRELATED ANATOMY AND ELECTROPHYSIOLOGY DURING SYNAPSE ELIMINATION IN FROG MUSCLE. K. Morrison-Graham. UCLA School of Med., Dept. of Physiol., Ahmanson Lab. of Neurobiology, and Jerry Lewis Neuromuscular Research Center, Los Angeles, CA. 90024.

Transient multiple innervation has been well documented, both anatomically and electrophysiologically, in developing skeletal muscle. However, it is not certain if all the inputs to one synaptic site respond to nerve stimulation and if they remain functional until the time of their elimination. This question was addressed in the cutaneous pectoris muscle of recently post-metamorphic bullfrogs (R. catesbiana) by correlating the number of components in the endplate potential with the number of NBT-stained axonal processes entering the same synaptic site. During the first three post-metamorphic weeks, when most synapse elimination occurs (Letinsky & Morrison-Graham, J. Neurocytol. 9: 321, 1980), no evidence for morphologically intact but functionally silent processes was found. Rather, the percentage of the total terminal length occupied by different axons at one site corresponded to the percentage of neurotransmitter released as measured by endplate potential amplitude or quantal content.

The endplates were also classified morphologically by the appearance of their pre-terminal axons and terminal arborizations. In general, thin pre-terminal axons formed small synaptic contacts which either shared the same synaptic gutter with thicker axon terminals or formed independent varicose contacts, and contributed less than 20% to the total nerve-evoked release. Similarly, when pre-terminal axons were the same thickness and their terminal expanses looked similar, their amounts of evoked release were comparable. Endplates were also found which had morphological characteristics intermediate to these two categories; these latter endplates had inputs with intermediate percentages of evoked release.

Staining for ACh receptors (using Rhodamine-labeled α-BGT) and/or AChE activity was also done on identified endplates which had been characterized electrophysiologically and anatomically. These post-synaptic components were well localized under the regions of thickened NBT in all endplates examined. However, small patches of post-synaptic membrane with ACh receptors and AChE activity, devoid of overlying nerve processes, were also found near NBT-stained varicosities formed by thin pre-terminal axons during the period of synapse elimination (Morrison-Graham, Soc. for Neurosci. Abstr. 6: 567, 1980).

These results suggest that axonal processes undergoing elimination may decrease their terminal arborization gradually while continuing to release transmitter in proportion to the amount of pre-synaptic membrane they contribute to the synaptic site.

This work was supported by USPHS grant NS13470.

24.3 PERSISTENCE OF MULTIPLE INNERVATION IN MOUSE MUSCLES PARALYSED NEONATALLY WITH BOTULINUM TOXIN. W. G. Hopkins*, M. C. Brown* and R. J. Keynes* (SPON: A. D. Smith).
University Lab. of Physiology, Oxford OX1 3PT, U.K.

The effect of botulinum toxin-induced paralysis on the elimination of neonatal multiple innervation of muscle fibers has been investigated using silver-stained, whole mount preparations of mouse muscles, the gluteus maximus and tensor fasciae latae. Toxin was injected into the muscles of 6 day old mice, when 20-50% of fibers are multiply innervated. Contralateral control muscles were excised at this time, and paralysed muscles on succeeding days up to one week later. It was found that the percentage of fibers contacted focally by more than one axon in the control and blocked muscles did not differ significantly throughout the period of block. Therefore there was probably neither retraction nor regrowth of axons in the blocked muscles.

The long-term effect of paralysis was investigated following injection of toxin at age 0-4 days. Focal multiple innervation assayed electrophysiologically and histologically persisted for at least several months in tensor fasciae latae, and there was also a stable ectopic innervation of gluteus muscle fibers from sprouted spindle motor axons. It is possible that myelination stabilises and thereby prevents elimination of these excess focal and ectopic axons.

(Supported by N.Z. and U.K. Medical Research Councils)

24.4 NEONATAL UNILATERAL ADRENALECTOMY AFFECTS THE NORMAL PATTERN AND DENSITY OF INNERVATION OF THE REMAINING ADRENAL MEDULLA. L. L. Ross, A. J. Smolen and J. Cherry*. Department of Anatomy, The Medical College of Pennsylvania, Philadelphia, PA 19129.

During the first month of life there is a striking reorganization of the pattern of preganglionic innervation to the rat adrenal medulla. Up to 3 weeks of age, the adrenal medulla is innervated by neurons located both ipsilaterally and contralaterally in the zona intermedia of the spinal cord. It is not known if some of these neurons project bilaterally to both adrenals. By the fourth week, there is a marked reduction in the total number of neurons projecting to the ipsilateral adrenal and a near-total loss of the neurons projecting to the contralateral adrenal. In a previous study, we demonstrated the importance of supraspinal input to the regulation of the development of the normal pattern of innervation of the adrenal medulla. An additional regulatory role may be played by interadrenal interaction mediated by the bilaterally-projecting neurons which may exist in the neonatal rat spinal cord. The present study was designed to assess the extent of such interaction by examining the effect of neonatal unilateral adrenalectomy on the development of innervation to the intact adrenal medulla.

On postnatal day 3 the right adrenals of rat pups were removed. On days 15 and 25 counts were made of the numbers of cells and synapses in the left adrenal medullas of some animals while HRP was injected into the left adrenals of other animals. On day 15 in the adrenalectomized animals the numbers of HRP labeled neurons in the spinal cord were reduced when compared with normal 15 day animals. Neurons on both sides of the cord were affected, but the contralaterally projecting neurons were more severely affected. By day 25 both experimental and control animals were the same with respect to number and distribution of labeled neurons. The number of synapses in the medulla of the adrenalectomized rat was 50% less than that of the controls at 15 days and 40% less at 25 days.

Thus, unilateral adrenalectomy results in a premature reduction of the total number of labeled neurons which innervate the remaining adrenal. The loss is particularly severe amongst the contralaterally projecting neurons. This preganglionic neuron loss is accompanied by a marked loss of the numbers of synapses in the remaining adrenal medulla. These findings support the concept of bilaterally projecting preganglionic neurons in the neonatal animal.

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24.5 AUTOMATED ANALYSIS OF SYNAPTIC SIZE AND NUMBER IN THE CEREBELLUM. D.E. Hillman, M. Chujo* and S. Chen*. Dept. Physiol. & Biophys., New York Univ. Med. Ctr., New York, NY 10016.

Recording of synaptic profiles from thin sections was accomplished by software for an interface between a scanning-transmission electron microscope and a computer (Hillman, et al SEM/I, pp. 125-139, 1980, Chicago, IL). Algorithms were implemented through software for analysis of shape, size and number of synapses. The method greatly improved the reliability and rate at which data could be collected. Software allowed arbitrary positioning of the scanning beam by feedback to the computer from the scanning detector. First, the beam was directed in a low resolution raster mode for locating dense objects as defined by a density threshold that was either manually or automatically set. Once located, the boundary of the objects were extracted as two-dimensional coordinates. The boundary data for each object was immediately processed for orientation, size and shape. Profiles of synaptic contacts on Purkinje cell spines were enhanced by staining and used for length determination. The computer defined the synaptic length along the contact curvature and accumulated the data for average profile length. Average contact area was estimated from profile length as diameter for a disc and then corrected by applying a factor from reconstruction of serial sections. The number of synapses was determined by stereological means utilizing a normalizing procedure to correct for varied shapes and distributions of profiles in thin sections. The sampling probe consisted of equally spaced parallel lines that were analyzed for the number of crosses made with the boundary of circles representing the area of each synaptic profile. Sampling bias due to patterns in the distribution of synapses was eliminated by random redistribution of these circles. Analysis of cerebellar tissue from controls, developmental conditions (such as malnutrition and genetic mutations) and deafferentation in the adult revealed marked increases in Purkinje cell spine contact size that was reciprocal to the deficit in the number of synapses on Purkinje cell spines.

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24.6 PARTIAL DEAFFERENTATION, SPROUTING AND REINNERVATION IN THE CRAYFISH CNS. R.J. Strandburg. Dept. of Psychology, UCLA, Los Angeles, CA 90024.

It has been amply demonstrated that following partial deafferentation, intact neurons will sprout to fill denervated muscle and CNS target cells. Moreover, in muscle tissue, regenerating axons can reinnervate fibers with sprouted inputs either by functionally replacing the sprouted terminals or by coexisting with them. Reinnervation by previous inputs is, however, not necessarily complete. In this study, the sprouting of sensory inputs to an identified target cell in the crayfish CNS (sixth abdominal ganglion) following partial deafferentation was examined, and the ability of regenerating axons to reinnervate that cell, to "silence" sprouted inputs, and/or to be excluded by them was assessed.

Specifically, the response of interneuron A (A) to water current activation of its third root (R3) sensory inputs was examined under a variety of experimental conditions. At the outset of each condition, all other (non-R3) sensory inputs to A were removed. In the first group, the efficacy of R3 inputs was tested immediately after this partial deafferentation to establish a baseline response in A. In the second group, 12 weeks elapse before testing to permit sprouting of R3 afferents. In the third condition, several roots are directed to the ganglion 12 weeks after the initial deafferentation, and an additional 12 weeks elapse permitting these roots to regenerate into the ganglion either to (1) reinnervate A and possibly displace or repress sprouted inputs or (2) be excluded from A by the sprouted terminals. Finally, a fourth group was run in which the regenerating roots were directed to the ganglion at the time of the initial deafferentation thus placing the regenerating axons and the sprouting collaterals in more equal competition.

It was found that, by physiological criteria, both sprouting and reinnervation by regenerating roots took place. Sprouting was most successful when unchallenged by regeneration. Regeneration was most successful when (presumed) sprouting had not previously occurred. Once sprouting had occurred, it largely survived subsequent invasion by regenerating afferents, though it appeared to be somewhat reduced by their return.

24.7 METAMORPHOSIS AND DESCENDING INPUT TO LUMBAR SPINAL CORD: AN INVESTIGATION USING TWO RETROGRADE TRACERS IN RANA CATESBEIANA. Cynthia J. Forehand and Paul B. Farel. Neurobiol. Prog. and Dept. Physiol., Sch. Med., Univ. N. Carolina, Chapel Hill, N.C. 27514.

The metamorphosis of tadpole to frog is accompanied by a dramatic change in the environmental challenges the animal must face. For example, the transition from aquatic to terrestrial life entails behavioral and electrophysiological changes in the mode of locomotion (Stehouwer and Farel, Neurosci. Abstr., 7, 1981). Whether such changes in the way the animal responds to its environment result from growth of new neural pathways or from a remodeling of existing connections during metamorphosis is unknown. A previous investigation (Forehand and Farel, Neurosci. Abstr., 6: 847, 1980) used the retrograde transport of injected horseradish peroxidase (HRP) to show that the same supraspinal areas project to lumbar spinal cord in tadpoles and juvenile frogs. In the present study, two temporally spaced retrograde tracers, HRP and tritiated, enzymatically inactive HRP (^{3}H-APO-HRP, New England Nuclear), were used to demonstrate that at least some projections to lumbar spinal cord arise from the same cells before and after metamorphosis.

An initial study showed that retrogradely transported HRP remains demonstrable in the neurons of tadpoles or juveniles after 30-40 day survival periods, although the number of labeled cells decreases. In the present study, HRP was injected into the lumbar spinal cords of Stage XVII Rana catesbeiana tadpoles and, after metamorphosis, ^{3}H-APO-HRP was injected into the lumbar cords of the same animals. Four days later, the animals were sacrificed and 15-µm cryostat sections of the brainstems were processed for HRP histochemistry and ^{3}H autoradiography.

Neurons that contained both HRP and ^{3}H-APO-HRP were unambiguously identified in the cerebellar nucleus, vestibular nucleus, brainstem reticular formation and rostral spinal cord. In addition to these doubly labeled cells, many neurons contained only the ^{3}H-APO-HRP, but relatively few neurons contained HRP alone. This result is expected because the HRP label diminishes with long survival times. However, nearly all cells in which HRP could still be histochemically demonstrated also contained ^{3}H-APO-HRP, indicating that many of the neurons which project to lumbar spinal cord in tadpoles retain this projection through metamorphosis.

These findings are consistent with the idea that long descending tracts are conserved through metamorphosis. The possibility thus remains that remodeling of existing neuronal connections occurs to mediate behavioral adaptations to terrestrial life.

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24.8 CHANGES IN STRUCTURE AND CABLE PROPERTIES OF DENDRITIC TREES FOLLOWING AXOTOMY OF LAMPREY SPINAL CORD NEURONS. B. N. Christensen and M. D. Christensen*. Dept. of Physiology and Biophysics, University of Texas Medical Branch, Galveston, TX 77550.

It has been reported previously that following spinal cord transection, the dendritic trees of lamprey reticulospinal neurons are structurally altered (Cohen, M.J. In: Simpler Networks and Behavior, 1976, p. 45). Individual dendrites decrease in length and there is an absolute loss of finer branches. We have investigated the dendritic tree response following axotomy of giant interneurons in the lamprey spinal cord. Our emphasis has been on a quantitative investigation of changes in physical structure and electrotonic length of dendritic trees and the effect of these structural changes on monosynaptic transmission.

At different survival times following spinal cord hemi- or transection, voltage transients produced by a brief current pulse applied to the soma through an intracellular microelectrode were recorded from individual interneurons. These transients were analyzed to determine the cable properties of the dendrites using the Rall model. The cells were then injected with HRP for later structural analysis. Labeled cells were reconstructed photographically from 10 µm thick transverse sections, and dendritic lengths and and diameters measured from photographic enlargements using a digitizing tablet. We found a marked change in the structural organization of the dendritic trees within 1 week following axotomy. These changes include loss of fine dendritic branches and shortening of major dendrites. These findings were correlated with a decrease in the electrotonic length of individual dendrites.

(Supported by grant DHEW-NS-14429)

25.1 DEVELOPMENTAL PROTEIN MALNUTRITION IN RATS: ADAPTIVE CHANGES IN BRAIN INDOLEAMINE METABOLISM IN SMALL-FOR-GESTATIONAL-AGE OFFSPRING. Maravene Miller, Rachelle Hasson* and Oscar Resnick*. Worcester Foundation for Experimental Biology, Shrewsbury, MA 01545.

Small-for-gestational-age (SGA) pups (> 20% reductions in body weights) were born to dams fed a very low protein diet (6% casein) during a 5 week pregravid period, pregnancy and lactation. The severe in utero malnutrition caused the SGA pups to show marked elevations for brain serotonin (5-HT), 5-hydroxyindoleacetic acid (5-HIAA), and tryptophan (TRP) at birth (Table below) compared to pups from dams maintained on a normal diet (25% casein) during the same time interval. After birth, the severe postnatal nutritional lacks caused the SGA pups to show an almost total failure of growth (> 60% reductions in body weights) which is characteristic of the onset of infantile marasmus. These lactational deficits were also responsible for the 40-180% greater than normal increases in brain indoleamine metabolism of the SGA-marasmic pups for days 3-21. Interestingly, these increases in brain 5-HT, 5-HIAA and TRP levels for the SGA-marasmic pups at birth and older ages were markedly higher (47-236%) than those reported by our group (Exp. Neur., 57: 142, 1977) for a milder form of developmental malnutrition in rats (use of an 8% casein diet). These data indicate that the adaptive responses shown by the developing brain to pre- and postnatal malnutrition are highly dependent not only on the duration of the protein lacks in the dam but also on the severity of these protein inadequacies.

Day of Birth (Mean ± S.E.)

Diets	6% (SGA)	25% (normal)
(n)	(8)	(8)
	5-HT ng/gm	
Telencephalon	844 ± 45[a]	361 ± 23
Brainstem	1482 ± 116[a]	537 ± 18
	5-HIAA ng/gm	
Telencephalon	1223 ± 31[a]	599 ± 18
Brainstem	1917 ± 55[a]	619 ± 16
	TRP ng/gm	
Telencephalon	19568 ± 1089[a]	8877 ± 266
Brainstem	25766 ± 547[a]	9333 ± 190

[a] $p < 0.001$, 2-tailed t-tests

Supported by grant HD 06364

25.2 SENSORIMOTOR EFFECTS OF PERINATAL DIETARY HISTIDINE SUPPLEMENTATION IN RATS, J. M. Bell*, P. K. Lundberg*, S. Finger and S. Henkel*. Dept. of Psych., Washington Univ., St. Louis, MO. 63130.

Lundberg (Diss. Abstr., 1981) has found that offspring of rat dams exposed to a 1.3% l-histidine-supplemented diet performed differently from control animals on certain neonatal reflexive behaviors. Negative geotaxis performance was superior to controls while swimming development was delayed. Additionally, tail flick latency data suggested that weanlings fed the histidine-supplemented diet showed an augmented analgesic response after morphine administration. Natelson, Janocko, and Jacoby (1981) have reported that rats fed tryptophan-deficient diets developed more gastric erosions after immobilization. Since histamine is a potent stimulus for gastric acid secretion, dietary histidine enrichment may predispose the animals to a greater incidence of stress-induced gastric erosions. Thus the goal of the present study was to compare the effects of pre-weaning (PRE), post-weaning (POST) or pre- and post-weaning (PRE/POST) dietary histidine supplementation upon various reflexive behaviors, open field behavior, analgesic response to morphine administration and the incidence of gastric erosions following restraint in the cold. Dams were randomly assigned to a 1.3% l-histidine-enriched diet (Nutritional Biochemicals) or a control diet 2 weeks prior to mating. Litters were sexed, weighed, and culled to 10 pups on postnatal days 1 (PN1), PN7, 14 and 21. Behavioral testing included surface righting (PN3-7), negative geotaxis development (PN6-12), pivoting activity (PN7, 9, 11), swimming development (PN6-20), and a battery of tests to assess neurological dysfunction (PN30). Histidine-exposed offspring (PRE and PRE/POST) again exhibited negative geotaxis latencies faster than those of controls and POST groups. Swimming development was also delayed. The mean percentage analgesia achieved in the PRE/POST group (59%) was greater than that of the PRE (11.5%) or POST-weaning groups (20.5%). The assessment of the ulcerogenic potential of dietary histidine supplementation is presently under way as is the determination of serum corticosterone levels.

25.3 FETAL ALCOHOL EFFECTS IN THE RAT HIPPOCAMPUS: A TIMM'S AND ANTEROGRADE HRP STUDY. J.R. West and C.A. Hodges-Savola*. Dept. of Anatomy, College of Medicine, University of Iowa, Iowa City, IA 52242.

In an attempt to determine the effects of prenatal ethanol exposure on the developing central nervous system, pregnant Sprague-Dawley rats were maintained on a liquid diet (Bio-Serv, PR-11) containing 35% ethanol derived calories from days 1-21 of gestation. Their ethanol consumption was approximately 12g/kg/day. Controls were either pair-fed the liquid diet in which isocaloric maltose-dextrin replaced the ethanol, or given lab chow and water. At birth all litters were culled to eight pups and the ethanol and pair-fed litters were cross-fostered to normal mothers. All were allowed to reach an age of 60-90 days at which time they were used for either Timm's or anterograde horseradish peroxidase (HRP) experiments. The modified Timm's sulfide silver method revealed dramatic changes in mossy fiber topography at midtemporal hippocampal levels in 9 of 10 ethanol-exposed rats. The alteration was in the form of an aberrant distal infrapyramidal terminal band in hippocampal subfield CA3a. To further examine the aberrant mossy fibers, small iontophoretic injections of HRP (Sigma type VI, 20% w/v in 2% DMSO) were made in the dentate gyrus at septotemporal levels where the aberrant Timm's staining occurred. Appropriate survival times (15-22 hrs) revealed the morphology of the mossy fiber axons. HRP-labeled mossy fibers were observed leaving the suprapyramidal bundle at several locations to penetrate the pyramidal cell layer and occupy a position similar to that of the aberrant staining in the Timm's material. The distal infrapyramidal mossy fibers possessed periodic swellings characteristic of the suprapyramidal mossy fiber axons. Therefore, although the prenatal ethanol exposure produced mossy fibers that terminated in an inappropriate area, the morphology of the axons appeared normal. The presence of abnormal brain circuitry in the absence of obvious external malformations suggests that these changes might best be termed fetal alcohol effects rather than fetal alcohol syndrome. Supported by grant AA 03884 from NIAAA.

25.4 MORPHOLOGIC ALTERATION IN HIPPOCAMPAL CA1 PYRAMIDAL CELLS FOLLOWING PERINATAL ETHANOL ADMINISTRATION. D. L. Davies and D. E. Smith. Dept. of Anat., Louisiana State Univ. Med. Ctr., New Orleans, LA 70112.

Elaboration of the dendritic arbor is a key developmental event which results from the interplay of numerous genetic and microenvironmental factors. The extent, intricacy and geometric pattern of the resulting dendritic array significantly influences the cell's capacity to interact with neighboring neurons, glia and the extracellular milieu.

The effects of ethanol on the dendritic arbor have been examined in postnatal mice following a relatively mild alcoholic insult timed to coincide with the birth and subsequent maturation of neurons in hippocampal CA1 sector. These cells are born during the last half of gestation in the fetal mouse (Angevine, Exp. Neurol. Suppl. 2:1, 1965).

Pregnant C57BL/6J mice were assigned to one of 3 groups: controls given free access to chow and water, experimentals supplied with a liquid diet containing 25% of its caloric value as ethanol (5.4% v/v) and nutritional-controls pair-fed a liquid diet with sucrose isocalorically replacing ethanol. This diet regimen was initiated on gestational day 12 and maintained until postnatal day 7. At birth, litters were culled to 6 pups to enhance maternal nurture; however, body weights of ethanol exposed pups remained below control values indicating possible attendant nutritional or metabolic derangement. Golgi impregnated material from litters sacrificed at 7, 14 and 21 postnatal days was assessed for changes in hippocampal CA1 pyramidal cells.

Preliminary examination of dendritic length and soma volume at 7 days revealed that both the controls and experimentals were at equivalent developmental stages. By 14 days, basal dendrites of controls were substantially expanded in size and complexity; whereas the dendritic arbors of ethanol-exposed experimentals exhibited a developmental delay. Measurement of these stunted dendrites showed a 20% reduction in total dendritic length with a concurrent decrease in soma size. By 21 days, the reduced arbor remained detectable, but was less dramatic, suggesting the possibility of neuronal recovery.

This investigation was supported, in part, by a research grant from the Distilled Spirits Council of the United States, Inc.

25.5 FETAL ALCOHOL EFFECTS ON ACTIVE AND PASSIVE AVOIDANCE. J.A. Holloway, and. M. Church*. Dept. Psychiat. & Behav. Sci., Univ. Okla. Health Sci. Ctr., Oklahoma City, OK 73190.

Children with Fetal Alcohol Syndrome are reported to have learning and memory deficits. Therefore, we examined if animals exposed to ethanol prenatally would also demonstrate such deficits. Female rats were exposed to a liquid diet containing ethanol (35% or 40% calories ethanol derived) from the 4th day of gestation until partuition. Pups born to these mothers were tested for retention of a passive avoidance task and learning and retention of an active avoidance task. Pair-fed sucrose (nutritional control) females and ad lib lab chow females were bred and their offspring used as controls. At 28 days of age offspring were put into the lighted compartment of a passive avoidance apparatus and their latency to enter a dark compartment where they received mild foot shock was recorded. Their retention of this task was assessed both 1 hr. post-training and 72 hr. post-training. Both offspring of ethanol exposed mothers and pair-fed sucrose controls showed passive avoidance retention deficits at both times tested when compared to the ad lib lab chow control offspring, although the ethanol exposed animals showed greater deficits. Therefore, the malnutrition effect of the diet produced by insufficient dietary intake of both the ethanol exposed mothers and pair-fed mothers may have contributed to these retention deficits in offspring.

From 58-70 days of age offspring of the 3 groups were trained in a step-up one-way active avoidance task and tested for retention 24 hrs. later. Only the ethanol exposed offspring demonstrated deficits in learning and retention of the task.

Thus, animals prenatally exposed to ethanol show deficits in both active and passive avoidance performance. The pattern of results is not consistent with the hypothesis that prenatal exposure to ethanol produces only response inhibition deficits. In that case, one would expect passive but not active avoidance deficits. These data do point to long term learning and memory deficits in offspring as a result of the mothers' prenatal intake of ethanol.

25.6

WITHDRAWN

25.7 BEHAVIORAL EFFECTS OF CHRONIC LOW LEVEL PERINATAL EXPOSURE TO BENZENE AND TOLUENE. J. Kostas* (SPON: W. Shain). Toxicology Institute, Div. of Labs and Research, New York State Dept. of Health, Albany, NY 12201.

The behavioral toxicity of chronic low level exposure to benzene and toluene was examined. Nylar mice were exposed to either benzene or toluene from conception to adulthood. Experimental dams were given drinking solutions of either 400, 80, 16, or 0 ppm benzene or toluene by weight in distilled water. The offspring were maintained on the same levels of solutions. Solution consumption of experimental dams and the offspring was not significantly different from control consumption. The exposed offspring showed no differences from controls in mortality rate, weight gain, or in the development of eye and ear opening, startle reflex and surface righting response. However, toluene exposure resulted in reduced habituation of activity in an open field (at 400 ppm) and depressed rotorod performance (16, 80, and 400 ppm). Benzene exposure resulted in a dose-dependent reduction of habituation of activity (16, 80, and 400 ppm), reduced center field crosses (females only at 80 and 400 ppm) and depressed rotorod performance (80 and 400 ppm). Toluene-exposed males also exhibited altered aggressive and social behaviors. A nonsignificant decrease was seen in blood leucocyte counts of benzene-, but not toluene-, exposed animals.

Results reveal several behavioral measures sensitive to exposure levels as low as 16 ppm. Moreover, the subsequent behavioral testing proved to be more sensitive to exposure to these substances than did the overt toxicological measures. While alterations in performance on these tasks indicate functional deficits, specific mechanisms have not been identified. For example, a lack of habituation in the open field may indicate deficits in utilization of sensory cues, alterations in emotionality or inhibitory processes. Nevertheless, the test profile has characterized the behavioral toxicity of benzene and toluene with regard to 1) dependence of effects on the particular task 2) differences between benzene and toluene on the pattern of effects observed and 3) dependence of effects on the level of exposure and, in at least one case, the sex of the animal.

25.8 DISRUPTION OF NUCLEIC ACID AND PROTEIN SYNTHESIS BY CHRONIC PRENATAL CARBON MONOXIDE EXPOSURE. L.D. Fechter, Dept. of Environmental Health Sciences, The Johns Hopkins University, Baltimore, MD 21205.

The consequences of acute asphyxia on the developing central nervous system have received substantial attention and both histopathological and biochemical sequelae have been documented. Despite the high frequency of occurence, less experimental attention has been focused on chronic, but mild hypoxia in the fetus although it represents both a serious environmental problem and therefore another legitimate model of prenatal hypoxia. Moreover, the pattern of CNS disruption produced by mild chronic hypoxia may well differ from that seen after asphyxiation. We have previously reported a variety of behavioral alterations following chronic prenatal carbon monoxide exposure including delays in development of simple sensory-motor tasks and permanent disruption of learning and/or retention of a conditioned avoidance task. Such effects are observed following exposure to carbon monoxide at concentrations far lower than those which disrupt neurotransmitter synthesis. We have reported, however, a reduction in whole brain protein concentrations at birth following such exposure. We have completed additional neurochemical experiments which indicate that prenatal CO exposure does not alter levels of nucleic acids at birth, but does decrease both content and concentration of protein. We have now measured nucleic acid and protein levels in brain regions of subjects at 21 days of age. We have seen a significant reduction in cerebellar protein content and concentration and, also, a significant reduction in the protein to DNA ratio in the cerebellum. No change in DNA content or concentration was seen in experimental subjects. If DNA levels are taken as a measure of cell number, the data suggest that prenatal CO exposure reduces mean cell size, but not cell number in the cerebellum.

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25.9 BRAIN GROWTH AND IRON CONTENT IN IRON-DEFICIENT ANEMIC RATS. Y. Ohira*, V.R. Edgerton, J. Hegenauer*, and P. Saltman*. (SPON: L. Goldberg). Department of Kinesiology, UCLA, Los Angeles, Calif. 90024, and Department of Biology, UCSD, La Jolla, Calif. 92093.

Severe iron deficiency was produced in female weanling Sprague-Dawley rats. Following five weeks on an iron-deficient diet.Mean body weight was 117 and 211 g ($p < 0.001$) in iron-deficient and normal control rats, respectively. Hemoglobin was 6.0 and 15.4 g/dl ($p < 0.001$), and plasma iron was 60 and 178 µg/dl ($p < 0.001$). Absolute brain weight was 1.67 and 1.89 g ($p < 0.001$) and relative weight to body weight was 1.52 and 0.92% ($p < 0.001$). Total iron content measured by an atomic absorption spectrophotometer was 14.5 and 20.2 µg/g ($p < 0.01$). After subtracting hemoglobin iron, however, no significant difference was observed between two groups (12.1 and 13.4 µg/g). Mitochondrial total iron was 20.9 and 25.0 µg/g protein ($p > 0.05$). Another set of rats was treated orally with tracer dose of ^{59}Fe and assimilation of ^{59}Fe was measured four days later. The ^{59}Fe found in each group was 6.2 and 3.8 ng/g ($p < 0.01$) for whole brain and 27.7 and 15.7 ng/g protein for mitochondria. Even though tissue iron was not depleted significantly, there was a greater iron uptake in the iron-deficient brain than found in the normal. These data also suggest that severe iron deficiency reduces the rate of general brain growth. This study was supported in part by a USPHS grant from National Institute of Arthritis, Metabolism, and Digestive Diseases, AM-12386.

25.10 **THE EFFICACY OF BRAIN STIMULATION REWARD IN PROTEIN MALNOURISHED RATS.** **Robert D. Hall. Worcester Foundation for Experimental Biology, Shrewsbury, Mass. 01545**

A persistent problem in animal studies of developmental malnutrition and its effects on learning has been the lack of an effective reward whose efficacy is clearly independent of dietary manipulations. One possibility that has not been examined is brain stimulation reward. At least two considerations, however, suggest that intracranial reinforcement might be more efficacious in malnourished animals than in well-nourished ones: (1) In several rat models of developmental malnutrition the levels of brain catecholamines and indoleamines are substantially higher than they are in well-nourished animals. To the extent that brain stimulation reward depends on the monoaminergic systems and the levels of their transmitters one might expect it to be more efficacious in malnourished rats than in well-nourished ones. (2) Animals reared and maintained on diets that restrict growth might exhibit an enhancement of intracranial self-stimulation rates like that resulting from acute food deprivation. This study was undertaken to evaluate these possibilities with a view towards using brain stimulation reward in studies of complex learning by developmentally malnourished rats.

Malnourished rats were born to dams maintained on an 8% casein diet from 5 weeks before mating until their pups were weaned at 21 days of age. The offspring were maintained on the same low protein diet throughout the experiment. Well-nourished rats were reared on an isocaloric 25% casein diet. The efficacy of medial forebrain bundle stimulation was assessed in an initial experiment by measuring self-stimulation rates on Fixed Ratio (FR) reinforcement schedules whose values were increased each day by 3 up to FR 12 at which value rats were run for 5-7 days. In the main experiment this paradigm was extended to FR30. Then in a single session FR values starting at 4 were increased every 5 reinforcements until an extinction criterion of more than 10 minutes between reinforcements was reached. Finally, abbreviated intensity functions with only 3 current values were determined.

There was no indication that the intracranial reinforcement was more efficacious for the malnourished rats than it was for the well-nourished ones. Measures of response rate under the various conditions and breakdown ratios on the increasing ratio test were comparable for the two groups of rats. This finding suggests that brain stimulation reward may indeed be a useful tool in the investigation of learning capacity in malnourished rats.

25.11 EFFECTS OF SHAKING STIMULATION ON PRENATAL CHICK BEHAVIOR. J. H. Gassler* and M. L. Kirby (SPON: R. Holt). Dept. of Anatomy, Med. Col. of Georgia, Augusta GA 30912.

In a previous report (Gassler et al., Neurosci Abstr. 6:641, 1980) we found that shaking stimulation accelerated hatch time significantly in chicks when applied intermittently during the second half of incubation. This report documents further behavioral studies on intermittently shaken chick embryos. We have examined Type III movements and pre-hatch positions in 16-19 and 16-21 day embryos respectively. We have added an acute group of shaken embryos (never shaken prior to the recording session) to our motility, Type III and pre-hatch position studies. In order to assess the effects of early versus late stimulation, two groups of eggs were incubated: one group received intermittent shaking stimulation from incubation days 10 through 16; the second group of experimental eggs received the same shaking stimulus from day 16 to pipping. This study showed that eggs shaken from day 16 to pipping had an accelerated hatch time of seven hours over controls. Eggs shaken on days 10-16 showed no acceleration in hatch time. Motility studies showed that, contrary to our previous report, chronically shaken embryos (shaken from day 10) increase their motility only on day 17 when the shaker was turned on. The motility dropped to resting values after two hours of shaking. Acutely shaken embryos (shaken once) showed no changes in motility on any day. Type III movements decreased after the shaker was turned on in acute and chronically shaken 16 day embryos. Type III movements increased on day 17 after two hours of shaking in the chronically shaken group only. Chronically shaken embryos on days 17, 20 and 21 showed significant differences from controls in pre-hatch positions, indicating accelerated development. Experimental 16, 18, and 19 day embryos showed no differences from controls. Because acutely shaken embryos show no changes in motility when subject to shaking, the motility changes in the 17 day chronically shaken embryos must be caused by exposure to more than one shaking cycle. It cannot be determined however, how much shaking must occur prior to day 17 in chronically shaken embryos to cause the above mentioned changes in overall motility and incidence of Type III movements. The hatching study mentioned above seems to indicate that shaking has no effect on behavior prior to day 16. We cannot rule out shaking effects prior to day 16 on motility or pre-hatching behavior until studies are carried out where shaking is begun on day 16 and motility, Type III movements and pre-hatching positions are examined on subsequent days.

26.1 ACTIVITY OF CEREBELLAR CORTICAL NEURONS DURING VOLUNTARY CO-CONTRACTION AND RECIPROCAL INHIBITION OF ANTAGONIST MUSCLES. Robert C. Frysinger*, Daniel Bourbonnais* and Allan M. Smith. Centre de recherche en sciences neurologiques. Université de Montréal, Québec, Canada.

This study was designed to examine the activity of single cerebellar neurons during the reciprocal inhibition as well as the co-contraction of antagonist muscles. To accomplish this, a Macaca fascicularis monkey was trained to perform two different motor tasks with the same hand. The first task consisted of maintaining an isometric precision grip of the thumb and forefinger on a hand-held strain gauge. This lateral pinch is achieved by a general co-activation of the antagonist muscles of the hand and forearm. In the second task the monkey was required to follow a visual cue in order to position the open hand within a 6° target zone by either flexing or extending the wrist. A torque motor added a 10 gram/centimeter force which the monkey was required to overcome in order to position and maintain the manipulandum within the target zone. Position correctly maintained for a one second period was rewarded with fruit juice. A study of the activity of the 6 muscles of the wrist during the flexion and extension movements indicated that antagonist muscles showed a pattern of reciprocal activity, as opposed to the pattern of co-contraction seen in the prehension task. During the performance of both motor tasks, recordings were made from single neurons located approximately in the culmen-simplex area of the ipsilateral cerebellar cortex. In accordance with previous studies, units were found which decreased firing frequency during prehension in spite of a general activation of all forearm muscles. Preliminary evidence from 15 units recorded during both prehension and wrist displacement suggests that neurons showing changes during grasping are activated in one movement direction in the wrist displacement task, and either show no change or a decrease in activity with movements in the opposite direction. In general, the results appear to support the suggestion that cerebellar cortical neurons play an important role in switching between reciprocal and co-active modes of muscle activation.

This research was supported by the Medical Research Council of Canada.

26.2 CHANGES IN PURKINJE CELL RESPONSIVENESS TO CF INPUT FOLLOWING PAIRING OF OLIVARY STIMULATION AND FOREPAW DEPLACEMENT. John F. Brons and Lee T. Robertson. Neurol. Sci. Inst., Portland, OR 97210.

It has been postulated that interactive effects of climbing fiber and parallel fiber systems on cerebellar Purkinje cells (P-cells) are important in motor learning. This study shows that a conditioning procedure can induce long-term changes in the pattern of spike responses associated with climbing fiber activation.

The activity of 26 P-cells was recorded extracellularly in the intermediate cortex of Lobule V. P-cells were identified by the occurrence of spontaneous complex spikes (CS) and simple spikes (SS). The interaction of climbing fiber activation and forepaw movement on P-cell activity was examined by a three step conditioning procedure: 1) stimulation of the contralateral inferior olive (IO), 2) pairing of IO stimulation with ipsilateral forepaw displacement produced by a computer controlled solenoid, and 3) stimulation of the contralateral IO. A minimum of 40 trials was used for each step.

The IO was electrically stimulated with single negative pulses (0.5 ms) at suprathreshold levels (100-400 uA) for eliciting a CS in each cell. The CSs were followed by pauses in SS activity, which ranged from 12 to 165 ms in duration (91 ± 36 ms, mean ± sd) During pairing, IO stimulation was followed by a 3 mm forepaw displacemennt with 100 or 150 ms interstimulus intervals.

In 14 cells, repeated stimulus pairing produced a greater uniformity in the duration of pauses of SS activity elicited by IO stimulation. Pauses of SS activity terminated 43 ± 27 ms (mean ± sd) prior to onset of paw movement. This uniformity resulted from longer pauses shortening in 7 cells and from shorter pauses lengthening in 7 cells. Resumption of SS activity after each pause appeared as a conditioned anticipatory response to the onset of paw movement. Similar results were seen with both 100 and 150 ms interstimulus intervals. The conditioned response persisted in half of the cells for at least 2 min during the final IO stimulation step.

These results suggest that stimulus pairing can alter the responsiveness of P-cells to climbing fiber input and that this is partially attributable to changes in the processses underlying the post-CS pause in SS activity.

26.3 EXCITABILITY CHANGES IN SIMPLE SPIKE PURKINJE CELL ACTIVITY FOLLOWING A CLIMBING FIBER INPUT. C. J. McDevitt*, T. J. Ebner, and J. R. Bloedel. (SPON: F. Torres). Depts. of Neurosurgery and Physiology, University of Minnesota, Minneapolis, 55455.

Many previous studies have suggested that a reduction of simple spike activity in a Purkinje cell follows the period of inactivation after a climbing fiber input to the same cell. However, the design of these studies did not enable an accurate assessment of the regularity with which this effect occurred following a spontaneous climbing fiber input in different Purkinje neurons. Therefore experiments were performed in decerebrate, unanesthetized cats to quantify the changes in the simple spike discharge of Purkinje cells following their spontaneously evoked response to a climbing fiber input. A total of 98 Purkinje cells identified by the presence of spontaneous complex spikes were recorded in the anterior lobe of the cerebellum with glass microelectrodes. Post stimulus time histograms (Σ =100) of the simple spike activity triggered on the occurrence of a complex spike were constructed and examined for changes in simple spike activity following the climbing fiber input. The average simple spike activity for 40 msec following the inactivation period was compared with the average background firing rate for each cell. In all cells an initial inactivation period (7-15 msec) was present. Following this inactivation, three different excitability profiles in the simple spike discharge were observed. For most cells (N=67) a brief increase in simple spike activity was observed, and for 14 Purkinje cells there was no change in simple spike activity when compared with the background. For the remaining cells (N=17), a decrease in simple spike firing rate was observed following the spontaneous climbing fiber input. These changes in simple spike activity following the spontaneous climbing fiber input could not be correlated with the average background firing rate of the cell. As has been shown previously, when many climbing fibers were synchronously activated by electrically stimulating the inferior olive, a reduction of the simple spike activity was evoked independent of the excitability change observed when the climbing fiber input occurred spontaneously. These results show that the most common change in impulse activity following the inactivation period evoked by a spontaneous climbing fiber input is an increase in the simple spike discharge rate. Although a decreased rate can occur, this effect can be most readily produced by electrical stimulation of the inferior olive. This work was supported by NIH Grant #2R01-NS 09447-10.

26.4 THE EFFECT OF DESCENDING PROJECTIONS ACTIVATED BY DENTATE EFFERENT PATHWAYS ON THE STRETCH REFLEX. J. L. Vitek*, T. J. Ebner, J. R. Bloedel. (SPON: C. Terzuolo). Departs. of Neurosurgery and Physiology, Univ. of Minnesota, Minneapolis, 55455.

Dentato-reticulospinal and dentato-rubrospinal projections have recently been demonstrated in the cat. These experiments were performed to examine the combined action of these systems on the stretch reflexes in decerebrate cats. The rectified, integrated EMG activity from the gastrocnemius-soleus (GS) and anterior tibialis (AT) muscles of the hindlimb were averaged during sinusoidal stretching (.1-5 mm, .25-15 Hz) of each muscle in a reciprocal fashion, i.e. GS was stretched as AT was shortened. Electrical stimulation of the dentate nucleus was applied with a stereotaxically placed monopolar stainless steel electrode (exposed tip of 25 u) or a small concentric bipolar stimulating electrode (interpose distance = 500 um). Capacitatively coupled current pulses (.2 msec in duration) at various combinations of current intensity (monopolar electrode 10-500 uA, bipolar electrode 400-1200 uA) and stimulus frequency (50-350 Hz) were used to stimulate the dentate. Electrode placement in the dentate was verified by histological sections and by monopolar recording of the field potential evoked at the fifth cervical segment. This field potential had been demonstrated previously to occur with a latency of 2-3 ms when the electrode is positioned within the dentate nucleus. Stretch reflexes in the decerebrate cat were characterized by extensor hyperreflexia and most commonly an attenuated or absent reflex in the flexor when it was stretched. However, co-activation of the flexor in phase with the stretch of the extensor was routinely present. Dentate stimulation produced changes in both the amplitude and the phase relationship of these proprioceptive reflexes. In almost all experiments, the GS stretch reflex as well as the co-activation response of the AT during stretch of the GS was reduced or even abolished. In contrast the flexor's response to stretch was increased, resulting in a reciprocal pattern of reflex activity in these muscles. In addition to demonstrating these specific effects, these experiments show that dentate efferent projections which do not include the thalamus or sensorimotor cortex can modify both the amplitude and organization of proprioceptive reflexes in the spinal cord. This work was supported by NIH Grant #2R-1-NS 09447-10 and NIH Contract #N01-NS-0-2338.

26.5 THE COMPARISON OF IPSILATERAL AND CONTRALATERAL EFFECTS OF DENTATE NUCLEUS INACTIVATION ON THE LATENCY, SPEED AND ACCURACY OF A GOAL-DIRECTED MOVEMENT IN MONKEYS. E. Trouche, D. Beaubaton and E. Legallet (SPON: C. Palmer). CNRS-INP, Marseille, France.

Experiments using exclusion or stimulation procedures demonstrate in monkey a predominant ipsilateral control of the dentate nucleus (DN). However, anatomical data give evidence of bilateral dentatofugal connections. The purpose of the present study was to compare the effects in both limbs of unilateral DN inactivation on spatio-temporal variables of a goal-directed movement in monkeys. The investigations were carried out on five baboons (Papio papio) performing in a cage designed to standardize the working posture. The animals were trained to press a lever situated on a vertical panel, to hold it until an illuminated target appeared, to release the lever and touch the target with one finger. The finger enterring into contact with the panel provided automatically the rectangular coordinated of the pointing response. Reaction time was defined as the time between illumination of the target and release of the lever, movement time as that between release of the lever and contact with the panel. Data analysis was carried out on the averages of reaction times, movement times and spatial errors. After the DN had been electrophysiologically located, unilateral lesions were made. In the arm ipsilateral to the lesion, DN exclusion brought about prolongation of reaction times, modification of movement times and the appearance of systematic errors. In the contralateral limb, only the movement times were impaired and a faster recovery was observed in this case. These results suggest that in monkey DN may exert a bilateral control of speed of movement. Conversely the control exerted by DN, on movement initiation and terminal accuracy is strictly ipsilateral.

26.6 LOCALIZATION OF IONIC CONDUCTANCES IN SOMA DENDRITIC REGIONS OF PURKINJE CELLS: AN IN VITRO STUDY IN GUINEA PIG CEREBELLAR SLICES. M. Sugimori* and R. Llinás (SPON: D. Chiarandini). Dept. Physiol. & Biophysics, New York Univ. Med. Ctr., New York 10016.

Electrical activity of guinea pig Purkinje cells has been demonstrated to consist of a Na^+-dependent action potential at the soma and Ca^{++}-dependent action potentials mainly, but probably not exclusively, in the dendrites (J. Physiol. 305, 197-213, 1980). A detailed study of the distribution of ionic conductances was recently obtained in the in vitro cerebellar slices by intrasomatic recording of Purkinje cells in the absence of extracellular Ca^{++} combined with an iontophoretic application of Ba^{++} at different levels along the vertical axis of the dendritic tree. This experimental paradigm demonstrated the electrical activity of Purkinje cell dendrites when Ba^{++} ions were released in the distal half of the molecular layer in the presence of TTX. Simultaneous recording with an extracellular electrode at dendritic level indicated a large extracellular negative field potential simultaneously with somatically recorded Ba^{++}-dependent action potentials. The action potentials quickly disappeared when the Ba^{++} electrode was removed from the vicinity of the dendritic tree. However, action potentials of smaller amplitude could be obtained when the Ba^{++} ejecting electrode was located at somatic level. At this level it was difficult to demonstrate whether the inward current was flowing at somatic level or at proximal dendritic level. This ambiguity was resolved when action potentials produced by Ba^{++} ejection at somatic level were seen to generate an extracellular positivity at that location, indicating that the soma served as a source of current and thus that Ba^{++} spikes were being generated mainly across the dendritic membrane. A similar set of results was then obtained for the normal Ca^{++} action potentials using a fire-polished extracellular current measuring microelectrode placed against the dendritic membrane. Here activation of the TTX-resistant action potentials recorded at somatic level produced all-or-none inward currents at dendritic level while at somatic level such action potentials were accompanied by outward current. The present results further indicate a spatial distribution for the Na^+ and Ca^{++} conductances in the Purkinje cell which confirm our proposal that Ca^{++} conductances are mainly dendritic while sodium conductances seem to be restricted to the somatic level. Supported by USPHS grant NS13742 from NINCDS.

26.7 EFFECTS OF EXTRACELLULAR POTASSIUM ON THE EXCITABILITY OF THE PARALLEL FIBERS. R. C. Malenka, J. D. Kocsis, and S. G. Waxman. Dept. of Neurology, Stanford Sch. of Med. and Veterans Administration Medical Center, Palo Alto, CA 94304.

Changes in extracellular potassium concentration ($[K^+]_o$), have been shown to effect several aspects of neuronal functioning. In this study, we examine the effects of $[K^+]_o$ on the excitability of the non-myelinated parallel fibers (Pfs) of the rat cerebellar cortex. Rat cerebella were continuously superfused with normal Ringer solution (NS) or with solutions containing varying concentraions of K^+ (5-30 mM). Local microstimulation of the Pfs was employed and the "on-beam" Pf field potential was recorded within 70µm of the cerebellar surface.

Double stimulation experiments have shown that the Pfs exhibit a pronounced supernormal period (SNP) following a single conditioning volley as evidenced by a decrease in the latency of the Pf volley. When the superfusate was changed from NS (3 mM K^+) to a 10 mM K^+ solution, the magnitude of the latency reduction during the SNP decreased as did the duration of the SNP. No SNP was exhibited in a 15 mM K^+ solution. Further analysis revealed that as the $[K^+]_o$ increased, the latency of the Pf volley continuously decreased while with further increases in $[K^+]_o$, the latency of the Pf volley increased beyond control values indicating a subexcitable state. With even greater increases in $[K^+]_o$ the Pf volley was obliterated indicating conduction block. During relatively high frequency stimulation (20-70 Hz) increases in $[K^+]_o$ are observed and consecutive Pf responses reveal a similar continuous change in excitability; an initial superexcitable state followed by a subexcitable one.

Increases in $[K^+]_o$ can also be recorded in areas lateral to the activated Pf beam. If the excitability of the Pfs is related to $[K^+]_o$ the excitability of adjacent nonactivated Pfs, where $[K^+]_o$ has increased, should change. Pairs of stimulating microelectrodes were placed on the cerebellar surface (100-500 µm apart) so that stimulation of the "off-beam" electrode elicited no Pf activity at the "on-beam" recording electrode. Following off-beam stimulation (50 Hz for 1.5 sec) the latency of the on-beam Pf volley decreased and its amplitude increased, indicating an increase in Pf excitability. The change in Pf excitability was only observed when the off-beam stimulating electrode elicited detectable $[K^+]_o$ changes at the on-beam recording site.

These results suggest that small increases in $[K^+]_o$ may contribute to an increase in Pf excitability while greater increases in $[K^+]_o$ lead to a decrease in Pf excitability. The extremely small diameter of these axons could make them particularly sensitive to changes in intra- or extracellular ionic concentrations.

26.8 DISTRIBUTIONS OF CHOLINE ACETYLTRANSFERASE AND ACETYLCHOLINESTERASE ACTIVITIES IN REGIONS AND LAYERS OF RAT CEREBELLUM. C.D. Ross, J.T. Smith* and D.A. Godfrey. Dept. of Physiology, Oral Roberts University, Tulsa, OK 74171

Two entire sagittal sections of cerebellar vermis from each of 5 rats were each microdissected into over 100 samples of molecular (ML), granular (GL) or white matter (WM) layers, the exact location of samples recorded onto a map of the section and assayed for choline acetyltransferase (ChAT) or acetylcholinesterase (AChE) activities. Highest activity of both enzymes was in the GL of the "vestibulocerebellum", the nodulus and ventral uvula (lobules X and IXc of Larsell). Differences of 14-(ChAT) and 7-(AChE) fold were found between lowest and highest average activities in samples of GL from lobules VIa (ventral) and X of the 5 rats. Data for samples from major folia were combined and averages determined (± S.E.M.) for 5 rats (ChAT: µmoles/kg dry wt/min @ 37°; AChE: mmoles/kg dry wt/min @ 37°).

FOLIUM		ML	GL	WM	entire folium
I	ChAT	27 ± 2	52 ± 4	34 ± 5	38 ± 3
	AChE	10 ± 0.4	35 ± 4	16 ± 1	21 ± 2
II	ChAT	21 ± 2	55 ± 7	29 ± 1	35 ± 4
	AChE	6 ± 0.4	28 ± 3	15 ± 2	15 ± 1
III	ChAT	21 ± 2	61 ± 3	34 ± 6	41 ± 3
	AChE	6 ± 0.3	25 ± 2	15 ± 3	15 ± 1
IV	ChAT	19 ± 2	55 ± 3	40 ± 4	37 ± 2
	AChE	6 ± 0.5	23 ± 2	12 ± 1	14 ± 1
V	ChAT	17 ± 2	52 ± 2	21 ± 3	33 ± 1
	AChE	5 ± 0.4	19 ± 1	7 ± 0.4	10 ± 0.5
VI	ChAT	15 ± 1	57 ± 3	20 ± 2	32 ± 1
	AChE	4 ± 0.3	26 ± 1	15 ± 2	14 ± 0.3
VII	ChAT	14 ± 1	71 ± 6	26 ± 10	35 ± 4
	AChE	5 ± 0.4	34 ± 2	17 ± 3	17 ± 1
VIII	ChAT	16 ± 2	53 ± 5	39 ± 6	34 ± 3
	AChE	7 ± 0.3	28 ± 3	20 ± 2	18 ± 1
IX	ChAT	35 ± 2	94 ± 4	73 ± 7	64 ± 2
	AChE	9 ± 1	43 ± 4	28 ± 2	26 ± 2
X	ChAT	52 ± 4	369 ± 30	263 ± 52	201 ± 13
	AChE	8 ± 1	85 ± 7	87 ± 17	46 ± 4
Average of all folia					total section
	ChAT	23 ± 2	85 ± 2		51 ± 2
	AChE	6 ± 0.5	34 ± 1		19 ± 1

(Supported by ORU intramural research funds)

26.9 **INCREASED NOREPINEPHRINE METABOLISM AND REDUCED β-RECEPTOR BINDING IN THE CEREBELLUM OF THE MUTANT MOUSE DYSTONIA MUSCULORUM.** **Donald Kay Riker, Ruben Zito*, Robert Roth, and Anne Messer. Depts. Pharmacology, Medicine, & Psychiatry, Yale Univ. Sch. Med. & West Haven VA Med. Ctr., New Haven, CT 06510 & NYS Dept. of Health, Albany, NY 12201**

The neurological mutant mouse dystonia musculorum (dt/dt) exhibits bizarre appendicular and truncal dystonia and clasping hyperflexion when suspended. No gross cerebellar histopathology has been reported, although red nucleus pathology and sensory polyneuropathy have been noted (Duchen & Strich, Brain 87:367, 1964; Messer & Strominger, Neurosci. 5:543, 1980). We evaluated striatal dopamine and cerebellar norepinephrine (NE) metabolism in this mutant and compared the results to those obtained from wild-type (+/+) control BALB/c or B6C3 backgrounds. Tyrosine hydroxylase (TH) activity and steady-state 3,4-dihydroxyphenyl-acetic acid and homovanillic acid levels were similar in the striata of mutants and controls. In the mutant TH activity and steady-state levels of the NE metabolite MHPG were elevated in the cerebellum 38% and 42-66% respectively. However, NE levels (~350ng/g) and a Purkinje cell (PC)-specific marker, cGMP-dependent protein kinase (cGMPK), were unchanged in the mutant cerebellum. Preliminary results suggest that ^{3}H-dihydroalprenolol β-receptor binding sites measured at saturation (18nM) are not significantly different in the neocortex or myocardium of mutants. On the contrary β-receptor binding appears reduced 65-70% in the cerebellum compared to controls.

We favor the hypothesis that there is a primary reduction in afferent information reaching the cerebellum during development. Secondarily, the locus coerulus, which supplies most of the cerebellar NE innervation, has responded to this decrease in impulse traffic by a compensatory increase in NE metabolism. This would seem to encourage afferent-evoked discharge of the PC (Woodward et al, Fed. Proc. 38:2109, 1979). A tertiary consequence might be "down-regulation" of cerebellar β-receptors. An alternative hypothesis, inherited loss of β-receptors, could be a primary event. However, their loss only in the cerebellum, the apparent normal density of PC's (ie-normal cytoarchitecture and cGMPK levels), and lack of evidence for reactive sprouting (ie-normal NE levels) suggest that cerebellar NE metabolism is activated in response to reduced impulse traffic, not target cell derangement. **(Supported by the Dystonia Medical Research Foundation, Beverly Hills, CA).**

26.10 THE DENSITY OF GRANULE CELLS AND MOLECULAR LAYER SYNAPSES IN THE RAT CEREBELLAR CORTEX. R. J. Harvey, R. M. A. Napper* and M. S. Phillips*. Dept. Anatomy, Univ. of Otago Med. Sch., Dunedin, New Zealand.

The cerebellar cortex is widely believed to be a flat uniform sheet which has been topologically deformed into the deeply folded form that it takes in an adult mammal. We have made some quantitative studies to determine the numbers of granule cells and the number of synapses which they make. We counted the granule cells in measured areas of granular layer from high-power optical micrographs of thin (1 or 1.5 μm) methacrylate sections of rat cerebellum, and used these values to obtain the volume density of granule cells (similar to the method used by Friedrich & Brand (Neurosci., 5:349-356, 1980) in the cat); the value obtained for fixed dehydrated material was $2.0 \times 10^6/mm^3$. The length of the "unfolded" cortical sheet measured along the Purkinje cell layer in parasagittal sections through the vermis is around 80 mm and the mean thicknesses of the granular layer and molecular layer are 180 and 270 μm respectively. From this, the number of granule cells underlying 1 mm^2 of cortex is 3.6×10^5. From transmission electron micrographs of the molecular layer cut in a plane perpendicular to the parallel fibres, preliminary results suggest that the mean density of parallel fibres is in the region of $4.2 \times 10^6/mm^2$. Thus the number of parallel fibres intersecting a section 1 mm long through the full depth of the molecular layer will be 1.1×10^6. These fibres are the axons of those granule cells lying beneath a strip of cortex 1 mm wide whose length is equal to the length of the parallel fibres. From this the mean length of the parallel fibres is 3.1 mm, a figure close to the middle of the range of lengths found by Schild (J.Physiol.(Lond.), 303:25P, 1980). Each parallel fibre makes about 600 contacts/mm length (Palay & Chan-Palay, The Cerebellar Cortex, Springer, 1974). This means that beneath 1 mm^2 of cortical surface, there are 6.6×10^8 parallel fibre synapses. The majority of these are believed to be with the long dendritic spines of Purkinje cells (Palay & Chan-Palay, op.cit.). There are around 1000-1200 Purkinje cells/mm^2 in the rat, so the above results imply that each must receive more than 3×10^5 parallel fibres. It is believed that a Purkinje cell spine rarely receives more than one contact from a parallel fibre, but the number of contacts suggested by our results is very much greater than the number of spines which has been estimated for rat Purkinje cells (1.8×10^4); this large discrepancy is under investigation.

26.11 DETAILS OF CEREBELLAR ORGANIZATION REVEALED BY DIGESTIVE PREPARATION FOR SCANNING ELECTRONMICROSCOPY. B. F. Reese* and D. M. D. Landis (SPON.: T. S. Reese). LNNS, NINCDS, NIH, Bethesda, MD 20205, and Dept. of Neurology, Harvard Medical School, Massachusetts General Hospital, Boston, MA 02114.

The scanning electronmicroscope (SEM) is a powerful technique for visualizing surfaces of cells and tissues at a level of resolution beyond the reach of the light microscope. However, its usefulness in examining the brain has been limited by the lack of a preparative method to expose regularly the true outer surfaces of neurons and glia. We present here an approach to preparation of the cerebellum for SEM which depends on acid digestion to weaken intercellular attachments. Adult rats were perfused with aldehydes and their cerebellum tissue-chopped at 600 μm. Slices were then digested in 4 percent osmium tetroxide (unbuffered) or in 4 N HCl at 40-60°C for 2-24 hrs (depending on temperature). These slices, which are quite fragile after critical point drying, were pulled apart to reveal neuronal and glial surfaces inside. The main advantage of these methods is that neurons and glia are not broken open, but instead separated along their natural surfaces to expose large three-dimensional fields of cerebellar processes which can easily be visualized in a SEM. Examples of cerebellar structures particularly well visualized are: 1. Complete granule dendrites including their interdigitations with adjacent granule dendrites. In contrast, the Golgi technique only shows one granule dendrite at a time while freeze-fracture shows only fragments of dendrites. 2. Glial sheaths and basket cell terminals remain intact over hemispheric areas which formerly abutted Purkinje cell bodies. The detailed views of these synaptic patterns, and their interrelationships with glia, may allow investigation of subtle changes during development or in different functional states. 3. Axon hillocks and ascending axons of granule cells. The organization of these axons into bundles is readily appreciated. 4. The mosaic pattern of glial processes abutting blood vessels on the surface of the brain. The pattern of glial processes at blood vessels differs from that on the cerebellar surface.

We began by applying this method to normal rat cerebellum because its structure was already well understood. So far, we have mainly confirmed earlier concepts of cerebellar structure. But a better and quicker method of seeing structures directly provides a means to look for subtle changes during development or after experimental manipulations, and to understand aspects of intercellular relationships in regions of the brain that are not so well understood.

26.12 VISUAL AND AUDITORY INPUT AND OUTPUT RELATIONS OF THE LATERAL CEREBELLUM: THE PARAFLOCCULUS. A. Azizi, R.A. Burne and D.J. Woodward. Dept. of Physiol., The Univ. Tx. Hlth. Sci. Ctr., Dallas, TX 75235.

Previous anatomical and electrophysiological evidence from this laboratory has demonstrated the existence of visual and auditory cortical inputs to the paraflocculus of the cerebellum. In this report we describe data indicating a convergence of natural visual and auditory stimulation onto parafloccular neurons, and in addition the existence of specific projections onto the deep cerebellar nuclear cells and output of these nuclei which influence the cerebral cortex. The techniques of 1) presentation of time locked tone and moving visual stimuli, 2) orthograde transport of labeled amino acids and 3) electrical stimulation of visual cortex were employed.

Single unit recordings of 32 units in the paraflocculus of unanesthetized immobilized rats showed evidence, through histogram analysis, for excitatory and/or inhibitory mossy fiber and climbing fiber inputs. Responses were elicited following presentation of pure tones (Sinusoidal waves 200 Hz to 20k Hz, 65-80 db. intensity) in auditory field and/or computer controlled visual stimuli (bars of light moving at specific orientations) in the visual field. Some units (11) showed evidence of facilitation when a combined auditory and moving visual stimuli were presented to the animal.

Hydraulic injections (.15-.3 μl) of ^{3}H-leucine (77 μCi/μl) into the paraflocculus resulted in afferent terminal labeling over specific regions of the ipsilateral lateral and interpositus nuclei of the cerebellum. Autoradiographic silver grains were primarily localized over the ventral caudal region of the large cell subdivision in the lateral nucleus and over the small and large cell subdivisions of the ventral caudal interpositus nucleus.

Single unit recordings were obtained from identified neurons in the lateral nucleus of halothane anesthetized rats. Histogram analysis showed evidence of excitatory and/or inhibitory inputs following electrical stimulation of the visual cortex (single pulse, .2 msec duration, -.07 to -.6 mA, 1-20 Hz) with latencies ranging from 3-10 msec. Electrical stimulation of lateral and interpositus nuclei evoked multiunit and single unit responses in the sensory and motor cerebral cortices.

The observations indicate a convergence of auditory and visual sensation in the paraflocculus. Further, specific projection areas in deep nuclear zones are identified which may have a role in relaying the output of visual-motor integration onto the brain stem and the cerebral cortical areas. Supported by grants NSF BNS77-01174, NIDA, DA02338-01 and the Biological Humanics Foundation.

26.13 PONTINE RELAY FROM VISUAL CORTEX TO UVULA AND PARAFLOCCULUS. F. Robinson*, J. L. Cohen*, J. May*, A. Sestokas*, M. Glickstein. Walter S. Hunter Laboratory of Psychology, Brown University, Providence, RI 02912.

Visual cells in the rostral pons of cat project to the uvula and paraflocculus of the cerebellum (Robinson et al., Abstract Soc. Neurosci. 6:510, 1980). The present study addresses two questions raised by these results. First, do pontine cells which project to uvula and paraflocculus relay information from cortical visual areas? Second, do uvula and paraflocculus receive their visual projections from anatomically separate populations of cells in the rostral pons? To answer these questions we compared the location of visual cortical terminals in the pons to the location of pontine cells that project to the uvula and paraflocculus.

Pontine terminals of visual cortical cells were labeled by injecting ^{3}H proline into the medial bank of the middle suprasylvian sulcus and into area 18. In the same animal, cells projecting to uvula or paraflocculus were labeled by making iontophoretic deposits of horseradish peroxidase (HRP) into one or the other cerebellar structure. Adjacent sections through the pons were alternately processed by autoradiography (ARG) and HRP. The locations of ARG labeled terminals or HRP labeled cells were marked on an outline of each section with an x-y plotter driven from the microscope stage. The overlap of labeled terminals and filled cells was studied by superimposing the outlines of adjacent sections.

We found that there was considerable overlap of ARG labeled terminals and HRP labeled cells in the rostral pons. The uvula and paraflocculus, therefore, both receive input from extrastriate visual areas via pontine relay cells. Further, the uvula receives its projections from cells clustered near (<400 µm) the pyramidal tract as it courses through the pons and from another group of pontine visual cells near the midline. The paraflocculus receives fibers from cells which are further away (>400 µm) from the pyramidal tract. Unlike uvula, the paraflocculus receives few fibers from the group of pontine visual cells near the midline. The uvula and paraflocculus thus appear to receive visual information from different sets of pontine relay cells.

26.14 FLUORESCENT DOUBLE-LABEL STUDIES OF THE PONTOCEREBELLAR SYSTEM. G.A. Mihailoff. Dept. Cell Biology, The Univ. Tx. Hlth. Sci. Ctr. Dallas, TX 75235.

In the course of deciphering the organizational features of the pontocerebellar system through HRP transport methods, one crucial question that has evolved concerns the degree to which individual pontocerebellar axons collateralize and distribute to multiple cerebellar cortical locations. This general issue of potential collateral branching can actually be divided into at least two components: 1) to what extent do pontocerebellar neurons distribute unilaterally to more than one cerebellar lobule; and 2) to what extent do they project bilaterally to corresponding lobules on both sides of the cerebellum. We have approached these two questions by employing double-label tracer studies involving the retrograde transport of two different fluorescent dyes injected in a restricted manner into individual lobules of the cerebellar hemispheres of pigmented (Long-Evans) rats. Two different dye combinations were nearly equivalent in their effectiveness. Nuclear Yellow and Propidium Iodide (Sigma); and Nuclear Yellow with Fast Blue (Illing). The latter combination was used more extensively because double-labeled neurons were more easily identified since both dyes fluoresce at the same excitation wavelength (360 nm). In order to determine the extent of unilateral pontocerebellar axonal branching, paired dye injections (.06-.08 µl) were made into individual hemispheral lobules using the following combinations: Crus I and Crus II; Crus I and Paramedian; and Crus II and Paramedian. Our findings, although only semi-quantitative, indicated that fewer (<10%) pontocerebellar neurons than expected (on the basis of previous HRP studies) were double-labeled and thus suggested that a relatively small number branched to adjacent hemispheral lobules. Often a restricted cluster of pontine neurons exhibited a mixture of singly labeled cells each projecting to separate lobules. This, however, was not the case for the adjacent nucleus reticularis tegmenti pontis (NRTP) where considerably more double-labeled neurons were noted. With regard to bilateral pontocerebellar axonal branching (paired bilateral dye injections in Crus I, Crus II, or Paramedian), the incidence of double-labeled cells was slightly greater (10-20% of labeled population) particularly in Crus I & Crus II cases. Similar to observations in paired unilateral injections, the number of double-labeled cells in NRTP after bilateral injections was slightly elevated over that of the pontine gray. Furthermore, our findings support previous HRP studies which demonstrated the existence of bilateral, mirror-image pontine cell groups projecting to a single cerebellar lobule. Supported by NS12644 (NIH), BNS8004853 (NSF), Biological Humanics Foundation. Thanks to Dr. Loewe for NY samples.

26.15 AN ANATOMICAL STUDY OF THE DISTRIBUTION AND MORPHOLOGICAL CHARACTERISTICS OF PERIOLIVARY RETICULAR NEURONS, G.A. Bishop and J.S. King, Department of Anatomy, The Ohio State University, College of Medicine, Columbus, Ohio 43210.

Anatomical, physiological and pharmacological studies have suggested that neurons in the reticular formation immediately adjacent to the inferior olive (IO) are involved in olivary circuitry. However, to date, conclusive anatomical confirmation of such a projection is lacking. In this study we have addressed three questions: 1) Are there neurons in the periolivary reticular formation (PRF) which project to the IO?, 2) if present, what is their distribution? and 3) what are the morphological characteristics of these neurons? The first two questions were addressed using extracellular injections of horseradish peroxidase (HRP) into the IO. In these experiments a ventral approach to the brainstem was used. Slow pressure injections of 0.03-0.05 µl of 30% HRP were made unilaterally into the IO. Data was analyzed from only those experiments in which the enzyme was confined to the olivary complex. Results indicate that neurons located dorsal to the IO, on or immediately adjacent to the midline project to the IO. Although the major portion of this projection is ipsilateral, there is also a contralateral component. Another population of PRF neurons, located dorsal and lateral to the IO are also retrogradely labeled with HRP. The morphology of PRF neurons was determined by using intracellular injections of HRP into single nerve cells. Results from these experiments confirm and extend the findings of the extracellular studies. Axons from single PRF neurons, located near the midline, distribute bilaterally to the IO. In addition, there are some neurons located immediately dorsal to the IO with dendrites that penetrate into the olivary neuropil. To date electron microscopic analysis of these dendrites indicate that they are postsynaptic. Finally, another population of neurons, located between olivary nuclei or ventral to the medial accessory olive also extend their dendrites into the neuropil of the IO. Thus, the present results suggest a potential anatomical substrate for the inhibitory responses recorded within the IO (Llinas et al '74). In addition, the labeled PRF neurons could represent, in part, the source of serotoninergic afferents to the IO (Wiklund et al '80 and King et al '81). It has been postulated that this system of afferents plays an inhibitory role in olivary circuitry (Wiklund et al '80). (Supported by funds from the Bremer Foundation, a Biomedical Research Support Grant and USPHS NS-08798).

26.16 WIDESPREAD SOURCES OF INPUT CONVERGE UPON MIDBRAIN NUCLEI KNOWN TO PROJECT TO THE INFERIOR OLIVE IN THE CAT. J.A. Saint-Cyr, Playfair Neuroscience Unit and Dept. Anatomy, University of Toronto, Toronto, Ontario, Canada.

In 12 cats anesthetized with Ketaset, either electrophoretic deposits of HRP (8 cats) or pressure injections of WGA-HRP (.1-.025 ul) (4 cats) were made unilaterally in and around midbrain cell groups previously shown to project heavily to the inferior olive (IO). These areas include: caudal-medial subparafascicular-parafascicular (sPf-Pf) nuclei; ventrally adjacent fields of Forel (FF); rostral extremity of the interstitial nucleus of Cajal (INC_R); nucleus of Darkschewitz (ND); caudal INC (INC_C); parvocellular red nucleus (RNp). In the cases of WGA-HRP, excellent anterograde labelling of fibres and terminal areas confirmed the projections to the IO and other brain stem nuclei. Anterograde labelling of lesser intensity was also seen in some HRP cases. Apart from a few local projections and interconnections within the midbrain groups injected, four main populations of neurons were retrogradely labelled. Care was taken to exclude cell groups labelled due to spread of the injection to the oculomotor complex. 1) Cerebellar nuclei-all contralateral: dentate projects to RNp and ND; interpositus posterior to INC and sPf-Pf-FF; infracerebellar to INC_C. 2) Vestibular nuclei: superior nucleus bilaterally but stronger ipsilaterally to INC_R, sPf-Pf-FF and ND; medial and descending nuclei weakly with strongest contralaterally to INC and ND. 3) Reticular formation: rostral to the superior olive (SO), mainly ipsilaterally to INC-ND and laterally adjacent tegmentum; between rostral pole of IO and SO, bilaterally but slightly more contralaterally to same midbrain area; between cervical cord and IO, bilaterally to midbrain. 4) The motor cortex projection is ipsilateral to the entire cell group and arises principally from layer V cells in area 6, and the rostral portions of areas 4 and 3a. Autoradiographic studies using tritiated amino acids have confirmed many of these projections.

Three additional retrogradely labelled cell groups identified as projecting to the midbrain were: 1) ventral horn of the upper cervical cord, strongest ipsilaterally, principally from laminae 7 & 8, 2) ipsilateral nucleus tegmento-peribrachialis surrounding the brachium conjunctivum, 3) ipsilateral zona incerta and the adjacent caudal hypothalamus.

Thus, the meso-diencephalic cell group receives converging afferents from widespread sources and projects to a limited number of brain stem and spinal centres, the most notable being the inferior olive. (Supported by MRC Grant MA 7209)

26.17 CEREBELLAR RESPONSES TO ELECTRORECEPTOR INPUT IN CATFISH. Shang-Liang Tong* and Theodore H. Bullock. (SPON: K. S. Cole). Neurobiol. Unit, Scripps Instit. Oceanog. and Dept. Neurosci., U.C.S.D., La Jolla, CA 92093.

Unit spikes from large cells in the cerebellum of curarized catfish (Ictalurus nebulosus), prepared under MS 222 or ice water, were recorded, and histograms made by computer, during stimulation by weak electric fields in the bath. Either homogeneous fields - transverse or longitudinal - or small dipole fields were used. Receptive fields and best dipole orientation and direction were noted, as well as integrative properties.

Electrosensory units are found mainly in the lobus caudalis pars lateralis, i.e. the posterior lateral corner including <5% of the cerebellum, between 0.3 and 1.5 mm deep; a few are encountered in the corpus. With our criteria we can not tell the cell type, e.g. Purkinje vs basal cells. Background firing varies from 10-60 spikes/sec. down to one spike every few seconds; some units are relatively regular, some quite irregular. A few burst at the respiratory rhythm.

Responses to long (250 ms) pulses of current in the bath may be phasic, phasic-tonic or apparently tonic; pure tonic responses are rare. 90% of units increase discharge at ON of inward current (external anode; cathode in the stomach) and decrease at OFF; others behave in the opposite way. Minimum thresholds are <1 μv/cm. Electrosensitive units are generally unimodal, i.e. do not respond to mechanical, acoustic or photic stimuli. 68% of units respond best in the 90° between head positive and ipsilateral side positive.

Hand held bipolar stimulating electrodes 2 mm apart moved around the fish close to the skin defined receptive fields. At each locus that elicits a detectable change from background in a histogram of several stimulus pulses, there is a best orientation and direction ($\vec{E}$ vector). Units vary widely: receptive fields may be small or large, unilateral or bilateral, bilateral symmetrical or asymmetrical, with foci of inward or outward current or two foci widely separated. Units vary also in latency, intensity function and dynamics with stimulus repetition rate.

Aided by grants to T.H. Bullock from NIH and NSF and to S.-L. Tong from the People's Republic of China through the Shandong College of Oceanology, Qingdao.

26.18 EFFECTS OF CEREBELLAR LESIONS ON SENSORY-MOTOR COORDINATION IN THE ELECTRIC FISH EIGENMANNIA SPEC. K. Behrend Inst. f. Zoologie Abt. Biophysik Univ. Mainz 6500 Mainz W-Germany

Electric fish evaluate their environment by measuring local disturbances of their own electric field caused by objects of different conductivity than the surrounding water.The role the cerebellum plays in a mottor act aimed to gain more precise information about these objects(Heiligenberg,W.,J.Comp.Phys.103, 247-272,1975)has been investigated by lesioning the electrosensory part in the lobus caudalis of the cerebellum(Bastian,J.,90,1-24,1974).The animals swam in their tankwhere metal rods of 4 mm diameter could be placed at various places,eliciting a "probing behavior" consisting of backwards swimming towards the object and bending the body around the object.The animals movements were recorded on video tape and analyzed frame by frame giving a time resolution of the movemnt of 20 msec.After the lesion besides other rather small disturbances the most striking difference was a "yaw" during the probing behavior and also during forward swimming giving the animal an eel like appearance.Since normally the head is kept very stable with respect to the object the yaw introduces a different field composition than normally which must impairthe object evaluation and,infact,lesioned animals are more apt to avoid the metal rod than to investigate it by the probing behavior.This suggests a role of the cerebellum in improving sensory evaluation of the environment by giving motor commands which in this case counteract the yawing of the head thus compensating effects on the electric field caused by the fish's own movements.

26.19 EFFECTS OF CEREBELLAR STIMULATION UPON MIDBRAIN SENSORY RESPONSES IN A TELEOST FISH. Luis Crispino*. (SPON: Theodore H. Bullock) Neurobiology Unit, Scripps Instit. of Oceanog. and Dept. Neurosci. U.C.S.D., La Jolla, CA 92093.

The effects of cerebellar stimulation upon midbrain evoked potentials and/or unit responses of different sensory modalities have been analyzed in curarized catfish (Ictalurus nebulosus).

Sensory stimulation consisted of flashes for the visual system and clicks, electric pulses and table tapping for the acousticolateralis system, all of arbitrary stimulus parameters. Flash evoked potentials were recorded from the optic tectum. Acoustic evoked potentials and acoustic, electrical, and mechanically sensitive units were recorded from torus semicircularis. Slow evoked wave averages and poststimulus histograms were obtained from the recorded data by means of a special purpose computer.

A train of square pulses (0.1 ms., 100/sec., for 150 ms., found by trial to be approximately optimal) was delivered to the cerebellum before the sensory stimulus, at various intervals. Stimulation was applied to the areas of the cerebellum receptive to each modality, according to S.L. Tong and L. Lee (pers. comm.), and to other areas.

The effects of such stimulation are an enhancement of the visual evoked potential and a decrease of the acoustic, electric and mechanical responses. The effect for all modalities is maximum at the interval of 15-20 ms. between the end of the cerebellar stimulation and the sensory stimulus and declines slowly to 100-120 ms.

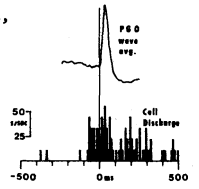
Attributing the action to peripheral inhibition can be rejected for the electric response since efferents are said to be absent (T.H. Bullock, Ann. Rev. Neurosci., in press). The response obtained by stimulation of the proximal stump of the posterior lateral line nerve (excluding receptor organs) was also affected by the cerebellar stimulation. Second order neurons recorded from the medulla following electrosensory activation did not show any change when the cerebellum was stimulated.

These results provide evidence of a central modulating action on the afferent responses seen in the midbrain, carried out by the cerebellum.

(Aided by grants to L. Crispino and to T.H. Bullock from NIH and NSF.)

27.1 PONTINE RETICULAR NEURONS AND PGO WAVE INITIATION: INTRACELLULAR RECORDINGS DURING NATURAL REM SLEEP. R. W. McCarley and K. Ito. Harvard Medical School, Boston, MA 02115.

PGO waves are characteristic EEG spikes occurring in Pons, Lateral Geniculate nucleus (LGN), and Occipital cortex during and just before REM sleep. Previous work in this laboratory has pointed to midbrain PGO burst neurons as responsible for the final step in brainstem to LGN transmission of PGO waves. We now report intracellular recordings (potassium citrate electrodes; stable DC potentials more negative than 45 mV) of pontine reticular formation (PRF) neurons whose inputs and discharge characteristics make them prime candidates for the initiators of PGO wave generation. Intracellular recordings during naturally occurring sleep-wake cycles in cats revealed long lead high coherence (LLHC) neurons whose depolarizing PSPs/discharges began about 100 msec prior to the onset of ipsilateral LGN PGO waves, as analyzed both by film and by computer generated cross-correlogram data. The figure shows the cross-correlogram for one LLHC neuron and the average of 10 PGO wave forms (discharge onset-to-PGO wave time is 80 msec; range was 70-110 msec). On the average a 12-fold increase in discharge rate occurred prior to PGO waves, with a high and reliable degree of coupling. A discharge acceleration occurred, on the average, before 86% of all isolated PGO waves. We have thus far recorded 5 neurons of this type. While histology is pending, our stereotaxic coordinates indicate recording sites in the dorsal portion of the rostral PRF about 1-1.4 mm lateral to midline, an area known to contain neurons involved in eye movement generation. All LLHC neurons received monosynaptic excitatory input from microstimulation sites in contralateral PRF and most from bulbar and mesencephalic reticular formations also. One neuron was antidromically activated from contralateral pons and one from midbrain. Carbachol stimulation at the recording site by passive diffusion (8μG/μL concentration in a pipette with a 15μM tip) markedly enhanced PGO wave activity while controls of diffusion with saline at the same site or more distant sites with carbachol did not produce such an enhancement. To our knowledge, this is the first report, either in extracellular or intracellular recordings, of a group of PRF neurons with the characteristics of such a long lead and such regularity of coupling of discharge to PGO waves. While more data are needed, the correlational data and the evidence from carbachol stimulation indicate these neurons are the best present candidates for cellular initiators of PGO waves.



27.2 CROSS-CORRELATION OF PGO BURST CELL FIRING WITH EYE MOVEMENT POTENTIALS OF THE ALERT CAT. J. P. Nelson, R. W. McCarley and J. A. Hobson. Laboratory of Neurophysiology, Department of Psychiatry, Harvard Medical School, Boston, MA 02115

PGO burst neurons are midbrain cells which fire in bursts of 4-6 spikes 12 msec before onset of ponto-geniculo-occipital (PGO) waves during REM sleep. We have shown that cells on one side of the midbrain fire when larger PGO waves occur in the lateral geniculate body(LGB) on the same side, but never fire when the larger wave occurs in the opposite LGB. They also fire preceding and during large rapid eye movements of REM sleep when they are to the ipsilateral side, but are almost always silent when the eye movements are to the opposite side. We have postulated that the burst cells act as output elements in the PGO generation system, and that during REM sleep they provide the forebrain visual system with information about the horizontal direction of upcoming REM's in the form of variation of PGO wave amplitude on either side of the brain.

We have thus far analyzed 11 of 44 burst cells in detail during wakefulness(W), using cross-correlograms(CC) of unit firing and averaged LGB and oculogram waveforms. During W these cells fire mostly single spikes with increased rates during periods of eye movement(EM) activity. Five of the 11 cells fired in relation to the LGB waves which often follow offset of saccades (eye movement potentials--EMP's). CC's of unit firing and EMP's showed that firing rates began to increase between 20 and 150 msec before onset of the EMP wave. The onsets were 20, 30, 30, 40, and 150 msec. The peak increase of firing rate over background levels ranged from 4 to 50 times, being for the individual cells 4, 6, 8, 10 and 50 times background.

Separate CC have been done on 3 cells for EMP's associated with rightward or leftward saccades. Two of these cells showed more increase of firing rate before EMP's associated with ipsilateral saccades than with contralateral saccades (7:4, 16:6 respectively), a finding of interest because it raises the possibility that waking EMP's may transmit information about EM direction.

The strong correlation shown here between firing of PGO burst cells and EMP's suggests that these cells likely play a role in generation of geniculate waves during wakefulness as well as during REM sleep. The cells on one side of the midbrain do not fire in unison in W as they do during REM sleep, but the more variable correlation in W is consistent with the greater variability of EMP's compared with PGO waves.

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27.3 COMPARISON OF THE PROJECTIONS FROM THE MESENCEPHALIC, ORAL PONTINE, CAUDAL PONTINE AND MEDULLARY RETICULAR FORMATION AND FROM THE LOCUS COERULEUS. T.Z. Yang and B.E. Jones (SPON: D.G. Lawrence). Lab. of Neuroanatomy, Montreal Neurological Inst., Dept. Neurology and Neurosurgery, McGill Univ., Montreal, Quebec, Canada H3A 2B4.

In order to assess quantitatively and qualitatively the efferent projections from the medial reticular formation and locus coeruleus in the rat, axonal transport of protein was studied by injection of ^{3}H-leucine (200μCi in 200μl, 40-60Ci/mM) into the following areas:mesencephalic, oral pontine, caudal pontine and medullary gigantocellular reticular formations and locus coeruleus. Two or seven days after injection, the rats were perfused and their brains processed either for autoradiography or liquid scintillation counting.

The mesencephalic reticular formation projected predominantly to the forebrain with a bilateral innervation to the following regions:basal forebrain, basal ganglia, entopeduncular nuc. subthalamic nuc., substantia nigra, zona incerta, and medial and intralaminar nuc. of the thalamus, the posterior nuc.,the pretectal area, the nuc. of the posterior commissure, and the interstitial nuc. of Cajal. In addition it sent bilateral widespread projections to the entire medial reticular formation of the brain stem and a small projection to the contralateral intermediate zone of the dorsal horn. The oral pontine reticular formation also sent significant though less dense projections to the same forebrain structures but more dense projections to the brain stem reticular formation. Caudally it projected the full length of the spinal cord, terminating predominantly in the ipsilateral intermediate zone of the ventral horn. The caudal pontine reticular formation sent a sparse ascending projection but provided a dense bilateral innervation to the brain stem reticular formation and vestibular nuc. It also provided the quantitatively most important innervation to the spinal cord, involving mainly laminae 7,8, and 9 of the ipsilateral ventral horn. The medullary gigantocellular reticular formation projected even more sparsely to the diencephalon but heavily innervated the brain stem reticular formation, the vestibular nuc., and the motor cranial nerve nuclei. Descending projections were bilateral onto the intermediate zone and lamina 9 of the ventral horns. In contrast to the reticular formation which thus provides a widespread but nonetheless differential innervation to the forebrain and spinal cord, the locus coeruleus innervates the entire central neuraxis including the cortex in a more diffuse and relatively homogeneous manner. (Supported by Canadian Medical Research Council Grant no. 6464).

27.4 STIMULATION OF MEDULLARY RETICULAR FORMATION DRIVES LUMBAR BACK MUCSLE EMG IN THE RAT. P.A. Femano, S. Schwartz-Giblin* and D.W. Pfaff. Rockefeller University, New York, NY 10021.

Previous studies have demonstrated that stimulation of neuronal activity within the pontomedullary reticular formation can exert both excitatory and inhibitory influences on motor mechanisms of the spinal cord via reticulospinal projections (see review by Peterson, B.W., Ann. Rev. Physiol., 41:127-140, 1979). For studying mechanisms of axial postural maintenance in the rat, we are investigating possible reticular influences on the lumbar axial musculature.

Adult female Sprague-Dawley rats were anesthetized with urethane and the axial muscles were exposed at vertebral levels L_2, L_4 and L_6. Recordings from motor units in either transversospinalis (TVS), medial longissimus (ML) or lateral longissimus (LL) muscle at each level were obtained by inserted pairs of twisted fine-wire tungsten electrodes fed directly into an LF356N FET as a first stage preamp. Bipolar stimulating electrodes consisted of a pair of twisted 75μ insulated tungsten wires. Electrodes were inserted stereotaxically into the medullary and pontine reticular formation ipsilateral and contralateral to the sites of EMG recording. Stimulation consisted of biphasic square wave pulses (leading phase cathodal) with a typical duration of 0.2 ms/phase. Frequency of stimulation varied from single pulses to trains of 1000 Hz, but typically trains of 200 Hz were used. Stimulus currents rarely exceeded 40μA. Conventional amplification, monitoring, stimulus, and recording circuits were used for on-line and post-experimental data analysis.

Stimulation of the medullary reticular formation ipsilateral to sites of motor unit recording elicited EMG driving at all vertebral levels investigated. Stimulation was especially effective in the region of nucleus reticularis gigantocellularis, Within this region effective currents were typically between 15μA and 25μA. Stimulation of the contralateral reticular formation at the same brainstem level could produce a strong inhibition of the EMG at currents comparable to those which elicited driving by ipsilateral stimulation. Repetitive stimulation of either side had a facilitating effect on the observed EMG responses. That is, a cumulative effect of repetitive trains of reticular stimulation occurred for both excitatory and inhibitory responses.

The results suggest that in the rat lumbar axial muscles receive a strong reticulospinal influence which could be important for the maintenance of posture and the initiation of certain behavioral responses.

27.5 PROJECTION FROM THE RETICULAR FORMATION OF THE MEDULLA TO THE FACIAL NUCLEUS. J. Courville, G. Goudreau*. Centre de recherche en sciences neurologiques, Département de physiologie, Université de Montréal.

Injections of small volumes (0.1 - 0.2 µl) of ^{3}H-L-leucine (conc. 150 - 200 µCi/µl) in the reticular formation of the medulla and the autoradiographic method were used in 22 cats to demonstrate the presence and distribution of the projections to the facial nucleus and other motor nuclei of the caudal brainstem. The reticular formation comprises a medial portion which corresponds to nucleus gigantocellularis in the rostral half of the medulla and to nucleus ventralis in the caudal half of this region (Brodal, '57). The lateral portion corresponds to nucleus parvicellularis. This part of the reticular formation projects to the facial nucleus. The projection is dense ipsilaterally and less dense contralaterally. Different positions of the injection centers within that area demonstrate distributions with different densities in various subgroups of the facial nucleus. It is not possible, however, to demonstrate a precise correspondence between reticular regions and the nuclear subdivisions. Nucleus gigantocellularis does not project to the facial nucleus while the medial portion of the caudal medulla sends a moderately dense projection distributed in all subdivisions of the nuclei. The ipsilateral side is slightly more labelled than the contralateral one. Horseradish peroxidase injections in the facial nucleus confirm the position of the cells of origin of the projections in the lateral and in the caudal and medial parts of the reticular formation. Pars caudalis of the spinal trigeminal nucleus projects to the lateral subgroup of the facial nucleus, ipsilaterally. The efferent fibers from pars caudalis travel within the spinal trigeminal tract while reticulo-facial fibers course within the lateral part of the reticular formation. Projections to the motor trigeminal, hypoglossal, retrofacial and ambiguus nuclei also originate from the lateral reticular regions.

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27.6 THE RETICULOSPINAL NUCLEI OF THE LIZARD AND RAT: A NISSL, GOLGI AND HRP COMPARISON. Donald B. Newman*, William L.R. Cruce and Rita Liu*. (SPON: R. Borke). Department of Anatomy, Uniformed Services University of the Health Sciences, Bethesda, MD, and Neurobiology Department, NEOUCOM, Rootstown, OH.

Present evidence (e.g., Cruce and Newman, '81) suggests that reptilian reticulospinal projections resemble those seen in mammals. Yet no one has compared reptilian reticular cell groups with those of mammals to ascertain whether these nuclei are similar in cytoarchitecture and neuronal morphology. Therefore, we have compared the organization of brainstem reticulospinal nuclei (RN) in Iquanid and Teid lizards with those of the rat using Nissl and Golgi techniques. In addition, we backfilled reticulospinal neurons with horseradish peroxidase (HRP) using large spinal injections and the tetramethyl benzidene (TMB) reaction. This enabled us both to precisely identify the location of reticulospinal cells and to compare their dendritic morphology in HRP and Golgi material. We found striking similarities. The rostral myelencephalic RN of the lizard can be subdivided into a dorsal portion (RID) vs. a ventral portion (RIV). RID contains large neurons with polygonal somata and dendrites which radiate in all directions. RIV contains neurons which possess fusiform somata and dendrites which course horizontally. Similarly, the rostral medullary RN of the rat can be subdivided into a dorsal portion (nucleus reticularis gigantocellularis) vs. a ventral portion (nucleus reticularis magnocellularis). Gigantocellularis, like RID in reptiles, contains large multipolar neurons whose dendrites ramify in all directions. Magnocellularis, like RIV in reptiles, contains neurons with fusiform somata and horizontally-coursing dendrites. The caudal metencephalic reticular field in reptiles (RM) resembles the nucleus reticularis pontis caudalis in the rat in that it also contains large multipolar neurons whose dendrites ramify in all directions. The dendritic arborizations of RM neurons, however, encompass a proportionately greater area of the brainstem than their counterparts in the rat. The rostral metencephalic RN of lizards (RS) resembles the nucleus reticularis pontis oralis of the rat in that it can be divided into a lateral area containing large cells (RSL) vs. a medial area containing mostly small cells (RSM). RSL cells of the lizard resemble the lateral nucleus reticularis pontis oralis cells of the rat in that the majority of their dendrites stream laterally as if to intersect the adjacent lateral lemniscus. Supported by USUHS Grant #919C07003 and NIH Grant #1R01.NS14346.

27.7 THE AFFERENT CONNECTIONS OF THE NUCLEUS VENTRALIS MEDIALIS THALAMI IN THE CAT,AS REVEALED BY THE HRP METHOD. J.Jiménez-Castellanos,Jr. and F.Reinoso-Suárez.Departamento de Morfologia.Universidad Autónoma de Madrid.Facultad de Medicina.Arzobispo Morcillo 2.Madrid 34.Spain.

The nucleus ventralis medialis thalami(VM)has been previously reported to provide an anatomical substrate for some nonespecific projection covering most of the cerebral neocortex.The aim of this repport is contribute to elucidate the origin of the afferent nervous impulses to the VM in the cat.Small amounts (25 nl)of a 16% solution of HRP(Sigma type VI)in distilled water were stereotaxically placed in the VM with a glass micropipette using an air-pulse delivery system.Forty eight hours postoperatively the cats were perfused and processed according to the method of Mesulam(1978)to reveal labeled neurons.The entire brain was surveyed for identifying HRP-labeled perikarya.At cortical levels labeled neurons were found in:gyrus proreus,sulcus praesylvius(medial and lateral banks and bottom),gyrus orbitalis gyrus frontalis,gyrus rectus,anterior limbic and cingular areae, gyrus suprasylvius(mainly in the anterior portion),sulcus ectosylvius anterior(both banks and bottom),gyrus sylvius anterior, sulcus rhinicus anterior(principally in the superior bank and bottom),sulcus cruciatus(mainly in the inferior bank and bottom) and sulcus rhinicus posterior.At subcortical levels the labeled neurons were situated principally in the claustrum,substantia innominata,nucleus entopeduncularis,amygdaloid complex,zona incerta,nucleus reticularis thalami,nucleus geniculatus lateralis (pars ventralis)and in dorsal,lateral and medial hypothalamic formations in the prosencephalon.The structures labeled in the brain stem were in all cases:substantia nigra,periaqueductal gray matter,mesencephalic and pontine reticular formations,deep layers of the superior colliculus,brachium conjunctivum area, locus coeruleus,nucleus reticularis gigantocellularis,nucleus spinalis nervi trigemini and deep cerebellar nuclei(mainly in the medial and lateral nuclei).These results show the forebrain as the main source of afferent connections to the nucleus ventralis medialis thalami.

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28.1 NEUROCHEMICALLY IDENTIFIED RETINAL NEURONS AS PROBES OF VERTEBRATE PHYLOGENY. R.E. Marc. Sensory Sciences Center, University of Texas Health Science Center, Houston, TX 77025.

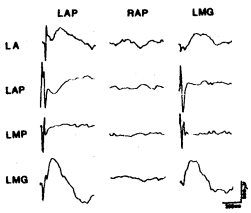
Two major uses of comparative neuromorphology are to investigate the validities of neural systems as mutual exemplars among vertebrates and to examine vertebrate evolutionary relationships. However, vertebrate phylogenies have rarely been evaluated at the level of neurochemically and morphologically identified neurons, though the primary unit of evolutionary change in the brain is indeed the neuron. Studying homologous neurons allows strong evaluations of phyletic models since neuron classes are generally robust characters not inclined to change dramatically except at major phyletic interfaces.

Using light and electron microscope autoradiography I have examined phylogenetic relations for identified GABA-ergic, dopaminergic and glycinergic retinal neurons in 36 species from 23 orders, and from all 7 classes. Each neurochemical class of cell shows either persistence or change that allows evaluation of events transpiring at different phyletic interfaces. GABA-ergic cone horizontal cells show continuity in all the modern "bony" groups(teleosts, holosteans, amphibians, reptiles, avians) except mammalians. This matches well with a model of mammalian evolution that posits a nocturnal or fossorial ancestor that lost or dramatically reduced cone vision. Since all GABA-ergic horizontal cells are cone-driven, their absence from mammalian eyes is consistent with the model. More evidence derives from the lack of homology between mammalian cones, which Walls (1943) considered to be secondarily derived, and all cones from other vertebrates. Dopaminergic neurons probe the osteichthyan/amphibian interface, where a major neural discrepancy exists: Anurans possess dopaminergic interplexiform cells as do modern teleosts, but urodeles possess, instead, a dopaminergic amacrine cell. The dopaminergic amacrine cell is the format found in urodeles, reptiles, avians, mammals and, significantly, the sarcopterygian fishes represented by the dipnoans, the lungfishes. The neurochemical and morphological organization of the retina of *Protopterus*, the African lungfish, is virtually identical to the retinas of urodele amphibians (*Necturus, Ambystoma, Amphiuma*), resurrecting the notion of Säve-Sodebergh (1934) that urodeles are the descendents of primitive dipnoans. That aside, my findings are in accord with the oft-suggested polyphyletic model of amphibian origins. Glycinergic amacrine cells appear to be archetypal in form and retinal distribution throughout the vertebrates, from Cyclostomata to Mammalia. They have apparently survived the events that drove the transformations of GABA-ergic horizontal cells and dopaminergic neurons.

28.2 FUNCTIONAL CONNECTIONS IN THE HUMAN LIMBIC SYSTEM. Michael Wang,* Charles L. Wilson, Thomas L. Babb, Eric Halgren and Paul H. Crandall. Brain Res. Inst., Reed Neurological Res. Cntr., and Dept. of Surg. (Neurol.), Sch. Med., UCLA, Los Angeles, CA 90024

In contrast to the animal literature, very little direct evidence exists on the functional pathways connecting structures within the human limbic system. The preliminary results reported here were obtained from 9 epileptic patients implanted bilaterally with chronic depth electrodes to localize temporal lobe seizure foci for surgical therapy. Sites included the anterior pes hippocampi (AP), middle pes hippocampi (MP), middle gyrus hippocampi (MG), posterior gyrus hippocampi (PG) and amygdala(A). These structures are homologous to ventral hippocampus, the temporal shoulder of dorsal hippocampus, entorhinal cortex, and basolateral amygdala, respectively. Bipolar recordings were from 40 uM platinum microwires which externded 5 mm beyond bipolar stimulating macroelectrodes stereotaxically placed under X-ray guidance within the same structure as the microwires. Single pulse, symmetrical, biphasic 100 usec square waves were delivered in turn, to macroelectrodes in each structure, and evoked field potentials were recorded from microwires in adjacent areas.

Results of tests in all electrode sites in 5 of the 9 patients indicate that the highest probability and lowest thresholds of response were from intrinsic macroelectrode stimulation within the same structure as recorded with local microwires. Next, in descending order of response probability came: ipsilateral response of amygdala to hippocampus, entorhinal cortex to hippocampus, hippocampus to entorhinal cortex and ipsilateral hippocampal sites to one another. In three cases hippocampal stimulation evoked responses in amygdala, and one amygdala site was responsive to entorhinal stimulation, but no entorhinal site responded to amygdala stimulation. Contralateral responses to stimulation of any limbic structure were totally absent. In the figure, single stimuli with a cathodal charge density of 7.0 uC/cm^2/ph were delivered to the sites across the top. The recording sites are labelled on the left, and responses are averaged from 4 stimuli. In response to LAP stimulation the shortest latencies are to intrinsic stimulation, then adjacent hippocampal (19 msec) and entorhinal cortex (25 msec) sites and longest, amygdala (37 msec). Contralateral response to RAP is absent.



Supported by NIH Grant NS 02808

28.3 ELECTRON MICROSCOPIC STUDIES OF THE PARABRACHIAL NUCLEI IN THE CAT USING HORSERADISH PEROXIDASE AND DEGENERATION METHODS: RECIPROCAL CONNECTIONS WITH THE AMYGDALA. Y. Takeuchi, J.H. McLean and D.A. Hopkins. Department of Anatomy, Dalhousie University, Halifax, Nova Scotia, B3H 4H7, Canada.

Recent anatomical studies have shown that the parabrachial nuclei (PBN) project to the amygdala which, in turn, projects back to the PBN. Initial studies have described the ultrastructure of four regions of the PBN (Takeuchi and Hopkins, Anat. Rec., 199: 252A, 1981) but there is no information on the synaptic relationships of these reciprocal projections. Therefore, in the present experiments, the ultrastructure of PBN projection neurons and amygdaloid afferents to the PBN were examined in the cat.

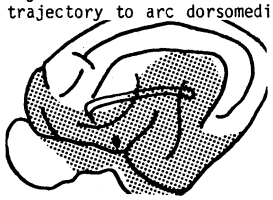
In order to identify the cells of origin of the PBN which project to the amygdala, horseradish peroxidase (HRP) was injected into the amygdala. After 2-3 days, cats were perfused with a 0.15M phosphate buffer containing 1.0% paraformaldehyde and 1.25% glutaraldehyde. Three-hundred-µm-thick sections of the PBN were first processed for HRP histochemistry using diaminobenzidine and then processed for electron microscopy. Retrogradely labelled neurons projecting to the amygdala were located mainly in lateral and ventral parts of the PBN. The labelled projection neurons were usually fusiform and ranged from 10-20 µm in diameter. They had a large nucleus which was often invaginated and had a moderately developed cytoplasm which contained many lysosomes.

In order to identify amygdaloid afferents, electrolytic lesions were made in the central nucleus of the amygdala. After 2 to 7 days, cats were perfused with the same fixative as in the HRP experiments. Degenerating axon terminals were found primarily in lateral and medial parts of the PBN and were characterized by swollen mitochondria and either round or pleomorphic vesicles in a dark matrix. Degenerating axosomatic terminals were occasionally found to contact soma of small-sized neurons particularly in the lateral part of the PBN. The majority of axodendritic terminals were 1-2 µm in diameter and were found mainly in lateral and medial parts of the PBN. The ultrastructural findings indicate that most small-sized neurons of the lateral and ventral PBN project to the amygdala. These neurons also appear to receive small numbers of axosomatic terminals from the amygdala but it remains to be determined to what degree they also receive axodendritic terminals. These reciprocal connections appear strongest in the lateral part of the PBN and provide further evidence for regional differences in the PBN.

Supported by the MRC of Canada and a Killam Postdoctoral Fellowship.

28.4 CORTICAL FIELD OF ORIGIN OF THE CAT'S ANTERIOR COMMISSURE. M. L. Jouandet. Institute of Anatomy, Univ. of Lausanne, 1011 Lausanne, Switzerland.

The extent of the neocortical field of origin of the anterior commissure (AC) of the cat, the cell types comprising this field, and the laminar and population distributions of these cells are not known. Following the complete transection, under deep Nembutal anesthesia, of the corpus callosum (CC) and hippocampal commissures (HC) of twelve cats, massive quantities of HRP (between 12 and 80 µL) were injected unilaterally in the right hemisphere in order to label completely the entire origins of the 3 branches of the AC in the left. Since the AC is the only interhemispheric telencephalic fiber system remaining following CC and HC commissurotomy, only the cells, fibers, and terminals of the AC should be labeled with HRP in the contralateral, uninjected telencephalon. Two additional cats, having their CC, HC, and AC aspirated, served as controls. All brains were cut coronally and processed by tetramethylbenzidine (TMB). HRP-labeled cells are found in the anterior olfactory nucleus, the prepiriform and piriform cortices, the nucleus of the lateral olfactory tract, the amygdala, and the olfactory tubercles. These findings agree with previous reports on other species. The neocortical field of origin of the AC is extensive. Deep layer V and VI polymorphic and pyramidal cells are apparent in high numbers in continuous laminae in the proreal, coronal, anterior ectosylvian, anterior sylvian, middle ectosylvian, posterior sylvian, posterior ectosylvian, and ventral portions of the posterolateral, gyri. More dorsal to a level horizontal to that at which the CC crossed the midline, the number of HRP-labeled cells drops off until only an occasional cell can be identified medially beyond the suprasylvian sulcus. The three major limbs, minor subsequent branches, and many individual axons of the AC system are labeled with HRP in the TMB-processed left hemispheres. Upon entering the hemisphere the posterior limb fibers sweep laterally to the ventral end of the external capsule and turn in tight bundles up into the external capsule. After leaving the dorsal end of the external capsule the axons then change trajectory to arc dorsomedially to recross the internal capsule at its dorsal end; arriving in the white matter just lateral to the dorsolateral tip of the lateral ventricle, the axons make sharp hairpin turns to then sweep directly lateral and fan out dorsally and ventrally into all those neocortical territories where HRP-labeled cells were found.



28.5 A MORPHOLOGICAL AND MORPHOMETRIC ANALYSIS OF THE SUBNUCLEAR STRUCTURE OF THE INTERPEDUNCULAR NUCLEUS IN THE RAT. G. S. Hamill* and N. J. Lenn. Dept. of Neurol., Univ. of Virginia, Charlottesville, VA 22908.

Nissl stained celloidin sections in three planes, 1μ plastic embedded sections, acetylcholinesterase (AChE) histochemistry, ^{3}H-leucine transport from injection sites in the habenular region and electron microscopy have been utilized to define the interpeduncular nucleus (IPN) and its major subdivisions in rat. Various parameters have been quantified using a Zeiss Videoplan image analysis system.

The cytoarchitectural boundaries of IPN are precisely congruent with the area labeled in the axoplasmic transport specimens. The volume of IPN so defined is 0.4 mm^3. Within IPN, there are 3 unpaired, median subnuclei: the rostral, central and caudal; and 3 pairs of bilateral subnuclei: the intermediolateral (IML), lateral and cap subnuclei. The myelin and AChE pattern, and crest synapse distribution suggest that IML consists of a rostral (IMLr) and a caudal (IMLc) division. The principal data are summarized in the Table.

Subnucl. Parameter	Rostral	Central	Caudal	IML (both)	Lateral (both)	Cap (both)
1. Volume(%IPN)	24	13	5	43	13	2
2. Cell diam.(μ)	8.13	11.53	--	12.3	13.3	12.8
3. Cells/area	18.5	8.33	--	12.8	4	--
4. Nissl stain	1+	3+	2+	2+	3+	4+
5. AChE stain	3+	2+	2+	1+	4+	2+
6. Density of label	3+	2+	--	2+	4+	--
7. Synapse type	S	--	--	Crest(IMLc)	--Dense-core	
8. Opiate recept.	3+	2+	--	0	1+	2+
9. Subst. P	--	--	--	Cell bodies	--	--
10. Afferents	Hab	Hab	DTN	Hab	Hab	Hab
11. Efferents	--	--	--	Hippocampus	--	--

2.p<.001 rostral vs all others; no other significant differences. 3. Denominator=7244μ^2. 4.-6. Rated 1+ to 4+. 7. Lenn, J.Comp. Neur.166,'76; Murray et al,J.Comp.Neur.187,'79. 8. Herkenham and Pert,Proc.Nat.Acad.Sci.77,'80. 9. Cuello et al, J.Comp.Neur.178,'78. 10. Hab=habenula; DTN=dorsal tegmental nucleus region, Herkenham & Nauta,J.Comp.Neur.187,'79;Contestabile & Flumerfelt,J.Comp. Neur.196,'81. 11. Baisden et al,Neurosci. Letters 13,'79.

The proposed scheme of subnuclei is based upon and supported by the above parameters. It is now clear that any detailed study of IPN will necessarily consider the subnuclear organization of this structure. Supported by NIH Grant # NS 16882.

28.6 DISTRIBUTION OF PRIMARY AFFERENTS TO THE OCTAVOLATERALIS AREA OF THE PIKE CICHLID. C.A. McCormick. Dept. of Anatomy, Georgetown University, Washington, D.C. 20007

The first order projections of the lateral line nerves were studied in the teleost fish Crenicichla lepidota using experimental degeneration methods. Either the posterior or the anterior lateral line nerve was transected at or proximal to its ganglion in a given specimen. Following survival times of 6-10 days at 28 C, brains were processed according to the Wittanen modification of the Fink-Heimer technique.

The posterior lateral line nerve enters the medulla as a single root just rostral to the entrance of the glossopharyngeal nerve and forms ascending and descending tracts positioned at the lateral edge of the medulla just ventral to the cerebellar crest. The single anterior lateral line nerve enters the medulla just caudal to the entrance of the sensory root of the facial nerve, and forms ascending and descending tracts which course ventral to those of the posterior lateral line nerve.

Some fibers in the ascending tracts of the posterior and anterior lateralis nerves terminate in the ipsilateral eminentia granularis of the cerebellum. The majority of primary lateralis fibers, however, terminate in two nuclei in the dorsal portion of the ipsilateral octavolateralis area of the medulla--nucleus medialis and nucleus caudalis. Nucleus medialis, which is the largest termination site of the lateralis nerves, is situated ventral to the cerebellar crest. Posterior lateral line nerve fibers terminate in the dorsal portion of this nucleus, while anterior lateral line nerve fibers terminate more ventrally. Some fibers in the descending posterior and anterior lateral line tracts terminate in the caudal-most portion of the dorsal octavolateralis area--nucleus caudalis.

In Crenicichla, the ventral portion of the octavolateralis area consists of five nuclei: the anterior octavus nucleus, nucleus magnocellularis, nucleus tangentialis, the descending octavus nucleus, and the posterior octavus nucleus. Preliminary studies indicate that all of these nuclei receive first order input from the eighth nerve. In addition to its primary eighth nerve input, nucleus magnocellularis also receives a light first order input from the posterior and anterior lateral line nerves, a connection similar to that seen in other fishes.

Supported by NINCDS 5 F32 NS06381-02 and NSF BNS77-26022.

28.7 PRIMARY PROJECTIONS OF THE LATERALIS NERVES IN THE SHOVELNOSE STURGEON, Scaphirhynchus platorynchus. J. G. New and R. G. Northcutt, Div. Biol. Sci's., Univ. Mich., Ann Arbor, MI 48109

Primary projections of the lateralis nerves were traced with HRP and Fink-Heimer methods. Adult sturgeon were anesthetized with MS222 and the anterior (ALLN) or posterior (PLLN) lateral line nerve exposed and unilaterally transected. A gelfoam pledget saturated with 40% HRP (Sigma VI) was inserted proximal to the transection in the HRP cases; the wound was packed with gelfoam and sealed with dental acrylic. After survival times of 6-16 days for the HRP cases and 14 days for the Fink-Heimer cases at 14°C, the animals were reanesthetized and perfused transcardially with phosphate buffer (ph 7.4) followed by 2% glutaraldehyde (HRP cases) or 10% formalin (Fink-Heimer cases). The brains were processed by a modification of the Mesulam HRP protocol using TMB as the substrate or by the Wiitanen modification of the Fink-Heimer method.

The ALLN of sturgeons possesses dorsal and ventral roots. Fibers of the dorsal root enter the medulla and form ascending and descending branches that terminate within the ipsilateral dorsal octavolateralis nucleus. Fibers of the ventral root of the ALLN, as well as fibers of the PLLN, enter the medulla ventral to the dorsal root of the ALLN where some of the fibers terminate among the dendrites of the magnocellular octaval nucleus. The bulk of the fibers form ascending and descending branches that terminate within the ipsilateral medial octavolateralis nucleus. A portion of the descending fibers continue more caudally to terminate in the ipsilateral caudal octavolateralis nucleus. Similarly, a portion of the ascending fibers continue more rostrally and terminate bilaterally in the cerebellar lateral lobes and granular layers of the corpus cerebellum. The dorsal root of the ALLN may possess additional projections, but experiments restricted to this root remain to be performed. The HRP cases also revealed retrogradely filled large neurons whose axons course peripherally in the lateral line nerves and are likely efferent to lateral line organs. These cells are situated in the medulla in the same rostrocaudal plane as the branchiomeric nuclei.

Thus there are marked similarities in the primary projections of the lateralis nerves in sturgeons and elasmobranchs, both of which possess ampullary electroreceptors; the direct cerebellar projections in sturgeons, however, appear to be unique.

This work was supported in part by NIH grants to RGN.

28.8 TECTAL AFFERENTS IN THE LONGNOSE GAR (HOLOSTEI). R. Glenn Northcutt. Division of Biological Sciences, University of Michigan, Ann Arbor, Michigan 48109.

Afferent pathways to the optic tectum of the longnose gar (Lepisosteus osseus) were determined by intratectal injections of HRP (Sigma VI). Volumes of 100-300nl of 20% HRP solution were delivered by micropipette pressure injections (3 cases), or the tectum was innoculated with HRP paste on the tip of a "00" insect pin (7 cases). Following survival times of 4-16 days at 27°C, the animals were reanesthetized and perfused transcardially with cold phosphate buffer (pH 7.4) followed by 2% glutaraldehyde in phosphate buffer. Brains were removed, embedded in 25% gelatin, and cut at 40μ on a sled microtome. Sections were reacted with o-dianisidine or tetramethyl benzidine following modified protocols of Coleman et al., 1976, or Mesulam, 1978.

Unilateral tectal injections resulted in retrogradely labeled cells in the following forebrain regions: ipsilaterally in the caudal portion of the rostral entopeduncular nucleus, anterior thalamic nucleus, ventrolateral and ventromedial thalamic nuclei, and periventricular pretectal nucleus; bilaterally in the rostral lateral zone of area dorsalis of the telencephalon, and in the central pretectal nucleus. At midbrain levels, retrogradely labeled cells were seen ipsilaterally in the torus longitudinalis, nucleus isthmi, and accessory optic nucleus; bilaterally in the torus semicircularis and a rostral tegmental nucleus. Pyramidal cells of the periventricular gray zone and fusiform cells of the central zone were also labeled in the contralateral optic tectum. Ascending tectal afferents from the brainstem arise bilaterally from the locus coeruleus, the superior, medial, and inferior reticular formations, and the nucleus of the descending trigeminal tract. Retrogradely filled cells were also observed in the inferior raphe nucleus, bilaterally in the eurydendroid cells of the cerebellum, and contralaterally in the medial octavolateralis nucleus and dorsal funicular nucleus and dorsal horn of the cervical spinal cord.

Analysis of these results and other connectional data for gars reveals that 1) There are two visual pathways to the telencephalon in these fishes, as in land vertebrates; 2) All pretectal nuclei except the superficial pretectal nucleus possess reciprocal connections with the optic tectum, thus suggesting that the superficial nucleus is not involved in tectal inhibition; 3) The gar tectum, like that of many other vertebrates, receives information from most sensory modalities that enter the brainstem.

(This work was supported by NIH Grant EY02485.)

28.9 ASCENDING AND DESCENDING SPINAL PATHWAYS IN THE PACIFIC HAGFISH. Mark C. Ronan* and R. Glenn Northcutt (SPON: M. S. Northcutt). Neurosciences Program and Division of Biological Sciences, University of Michigan, Ann Arbor, Michigan 48109.

Ascending spinal pathways and cephalic cell groups projecting to the anterior spinal cord in the Pacific hagfish, Eptatretus stouti, were examined with HRP histochemistry. Rostral cord lesions (5th-10th spinal segments), ranging in extent from hemisections to near transections, were made in seven adult hagfishes (29-41cm in length) and packed with gelfoam saturated with 40% HRP (Sigma VI). Animals survived 6-15 days in 14°C seawater. Transverse sections of the brain and rostral cord, 40μ thick, were processed according to a modification of the Mesulam, '78 TMB protocol. Staining of degenerating axons after rostral cord hemisections by the Wiitanen modification of the Fink-Heimer technique provided confirmation of ascending spinal pathways observed in HRP material.

The ascending pathways consist of a dorsal funiculus (DF) and a spinal lemniscus (SL). A large, well-defined DF ascends adjacent to the dorsal midline in the cord and then curves laterally in the diverging horns of the rostral medulla. The DF arches dorsally over the octavolateralis area and the trigeminal complex as it travels in the peripheral margin of the medulla before ending near its rostrolateral extent. The SL spans the ventral and lateral funiculi of the cord and ascends through the magnocellular divisions of the caudal and rostral medullar reticular formations. In the rostral medulla, fibers swing dorsomedially from the lateral SL and terminate in the ipsilateral caudal tectum.

Retrograde transport of HRP labels neurons in the nucleus of the MLF and mesencephalic reticular formations ipsilateral to the implant. In the medulla, HRP-filled cells of the rostral reticular formation included the entire ipsilateral and the anterior contralateral magnocellular divisions plus scattered cells of the ipsilateral parvocellular division. In the caudal reticular formation, magno- and parvocellular elements were labeled bilaterally with the ipsilateral side predominating in the latter division and the contralateral in the former, particularly in its posterior half. Large bipolar neurons in the ipsilateral octavolateralis area and numerous small cells in the perivagal nucleus were also labeled. Most filled cord cells were found in the intermediate contralateral spinal gray.

Pacific hagfish thus possess interstitiospinal and extensive reticulospinal pathways. A vestibulospinal system may be present. Ascending pathways include spinoreticular and spinotectal systems.

(Supported by NIH grant EY02485.)

28.10 CENTRAL PROJECTIONS OF THE OCTAVOLATERALIS NERVES IN GYMNOTIFORM FISH. Catherine Carr and Joanne Matsubara. Neurobiology Unit, A-002, S.I.O., UCSD, La Jolla, California,92093.

The afferent and efferent projections of the mechanoreceptive posterior and anterior lateral line nerves and the eighth nerve were investigated in two species of South American weakly electric fish (Gymnotiformes). The central cut ends of nerves were incubated in 30% horseradish peroxidase and reacted after a 3-10 day survival time using tetramethyl benzidine and Hanker Yates reagent as chromagens. Nomenclature was based on the work of McCormick (1981).

Mechanoreceptors of the posterior lateral line nerve (trunk) and the anterior branch of the anterior lateral line nerve (head) project topographically to the anterior lateral line lobe (ALLL), a probable field homologue of the medial octavolateralis nucleus, and to the caudal octavolateralis nucleus. Mechanoreceptive primary afferents also terminate on the large cells of the dorsal portion of the magnocellular octaval nucleus. Primary afferents send collaterals to the ementia granularis and the caudal lobe.

Eighth nerve fills produce terminal fields, from rostral to caudal, in the anterior octaval nucleus, the ventrolateral border of the mechanoreceptive ALLL, the magnocellular octaval nucleus, the tangential octaval nucleus, the descending octaval nucleus and the posterior octaval nucleus.The eighth nerve also projects to the ementia granularis of the cerebellum. Considerable overlap of eighth and mechanoreceptive lateral line afferents occurs at the ventrolateral border of the ALLL and adjacent dorsomedial border of the octaval column. There is no overlap between the ALLL and the tangential octaval nucleus.

Efferent cells of both the eighth and mechanoreceptive lateral line nerves are found bilaterally in a motor column which extends from caudal to the Mauthner cells to the level of the pacemaker nucleus. An additional small cluster of cells efferent to the lateralline are found closely apposed to the axons of the Mauthner cells at the level of eighth nerve entry. Only a small proportion of all the efferent cells are contralateral. Some project to both lateral line receptors and to the ear, as is supported by the observations that the same efferent cells appear to be backfilled by injections into either the lateral line nerves or the eighth nerve, and that an injection into the lateral line nerve produces a few well labelled fibers in the eighth nerve root.

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28.11 ELECTROSENSORY, AUDITORY AND LATERAL LINE NUCLEI IN THE MIDBRAIN OF A GYMNOTIFORM WEAKLY ELECTRIC FISH, STERNOPYGUS. J. Matsubara T.E.Finger, L. Maler, C. Carr. Scripps Inst. Ocean., UCSD, La Jolla, Cal. 92093; Dept of Anat, Univ Colo Med Ctr, Denver, Co. 80262; Dept of Anat, Univ Ottawa Fac Med, Ottawa, Canada, K1N9A9

The octavolateralis system of teleosts is composed of the lateral line, auditory and vestibular sensory systems. In the Gymnotiformes, weakly electric fish of South America, the lateral line system includes both mechanosensory and electrosensory modalities. The primary afferent fibers of the mechanoreceptive, electroreceptive and inner ear sense organs enter the brain via the anterior lateral line nerve (electroreceptive and mechanoreceptive fibers), the posterior lateral line nerve (mechanoreceptive fibers) and the 8th cranial nerve (vestibular and auditory fibers). The first-order termination sites of the octavolateralis system are segregated on the basis of modality. The electroreceptive input projects to the posterior lateral line lobe, mechanoreceptive input projects to the anterior lateral line lobe and the 8th nerve inputs project to a collection of 8th nerve nuclei (Maler et al 1974; Carr and Matsubara, in prep.).

In catfish, the second-order octavolateralis nucleus in the midbrain, the torus semicircularis, is also segregated on the basis of modality (Knudsen, 1977). However, in gymnotiforms the torus semicircularis is a complex laminated structure and the relationship between the electrosensory and other modalities has been unclear.

Horseradish peroxidase (HRP) was used as an anterograde and retrograde tracer to determine connections of the various octavolateralis nuclei in Sternopygus. Injections were made in the anterior lateral line lobe (ALLL), the octaval nuclei and various regions of the torus semicircularis (TS). These injections reveal two distinct regions of TS: a ventral nucleus which receives bilateral input from the mechanosensory lateral line and the octaval nuclei, and a laminated dorsal nucleus which receives bilateral input from the electrosensory system via the posterior lateral line lobe (Carr et al submitted). Furthermore, within the ventral nucleus there exists some segregation of mechanosensory lateral line and octaval inputs: efferents to the rostral ventral TS arise largely from the anterior octavus nucleus whereas efferents to the remainder of the ventral TS arise mainly from the crest cells of the ALLL. In addition to the projection to TS, the ALLL projects to the deep layers of the optic tectum and receives input from granule cells of the caudal cerebellum as well as from cells of the ventral region of nucleus praeementialis.

28.12 RETINAL PROJECTIONS IN THE GREEN SUNFISH: A CASE OF HOMOPLASY. Ann B. Butler and R. Glenn Northcutt. Dept. Anat., Georgetown Univ., Washington, D.C. 20007 and Div. Biol. Sci., Univ. of Michigan, Ann Arbor, Mi. 48109.

Eight adult green sunfish, Lepomis cyanellus, received unilateral intraocular injections of tritiated proline (spec. act. 139 Ci/mmol, conc. 50 μCi/μl) under MS222 anesthesia. After 1,3, 6,12 and 35 days, animals were reanesthetized and perfused. 15μ sections were coated with Kodak NTB3 and exposed 45 days at 7°C.

After complete decussation of fibers in the optic chiasm, two distinct fascicles enter the contralateral suprachiasmatic nucleus after arising from the medial edge of the marginal optic tract (MaOT). At more caudal levels, the axial optic tract divides into 3 main components: a medial fascicle that innervates the contralateral preoptic area (PO) and then redecussates to innervate the ipsilateral PO, a ventral fascicle that bilaterally terminates in the anterior tuberal region of the hypothalamus, and a lateral component that rejoins the MaOT at the level of the pars magnocellularis of the superficial pretectal nucleus (SPt). As the MaOT courses caudally it divides into dorsal (DOT), ventral (VOT) and medial (MOT) optic tracts. The MOT courses toward the periventricular cell groups of the diencephalon where rostrally it terminates in the anterior (A), ventrolateral (VL) and ventromedial (VM) thalamic nuclei and in a dorsal portion of the preoptic area. More caudally, the MOT terminates in the periventricular pretectal nucleus (PPt) and posterior tuberculum and contributes fibers to the deep white zone of the optic tectum (TeO) and posterior commissure (PC). As the MaOT is traced caudally it first gives off terminals to the pars parvocellularis of the SPt and then divides into DOT and VOT with most fibers coursing around or through the pars magnocellularis of the SPt. The DOT and VOT terminate in nucleus corticalis and the central pretectal nucleus and enter TeO to distribute fibers to the deeper portions of the marginal layer, the superficial white and grey zone, and the deep white zone. At more caudal levels, VOT contributes fibers to a basal optic nucleus, an accessory optic nucleus, VL, VM, the periventricular pretectal nucleus (PPt) and to the PC. Fibers of the MOT and VOT cross in the PC to innervate the ipsilateral deep white zone of TeO, PPt, VL, VM and A. Thus, the sunfish possesses bilateral primary retinofugal projections to the diencephalon and tectum; however, the ipsilateral projections decussate in the posterior and preoptic commissures rather than in the optic chiasm, suggesting that these ipsilateral pathways are homoplastic to the ipsilateral retinofugal pathways of other vertebrates.

Supported by NSF grant BNS 77-26022 to ABB and NIH grant EY02485 to RGN.

28.13 LOCALIZATION OF ENKEPHALIN-LIKE IMMUNOREACTIVITY IN THE BRAIN OF THE LAMPREY, M.R. Gold and T.E. Finger, Depts. of Physiol. and Anat., Univ. Colo. Med. Sch., Denver CO 80262

Enkephalin-like immunoreactivity (ELI) has been reported in the nervous system of a wide variety of vertebrates. In the few amniote species examined, there appears to be marked similarities in the distribution of immunoreactivity. In order to assess the consistency of these patterns throughout the vertebrate phylogeny, we examined the distribution of ELI in a species of lamprey. The brain and pituitary of adult brook lampreys, Lampetra lamottei, were removed from the braincase and fixed by immersion in periodate-lysine-paraformaldehyde solution for 2-6 hours. Following wash in phosphate buffer, the tissue was sectioned in the transverse plane and reacted with unlabeled L-enkephalin antiserum (supplied by R. Miller). Both fluorescence and PAP techniques were used. All immunoreactivity was abolished by preabsorbing the antiserum with L-enkephalin (10 μM).

As in other vertebrates, immunoreactive somata and fibers occur in discrete areas throughout the length of the neuraxis. In the brainstem, a substantial plexus of ELI fibers courses through the ventralateral portion of the medullary neuropil. Immunoreactive fibers in this plexus turn dorsolaterally to end in the vicinity of the dendrites of the vagal and facial, but not trigeminal, motor nuclei. A second immunoreactive fiber bundle is located just ventral to the MLF in the rostral rhombencephalon. In addition, ELI fibers and cell bodies are located in the vicinity of n. solitarius and comm. nuc. of Cajal. Scattered ELI fibers enter the cerebellum. Throughout the metencephalon and mesencephalon ELI fibers run adjacent to the ependyma. Both the optic tectum and torus semicircularis show a laminar pattern of ELI fibers. None of the large identifiable reticulospinal neurons exhibit ELI.

In the prosencephalon, a dense plexus of ELI fibers extends through out the ventromedial hypothalamus and preoptic area. ELI somata occur throughout the hypothalamus. Both the median eminence and intermediate zone of the pituitary exhibit heavy ELI. Comparatively less ELI is present in the dorsal thalamus although substantial numbers of ELI fibers occur in portions of the habenular complex. In the telencephalon, ELI fibers are present mostly in ventromedial portion but certain pallial fields also receive a significant ELI input.

Comparison of these findings with previous reports indicates substantial similarities in the distribution of ELI among widely divergent vertebrate classes. This suggests marked conservation of enkephalinergic systems throughout the vertebrate phylogeny. Accordingly, peptide localization may prove to be a powerful means to indicate homologous systems.

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28.14 CONNECTIONS OF CEREBRAL CORTEX IN THE TURTLE (PSEUDEMYS SCRIPTA ELEGANS). P. Desan*. (SPON: R. Baughman). Dept. of Neurobiology, Harvard Medical School, Boston, MA 02115.

The afferent and internal connections of the turtle cerebral cortex were delineated by anterograde and retrograde transport methods. This cerebral cortex is divided into four rostrocaudal strips, termed lateral, dorsal, dorsomedial and medial cortex. Injections of tritiated proline in the olfactory bulb label a dense plexus of afferent terminals in the superficial 100μ of the entire lateral cortex on both sides of the brain. Large injections of tritiated proline in the thalamus label a similar superficial lamina in dorsal cortex. After intraocular injections of tritiated proline, such labeling is restricted to a lateral, histologically distinct subdivision of dorsal cortex, presumably reflecting the transneuronal transport of the amino acid through a visual relay nucleus in the thalamus.

Injections of 15% HRP/2% lysolecithin or 2% wheat germ agglutinin-HRP conjugate (Sigma L-2384) were made across the rostrocaudal extent of dorsal cortex. These injections retrogradely label cell groups of the lateral thalamus adjacent to the optic tract: the nucleus ovalis and associated nucleus dorsolateralis anterior, the rostral and caudal parts of nucleus geniculatus lateralis. Rostral injections label caudal portions of this complex; caudal injections label rostral portions, in both ipsilateral and contralateral thalamus. Furthermore, injections in dorsal or lateral cortex label cells in medial cortex; injections extending into dorsomedial cortex label cells in medial and dorsomedial cortex on both sides of the brain.

Both types of HRP injections yielded vigorous anterograde transport. Olfactory cortex projects densely to a narrow superficial lamina of medial cortex, dorsal cortex projects to a deeper lamina of medial cortex, and injections extending into dorsomedial cortex additionally label the remaining deepest lamina of medial cortex (a similar pattern has been described in snake cortex- Ulinski, P.S., J.Morph., 150: 463-483, 1976).

These experiments suggest that visual and non-visual nuclei of the thalamus and the olfactory bulb project to different areas of the cortex, and these areas, with dorsomedial cortex, project convergently to medial cortex and receive reciprocal projections from it.

28.15 ANATOMY OF THE TRIGEMINAL MOTOR NUCLEUS OF THE TEGU LIZARD, G.A. Iwamoto and G.S. Throckmorton*, Dept. of Cell Biology, The Univ. Texas Hlth. Sci. Ctr., Dallas, TX 75235

Previous studies (Throckmorton, Archs Oral Biol 25:225-233 1980 and Bramble, Amer Zool 20:931 1980) have shown that jaw and tongue movements during the chewing cycle in reptiles are remarkably similar to those of mammalian species. Reports on several mammalian species (See Matsuda et al Neurosci Lett 8:1-4 1978) have shown a characteristic topographical organization within the trigeminal motor nucleus. Our investigation was undertaken to determine if a similar nuclear organization was also present in the lizard. Adult tegu lizards were anesthetized with ketamine hydrochloride (80 mg/kg) and sodium pentobarbital (15 mg/kg) and the muscles of the trigeminal nerve exposed. Injections of a 10% solution of horseradish peroxidase (Sigma Type VI) were administered in 1 μl aliquots (5-15 μl total per individual muscle) to all trigeminally innervated muscles on one side followed by injection of a selected muscle on the contralateral side. Selected muscles included: Superficial Portion of the External Adductor, Pterygoideus, Middle Portion of the External Adductor and Pseudotemporalis. The animals were re-anesthetized after a survival period of 48-72 hrs and perfused with 0.4 paraformaldehyde 2.5% glutaraldehyde following a saline wash. The brainstems were removed and following a 12 hr post-fixation period, placed in 30% sucrose-phosphate buffer for 48-96 hrs at 4°C. The brainstems were cut in 50 or 100 μm transverse sections, reacted with tetramethyl benzidine, counterstained with Neutral Red and microscopically examined. Labeled cells ranging from 15-50 μm were noted as to size and location. In agreement with Ebbesson (Brain Res 5:178-206 1967) it was found that the trigeminal motor nucleus may be grouped at least into major dorsal and ventral divisions. The muscles were represented as follows: the Superior Portion of the External Adductor occupied much of the ventral division with Pterygoideus occupying a smaller area on the ventral aspect of this division. Remaining portions of the External Adductor occupied the dorsomedial aspects of the nucleus comprising some of the dorsal division. Finally, Pseudotemporalis is represented in the most dorsomedial portion of the dorsal division. Comparisons with results from mammalian species suggest similarities in distribution of motor neurons between lizards and mammals for the following muscles: Superficial External Adductor-Masseter, Pterygoideus-Medial and Lateral Pterygoid muscles, Middle External Adductor and Pseudotemporalis-Temporalis. The data suggests that the anatomical organization of the trigeminal nucleus of the lizard is similar to that of mammalian species and may indicate that the neural apparatus controlling mastication had an early phylogenetic origin. This study was supported by NSF Grant No. DEB78-05330.

28.16 THALAMOCORTICAL ORGANIZATION IN TWO LIZARDS. Laura L. Bruce and Ann B. Butler. Dept. Anatomy, Georgetown Univ., Washington, DC 20007.

Thalamic projections to three divisions of telencephalic cortex were studied with retrogradely transported wheat germ agglutinin - horseradish peroxidase complex (Sigma WGA-HRP Type VI). In two lizards, Iguana iguana and Gekko gecko, crystals were deposited into the medial cortex, the dorsal cortex, and into a rostral area which included the lateral cortex and the pallial thickening. After six day survival periods, the animals were perfused and the tissue was processed according to the deOlmos and Heimer protocol (Neurosci. Lett., 1977). The characteristics of the retrogradely labeled cells were identical in both species.

Depositions of HRP which were confined to the medial cortex resulted in retrogradely labeled cells limited to the large-celled portion of the nucleus dorsolateralis anterior (DLA) of the thalamus. Labeled cells were present bilaterally in equal numbers. After depositions of HRP confined to the dorsal cortex, retrogradely labeled cells were observed only in the small-celled portion of the DLA. Slightly more labeled cells were present ipsilaterally than contralaterally. When depositions of HRP were confined to the lateral cortex and the pallial thickening, a small number of heavily labeled cells were present in the large-celled DLA, principally the ipsilateral side. The small-celled DLA contained lightly labeled cells bilaterally, possibly due to incorporation of HRP by broken fibers of passage. A small number of lightly labeled cells were present ipsilaterally in the dorsomedial nucleus and the perirotundal cell group. Labeled cells were present throughout the ipsilateral nucleus intercalatus. This nucleus was also labeled after HRP injections restricted to the pallial thickening and the dorsal ventricular ridge. Nucleus intercalatus receives a retinal projection from the contralateral eye in both iguana and gecko (Butler and Northcutt, Brain Res., 1978). Thus the pallial thickening is the recipient of a retino-thalamo-cortical pathway.

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29.1 ONTOGENETIC DIFFERENCES IN THE RESPONSE OF THE CHOROID PLEXUS pH TO ACETAZOLAMIDE. Conrad E. Johanson and Zahra Parandoosh*. Dept. Pharmacology, Univ. of Utah, Salt Lake City, UT 84132

The carbonic anhydrase (CA) in choroid plexus (CP) tissue may participate in the regulation of pH of the CP-CSF system. A previous study of adult rats (Johanson, The Pharmacologist 21, p. 242) demonstrated that the CA inhibitor, acetazolamide, 20 mg/kg I.P. for 1 hr, markedly elevated the pH of the lateral ventricle choroid plexus (LVCP), from 7.0 to 7.5. We have expanded the investigation to include immature rats, and fourth ventricle choroid plexus (4VCP) as well as LVCP.

Immature Sprague-Dawley rats, 1 and 3 wk, were etherized and bilaterally nephrectomized and injected I.P. with DMO (dimethyloxazoladinedione), as tracer ^{14}C (20 $\mu C/100$ g) and as carrier (15 mg/kg). After 1 hr, samples were taken from arterial blood (AB), cisternal cerebrospinal fluid (CSF), and choroid plexuses. The pH of AB was analyzed on an IL 213, and the pHs of CSF, LVCP and 4VCP were calculated from the steady-state distribution of ^{14}C-DMO. Means (± SEM) for n = 6 are given below for control (C) and treatment (T), 20 mg/kg acetazolamide.

	pCO_2	pH values:			
	AB	AB	CSF	LVCP	4VCP
1 wk (C)	29(1)	7.43 (.03)	7.41 (.03)	7.05 (.07)	7.21 (.04)
1 wk (T)	47(5)*	7.18 (.03)*	7.24 (.06)*	7.03 (.05)	7.24 (.08)
3 wk (C)	22(1)	7.52 (.02)	7.44 (.02)	7.11 (.04)	7.25 (.04)
3 wk (T)	38(1)*	7.30 (.02)*	7.45 (.04)	7.30 (.04)*	7.33 (.02)*

Each asterisk denotes $P < 0.05$ by t test.

For the first time, control pH values are presented for the immature CP. The 1-wk rat LVCP, across which there is negligible turnover of CSF, has a control pH (7.05) not significantly different from that previously determined in adults. Moreover, choroid cell pH in 4VCP is consistently 0.15 pH unit greater than in LVCP. In 1-wk rats, acetazolamide does not alter cell pH. However, in 3-wk animals the CA inhibitor significantly elevated cell pH in both LVCP and 4VCP. The magnitude of the acetazolamide-induced change in cell pH in 3-wk rats (0.1 to 0.2 pH unit) was less than the corresponding induced change noted previously in adult rats (i.e., 0.5 pH). The increase in CP pH occurred in spite of a marked elevation in arterial pCO_2, and it is likely due to a build-up in the concentration of OH^- and HCO_3^- subsequent to acetazolamide effects on choroid cell function. Acetazolamide treatment did not alter CSF pH in the 3-wk rats; however, CSF pH was not effectively buffered in the 1-wk animals. This work was supported by USPHS Grant 1 R01 13988, NINCDS and by a RCDA to C.E.J.

29.2 SALICYLATE EFFECTS ON ORGANIC ACID HERBICIDE TRANSPORT BY CHOROID PLEXUS. C. S. Kim,* L. A. O'Tuama* and C. R. Roe* (Spon. G. D. Frye) Univ. North Carolina Sch. of Med., Chapel Hill, NC 27514 and Duke Univ., Durham, NC 27706.

We have shown that the uptake of 2,4-dichlorophenoxyacetic acid (2,4-D) by adult rabbit choroid plexus (CP) is saturable, energy and temperature dependent (Kim et al., Soc. Neurosci. Abs. 6:291, 1980) and have also described differential effects of salicylate and acetaminophen on the uptake system. The present study was undertaken to investigate whether these drugs also have differential effects on neonatal rabbit CP and to study interaction of salicylate metabolites with the carrier. Lateral and fourth ventricular CP (LVCP, FVCP) of 3 day and 30 day old rabbits were incubated for 10 min in control and experimental media containing ^{14}C-2,4-D 0.02 μCi/ml (sp. act. 28 mCi/mmol). The uptake of ^{14}C-2,4-D at 10 min was reduced by 52% and 66% respectively in FVCP and LVCP of neonatal rabbits incubated with 10^{-3} M sodium salicylate (SS) ($p < 0.01$). However, treatment with acetaminophen at the same concentration did not alter ^{14}C-2,4-D uptake by either LVCP or FVCP of neonatal rabbits. The major metabolite of salicylate, salicyluric acid (SU) has stronger inhibitory effects than its precursor salicylate. ^{14}C-2,4-D accumulation was reduced 66% and 84%, respectively, in adult FVCP and 53% and 83%, respectively, in LVCP incubated with SU (10^{-4} M, 10^{-3} M) ($p < 0.01$, $p < 0.001$). Gentisic acid was the weakest inhibitor of uptake. Recent studies suggest that high pesticide exposure and salicylate consumption contribute to the pathogenesis of Reye's syndrome (RS) (Starko et al., Peds. 66:859, 1980). This finding strengthens the view that embarrassment of cellular energy-linked functions is a critical event in the development of RS. This study suggests that salicylate intoxication to CNS function via impairment of the organic acid transport system in CP can provide a direct mechanism for the development of encephalopathy in RS. Supported by USPHS Grant HD-03110.

29.3 INSULIN EFFECTS ON SODIUM TRANSPORT BY CHORIDE PLEXUS. Yoshitaka Saito* Helen Wong and Ernest M. Wright. Dept. of Physiol., UCLA School of Medicine, Los Angeles, Ca. 90024.

CSF secretion by the choroid plexus is driven by Na/K pumps in the brush border membrane. Since little is known about the control of CSF secretion we have studied the effects of insulin on the Na pump in the frog choroid plexus.

Tissues were loaded with ^{22}Na in K-free, cold buffer solution. The efflux of ^{22}Na from the plexus was monitored at room temperature and the rate constant (k) was estimated by the method of Keynes (J. Physiol. 178:305, 1965). The pump component was obtained from the change in rate constant induced by the addition of 2 mM KCl to the Ringer solution (Δk). Ouabain (1 mM) completely blocked this K-induced Na efflux. Na pump rate ($\Delta k \times (Na_i)$) was a sigmoidal, saturable function of intracellular Na (Na_i) and extracellular K (K_o) concentration. The data suggested that the pump has two sites for K_o and three sites for Na_i. The K_m's and V_{max}'s were 1.1 mM and 7 mM and 8 μmoles/cm^2·h and 13 μmoles/cm^2·h for K and Na transport, respectively.

Addition of insulin (10^{-7}-10^{-5}M) to the washout medium significantly stimulated sodium pumping by 24-140%. This hormonal effect on the Na/K pump was also monitored by transepithelial Na flux measurement. We observed that insulin (10^{-6}M) increased net sodium secretion. Insulin (1×10^{-7}M) increased the guanylate cyclase activity of the epithelium by 109%, while there was no substantial change in adenylate cyclase activity. Furthermore, we found that addition of dibutyryl cyclic-GMP (1~3 mM) increased the Na pump rate by 42~219% while the effect of dibutyryl cyclic-AMP was marginal.

These results suggest that Na secretion by the choroid plexus is modulated by insulin via effects on Na/K pump, and the stimulatory effect of insulin may be mediated by increases in intracellular cyclic-GMP levels.
Supported by a grant from the USPHS (NS09666).

29.4 STUDIES OF THE BLOOD RETINAL BARRIER IN RATS WITH INHERITED RETINAL DYSTROPHY. Ruth B. Caldwell, Lou G. Boykins* and Barbara J. McLaughlin. Department of Anatomy. University of Tennessee Center for the Health Sciences, Memphis, TN 38163

Retinal degeneration in the Royal College of Surgeons rat occurs between 12 and 70 postnatal days with a gradual decline in phagocytic activity by the defective pigment epithelium (PE) as the photoreceptors degenerate. Another change in the dystrophic retina which can be attributed to a defect in the PE cell layer is a breakdown in the blood-retinal barrier. Between 72 and 116 days, the PE cell junctions, which form part of this barrier, become permeable to extracellular tracer (Bok, 1979) and corresponding intramembrane changes in cell junction structure are observed (Caldwell et al., 1981). In this study we have used tracer and freeze-fracture techniques to study the progression of these changes during retinal degeneration in dystrophic and normal rats from 3 wk to 1 1/2 years old. Eyecups were incubated with horseradish peroxidase (HRP) (Sigma, Type VI, 20%) for 30 min-1 hr and processed for EM. The other eyes were prepared for freeze-fracture. As early as 6 wks, PE cell junctions become permeable to HRP. By 72 days there are striking changes in PE cell junctions. Numerous annular gap junctions which are thought to be a means of disposing of cell junctional membrane (Albertini et al., 1975), are seen in the PE cytoplasm. In addition, some PE cells appear to have detached completely from each other while others remain joined but their junctions are displaced from their normal position. In these abnormal areas, HRP bypasses the cell junctions and is present on the baso-lateral membrane surface of some PE cells. Freeze-fractured tight junctions between normal PE cells are found to consist of 8 to 12 anastomosing ridges on the cytoplasmic membrane leaflet (P-face) and 8 to 12 complementary grooves on the external membrane leaflet (E-face). Gap junctional aggregates of hexagonally packed P-face particles and complementary E-face pits are enclosed within the tight junctional strands. In dystrophic rats younger than 42 days old, all PE junctional areas are indistinguishable from those of normal retinas. After 113 days dramatic alterations are observed: the tight junctional strands appear unraveled and to be broken up into short segments; the number of strands and the number of gap junctions are often reduced; and gap junctions are no longer encircled by the tight junctional strands and exist as isolated particle aggregates. In summary, our results show that permeability changes in the blood retinal barrier between PE cells occur as early as 42 days. These changes in PE cell junction permeability are found before any changes in junctional intramembrane structure are discernable by freeze fracture. Supported by USPHS EY 02853.

29.5 ASSESSMENT OF NEURAL TRANSPORT OF LOW DOSE INORGANIC LEAD. L. A. O'Tuama*, C. S. Kim* and R. N. Johnson* (Spon. P. Morell). Univ. N. Carolina Sch. of Med., Chapel Hill, NC 27514.

Concerns about possible effects of "low level" exposure to toxic metals, e.g., lead (Pb) (McCabe, Env. Health Perspect., 29: 29, 1979) indicate a need for detailed information on the neural distribution of such compounds in tracer amounts. These studies are complicated by the low entry rate of toxic metals into brain (O'Tuama et al., Toxicol. Appl. Pharmacol., 36:1, 1976). Arterial integral analysis (Blasberg, Radioscience 14:335, 1979) allows highly sensitive measurement of cerebrovascular permeation of tracers whose flux from plasma to brain corresponds to model assumptions. These assumptions include first-order transfer from plasma to tissue, initial permeation into a tissue space permitting free diffusion and final location in a tissue compartment whence no back-flux occurs. We have tested this model in the study of distribution of tracer amounts of inorganic Pb in peripheral and neural tissues. Young adult rabbits received radiolabeled $Pb(NO_3)_2$ [^{210}Pb] by iv bolus (1.2 μCi/kg; S.A. ≥ 1 mCi/mg). Serial arterial plasma ^{210}Pb activity was sampled from an arteriovenous anastomosis and compared with the activity in representative peripheral and neural tissues. The plot of the ^{210}Pb plasma concn. x time integral vs. tissue levels was linear for the peripheral organs, supporting model assumptions. Unidirectional rate constants for plasma-to-tissue transfer of metal ranged from 1.2 x 10^{-3} ml/g.min. (liver) to 2.5 x 10^{-3} mg/g.min. (bone). In contrast, for neural tissues, the integral vs. tissue plot was non-linear. The results show that plasma-to-neural transfer of tracer dose Pb involves mechanisms that are qualitatively different from those subserving uptake by peripheral tissues and appear substantially more complex than can be explained by a two compartment model. Preliminary analysis suggests that in brain there is a rapid initial uptake of metal followed by redistribution to peripheral organs. This pattern appears similar in several neural areas (cerebellum, caudate nucleus, occipital cortex, etc.) and may involve an anatomical structure common to all regions, e.g., the capillary endothelium tight junction. Supported by USPHS Grant ES-01141.

29.6 THE EFFECT OF LEAD ON CSF TRANSPORT IN THE ISOLATED SUPERIOR SAGITTAL SINUS OF THE DOG. J. D. Charlton*, S. L. Cookson*, N. E. Pederson*, R. N. Johnson and J. D. Mann. Dept. of Neurology, Univ. North Carolina, Chapel Hill, N.C. 27514.

We have previously shown that exposure to lead early in development results in impaired absorption of cerebrospinal fluid (CSF) in vivo. In the present study, we have assessed the effects of lead on CSF transport across the arachnoid villi in the isolated superior sagittal sinus of the adult dog.

Twenty adult mongrel dogs of either sex weighing 12.5 ± 2.8 kg were anesthetized with sodium pentobarbital. The superior sagittal sinus and a portion of the surrounding dura were removed from each animal and placed in cold, buffered artificial CSF. The superior wall of the sinus was opened longitudinally over a length of 2-3 cm and residual blood was removed. The sinus was mounted in a lucite chamber and perfused with oxygenated, buffered artificial CSF (10 mM glucose, 308 mOsm). The sinus was positioned to divide the chamber into two separate compartments (blood and CSF), with the sinus membrane providing the sole barrier between them. Pressure across the sinus was regulated by adjusting the relative heights of reservoirs of fluid circulating through either side of the chamber. A drop counter technique was used to measure the volume of CSF transported per unit time between compartments. Bulk flow was studied in response to changes in temperature, CSF composition, and differential pressure across the membrane. Movement of fluid into the CSF compartment from the blood side was negligible under all conditions studied.

Bulk transport of CSF to the blood side of the membrane was found to be both temperature and pressure dependent. Addition of uM quantities of KCN or ouabain significantly decreased transport. The addition of lead acetate (10-50 uM final concentration) resulted in a mean decrease of 37% (± 18% S.D.) in bulk CSF transport to the blood compartment as compared to baseline and Na acetate control conditions ($p < .001$). The effect was partially reversible when lead-free CSF was returned to the chamber. Nevertheless, lead clearly has an adverse effect on the bulk fluid transport capacity of tissues mediating absorption of CSF into the venous circulation. This study suggests that low level lead exposure results in a loss in intracranial pressure buffering capacity, thereby enhancing the risk for the development of intracranial hypertension and brain edema.

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29.7 CHARACTERISTICS OF ZINC METABOLISM IN RAT BRAIN. E. J. Kasarskis* (SPON: A. C. Lastimosa). Dept. of Neurology, VA Hospital and Univ. Kentucky Med. Ctr., Lexington, KY 40536.

Zinc is the second most abundant trace metal cation in brain. However, the characteristics of zinc metabolism in the CNS have not been fully investigated to date. Zinc uptake and turnover in 7 brain regions, choroid plexus, and CSF have been determined and compared to plasma and non-neural tissues.

Groups of adult male Sprague-Dawley rats were maintained on zinc-adequate diets and received a single intraperitoneal injection of $^{65}ZnCl_2$ (25 μCi/100 g Body weight; 1.3 μCi/1 μg Zn). Representative animals were killed in groups of 3 at various times between 15 minutes and 28 days post-administration after pentobarbital anesthesia. The brain was perfused free of blood prior to dissection and assay of ^{65}Zn content.

Turnover of ^{65}Zn from plasma was rapid; less than 12% of the initial plasma concentration remained by 24 hrs. Uptake of ^{65}Zn by liver and other non-neural soft tissues was maximal by 6 hrs. In contrast, ^{65}Zn uptake into the CNS was exceedingly slow. Less than 0.4% of the administered dose was present in whole brain at 24 hrs. Moreover, the maximal accumulation of ^{65}Zn in whole brain did not occur until 7 days post-administration, remained relatively constant until 14 days, and declined slowly thereafter. The biological half-life of ^{65}Zn in brain varied from 13.0 days (optic nerve) to 26.6 days (hippocampus).

Initial transport into brain was greatest in cerebellum, optic nerve, and brainstem. Serial sectioning of brainstem revealed highest ^{65}Zn concentration at the level of the caudal IV ventricle. The concentration of ^{65}Zn in choroid plexus and the meninges was 10-100 fold higher than plasma throughout the 28 day period. Cerebrospinal fluid ^{65}Zn content averaged 0.5-1.5% of plasma and 9% of plasma ultrafiltrate (Pellicon PSAC membrane).

These studies demonstrate that the uptake and turnover of zinc in the CNS is protracted, especially when compared to metabolizable substrates. The rapid uptake of zinc by the choroid plexus suggests that it may be involved in zinc homeostasis within the CNS. The pattern of zinc metabolism in the CNS is qualitatively similar to other micronutrient cofactors. (Supported by a research grant from the Veteran's Administration and by USHS grant NS 16009).

29.8 EXPERIMENTAL SPINAL CORD TRAUMA: EARLY ALTERATIONS IN FLUID AND ELECTROLYTE LEVELS. NR Clendenon, WA Gordon* and JN Allen, Jr.* Neurochem Lab, Div of Neurol, Ohio State Univ, Columbus,O 43210

Edema of both gray and white matter was recognized as a prominent pathological finding in early morphological studies on experimental spinal cord trauma (Ducker TB et al, J Neurosurg 35: 700, 1971). Most biochemical studies of edema formation focused on intervals of 6 hrs or greater following impact injury. Yashon et al (J Neurosurg 38:693,1973) reported an average 2.6% increase in spinal cord water in trauma vs control samples as early as 5 min following a 300 g·cm impact injury to monkeys, reaching a maximum of 7.5% at 5 days. The present investigation used the rat model of cord injury and studied the sequence of edema formation within the initial 2 hrs following trauma.

Water and electrolyte levels of normal and traumatized spinal cord were assayed in laminectomized Sprague-Dawley rats weighing 250-300 gms using a section of thoracic cord approx 1 cm in length. Trauma was induced by applying a 90 g·cm force to the exposed cord by the weight drop method. Cord sections were cleaned of excess blood, weighed, dried at 95-100°C for 48 hrs and re-weighed. For electrolyte determinations, dried samples were homogenized in double distilled demineralized water. An aliquot, digested with concentrated nitric acid, was used for sodium and potassium determinations by emission flame photometry. Chloride was assayed using a protein-free filtrate of the remaining aqueous extract by an automated titration technique. Serum electrolyte assays followed established methods. Results were expressed as milliequivalents (mEq)/kg wet or dry weight of tissue and as mEq/l of serum.

Laminectomy alone produced a slight non-significant increase in tissue water and electrolyte levels over values obtained by the rapid removal method of DeSousa and Horrocks (Dev Neurosci 2: 115, 1979). Water content 5 min post trauma was not significantly different from laminectomized controls. Increased tissue water was evident by 10 min($p<0.05$) and continued to rise gradually throughout the period studied, reaching 4.5% of control at 120 min post injury. When tissue electrolyte levels were expressed on the basis of dry weight, a net gain in sodium($p<0.01$) and chloride ($p<0.001$) was evident by 15 min along with a slight but non-significant gain in potassium. Not until 30 min post injury was a significant decrease($p<0.02$) in net potassium observed, a time when serum levels were highest. Between 30 and 60 min, tissue sodium and chloride plateaued at levels approx 30% higher than laminectomized controls while potassium was 20% lower. These data suggest a vasogenic contribution to the edema formation occurring as early as 15 min post spinal cord injury and possibly a cytotoxic influence at later time intervals. (Supported by NINCDS #NS-10165).

29.9 DIETARY CHOLINE AND THE PERMEABILITY OF THE BLOOD-BRAIN BARRIER. R. Sankar*, B.A. Trommer*, K.L. Parrish, F.R. Domer and L. Wecker Depts. of Pharmacology, Tulane and Louisiana State Univ. Med.Ctrs. and Xavier Univ. College of Pharmacy, New Orleans, LA 70112.

We have examined the effect of dietary choline availability on the permeability of the blood-brain barrier (BBB) to a large molecular weight protein and a small anion by using radioiodinated serum albumin (RISA) and the ^{99m}Tc-pertechnetate anion(TcO_4^-), respectively. Male Sprague Dawley rats (final weight 250g) housed in groups of 2 and maintained on a 12 hour light/dark cycle with food and water available ad libitum were used for all studies. Rats were randomly divided into 3 groups and maintained for 12 or 24 days on: a) choline deficient chow (no free choline), b) basal choline chow (0.2% choline chloride) or c) choline supplemented chow (2.0% choline chloride). The rats were anesthetized with pentobarbital (60 mg/kg) and either RISA or pertechnetate was administered via the lateral tail vein.A cardiac blood sample was drawn 30 minutes after administration of the tracer , followed by perfusion with normal saline through the left ventricle to clear the cerebral vasculature. The brain was removed and the radioactivity in the brain was compared to that in the blood. Rats maintained on the 3 dietary regimens for 24 days did not exhibit any significant differences in the entry of RISA into the brain. The brain levels of the pertechnetate anion however, increased in the rats maintained on the choline deficient diet as compared to basal rats. Rats on the deficient diet for 12 days showed a 28% increase, while those maintained for 24 days showed a 50% increase increase in the brain to blood ratio of pertechnetate, indicating a time-dependency. Rats maintained on the choline supplemented diet did not exhibit significant differences in pertechnetate entry compared to rats on the basal diet. TcO_4^- is known to mimic I^- for many uptake processes and active removal of I^- from the CSF at the choroid plexus has been demonstrated. Our results indicate that dietary (chronic) choline deficiency leads to a compromised performance of this process. (Supported by NIMH grant # 33443.)

29.10 COMPARISON OF LIPID-MEDIATED BLOOD-BRAIN BARRIER PERMEABILITY IN THE ADULT AND NEWBORN BRAIN. L.D. BRAUN*, E.M. CORNFORD AND W.H. OLDENDORF (SPON: S.Diamond). Research Service, Brentwood VA Medical Center, Los Angeles, CA 90073 and Department of Neurology, UCLA School of Medicine, Los Angeles, CA 90024.

In many different membrane systems, oil:water partition coefficents of nonelectrolytes have been positively correlated with membrane permeability in a variety of in vitro studies. In this laboratory, it was demonstrated that measurement of brain uptakes could be correlated with olive oil:water partition coefficients (Oldendorf: Proc Soc Exptl Biol Med 147: 813, 1974) to extend this phenomenon to in vivo BBB function. Recently, we have demonstrated dramatically higher newborn brain uptakes for certain metabolites which cross the BBB by way of carrier-mediated systems as compared to the adult (Braun et al, J. Neurochm 34: 147, 1980). Preliminary studies also suggested brain uptakes of the anticonvulsants dilantin and phenobarbital was higher in the newborn than in the adult, and precluded a possibility of carrier-mediation. Because brain myelin content increases significantly in the neonatal period, the present study was undertaken to determine whether or not newborn brain uptakes of non-electrolytes could be correlated with octanol:saline partition coefficients. Brain uptake indices (BUI's) of cytosine, mannitol, urea, thiourea, ethylene glycol, propylene glycol, acetamide, methanol, ethanol, butanol, benzyl alcohol, phenobarbital, dilantin, antipyrine, caffeine and heroin were determined in the present study of newborn rabbits. Pardridge and Mietus (Endocrinology 107: 1705, 1980) have also determined newborn BUI's of estradiol and testosterone. Linear regression analyses indicated that the relationship of these 18 BUI's and octanol:saline partition coefficients (PC) in the newborn rabbit brain could be defined: (log BUI)x(Sq. root molec wt) = 6.3 (log PC) + 17, R= 0.86. In the adult rat BBB, on the basis of measuring BUI's of more than 45 compounds, and an almost identical relationship was derived: (log BUI)x(Sq. root molec wt) = 6.0 (log PC) + 14.5, R= 0.86.

Conclusion. These studies emphasize that despite developmental alteration in cerebral blood flow rates, and biochemical composition of the brain, in vivo function of the plasma: brain capillary interface is the same in the newborn and adult brains with respect to lipid-mediated penetration.

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29.11 CHLORIDE MOVEMENT ACROSS THE BLOOD-BRAIN BARRIER: EVIDENCE FOR PASSIVE-DIFFUSIONAL TRANSPORT. Quentin R. Smith and Stanley I. Rapoport. Laboratory of Neurosciences, Gerontology Research Center, National Institute on Aging, Baltimore City Hospitals, Baltimore, Maryland 21224.

The mechanism of chloride transport across the in vivo rat blood-brain barrier was investigated to determine whether passive diffusion, facilitated diffusion, or active transport are involved in CNS Cl regulation. The concentration dependence of Cl-36 unidirectional influx (plasma to brain) and of brain chloride content was measured at the steady state after partial replacement of body Cl with nitrate. Briefly, Osborn-Mendel rats were anesthetized with 30 mg/kg Na pentobarbital. Plasma [Cl] was set at 50 ± 2, 72 ± 2, 97 ± 1 or 114 ± 1 mmol/l by peritoneal dialysis (3 cycles, 45 min/cycle) with rat artificial interstitial fluid containing 0, 50, 110 or 180 mmol Cl/l, respectively. Then, bilateral nephrectomy was utilized to maintain constant plasma [Cl] for 6 hr, during which time brain Cl content reached a steady-state distribution. At 1/2 hr prior to sacrifice, Cl-36 (6 μCi) and H-3 inulin (30 μCi) were injected into the femoral vein and plasma activity monitored until the rat was killed. Samples from 9 brain tissue regions, cisternal CSF, and plasma were analyzed for radioactivity and Cl content. When plasma [Cl] was varied over a 64 mmol/l range, changes in brain tissue Cl content were proportional to those in the plasma. In contrast, CSF [Cl]/plasma [Cl] decreased significantly from 1.62 ± 0.06 at 50 mmol/l to 1.21 ± 0.02 at 114 mmol/l. Cl-36 unidirectional influx from plasma to brain was linearly related to plasma [Cl] and extrapolated to the origin over a plasma [Cl] range of 50-114 mmol/l. Significant regional differences in blood-brain barrier permeability to Cl were detected in control rats. For example, the permeability surface-area product (PA) to chloride of cerebral cortex (1.68 ± 0.11 l/sec) was markedly less than the PA of cerebellum (3.03 ± 0.20), hippocampus (3.51 ± 0.43), and thalamus-hypothalamus (3.23 ± 0.25). The cisternal CSF PA to Cl was approximately 10 times greater than that of the cerebral cortex. In conclusion, the constancy of brain tissue Cl content/plasma [Cl] at the steady state and the linear relationship between Cl-36 influx and plasma [Cl] are consistent with passive-diffusional transport of Cl across the blood-brain barrier.

29.12 DEVELOPMENTAL CHANGES IN CALCIUM CONCENTRATION IN VARIOUS REGIONS OF THE RAT CNS, Scott A. Burton* and Conrad E. Johanson (SPON: S. Turkanis). Dept. of Pharmacology, Univ. of Utah College of Medicine, Salt Lake City, Utah 84132

Preparatory to an ontogenetic study of fluxes of radioactive Ca among various CNS compartments, we have analyzed the stable calcium content of cerebrospinal fluid (CSF), serum (SER), lateral ventricle choroid plexus (LVCP), fourth ventricle choroid plexus (4VCP), pineal body (PIN), cerebral cortex (CC), hippocampus (HIP), thalamus (THAL), cerebellum (CER), and medulla (MED) in infant (1 wk) and adult (8 wk) Sprague-Dawley rats. Blood taken from the inferior vena cava of lightly etherized rats was allowed to clot and then centrifuged to obtain serum. After exsanguination, CSF was taken immediately via puncture of the cisterna magna. Minced tissues were extracted for 2 days with 0.5 N HNO_3 and analyzed for Ca by atomic absorption. Mean values (± SEM), as mEq Ca/kg fluid H_2O (or kg wet tissue), for n = 6 are given below; because of developmental differences in tissue (tiss) H_2O content, Ca concentrations are also given per dry weight.

	One week	Eight weeks
SER	6.55 (.03)	6.03 (.02)
CSF	2.34 (.06)	3.36 (.03)

Total calcium in serum and CSF significantly decreases and increases, respectively, after 1 wk; CSF/SER Ca is greater in the adult (0.56) than in the infant (0.36).

	One week		Eight weeks	
	mEq Ca / kg wet tiss	mEq Ca / kg dry tiss	mEq Ca / kg wet tiss	mEq Ca / kg dry tiss
LVCP	2.29 (0.08)	13.45 (0.48)	1.84 (0.09)	9.20 (0.44)
4VCP	2.79 (0.13)	16.39 (0.76)	1.90 (0.05)	9.50 (0.23)
PIN	2.32 (0.15)	19.33 (1.25)	2.16 (0.05)	11.37 (0.26)
CC	2.12 (0.06)	18.74 (0.53)	2.39 (0.01)	12.58 (0.06)
HIP	2.05 (0.02)	18.14 (0.18)	2.45 (0.03)	12.89 (0.16)
THAL	2.10 (0.16)	17.50 (1.29)	2.38 (0.04)	11.90 (0.20)
CER	1.55 (0.05)	11.74 (0.38)	2.21 (0.02)	10.23 (0.09)
MED	1.68 (0.11)	12.44 (0.81)	2.48 (0.06)	9.65 (0.23)

In all regions analyzed, CP and brain tissue [Ca] (dry weight basis) is substantially greater in infants than in adults, particularly so (by 1/3) in 4VCP, PIN, CC, HIP and THAL. Since CSF [Ca], i.e., extracellular fluid [Ca], displays a trend with age opposite to that in tissue, the data strongly suggest that [Ca] in choroid epithelial cells and in brain cells (neurons and/or glia) of the rat undergo a significant reduction after 1 wk.

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29.13 THE BLOOD-SPINAL CORD BARRIER FOLLOWING TRANSECTION IN THE RAT. Linda J. Noble* and D.S. Maxwell. Dept. of Anatomy, School of Medicine, UCLA, Los Angeles, CA 90024.

Following spinal transection in the rat, the blood-spinal cord barrier was studied at the ultrastructural level using Horseradish Peroxidase (HRP). Animals were sacrificed at 1 hour, 12 hours, and 3 days following surgery. Prior to sacrifice, HRP was administered intravenously and allowed to circulate 15-30 minutes. A 2 cm segment of spinal cord, distal to the transection, was removed and fixed by immersion. From the 1 hour and 12 hour timepoints, samples were processed by the method of Reese and Karnovsky. The tissue was examined electron microscopically. Vessels with reaction product were classified in 3 categories: Type I - no evidence of leakage but vesicles, containing reaction product, present in the endothelium, Type II - leakage of HRP into the basal lamina and surrounding interstitium without any apparent vesicles, Type III - presence of vesicles in the endothelium combined with HRP in the basal lamina and adjacent regions.

The present findings suggest barrier leakage at 1 and 12 hours which is not apparent at 3 days. In general, the vascular response was not consistent at each level and timepoint. In areas where leakage was noted, the endothelial tight junctions appeared intact. At 1 hour there was HRP in the endothelium, basal lamina and surrounding interstitium up to 18mm distal to surgical transection. Of the vessels analyzed, 56.5% were Type I, 22% were Type II, and 22% were Type III. At 12 hours, 75.5% were Type I, 2.0% Type II, and 22.4% were Type III. The preliminary evidence at 3 days demonstrates an intact barrier, although vesicles were present in the endothelium.

The barrier breakdown present at 1 hour and 12 hours following transection appears to be either absent or considerably diminished at 3 days.

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29.14 CEREBROSPINAL FLUID DYNAMICS IN THE CAT UNDER HALOTHAN AND PENTOBARBITAL ANESTHESIA. K.E. VanLandingham*, C.J. Maffeo*, H. Jackson, and A. B. Butler. Depts. Neurosurg., Anes., and Biom. Engr., Univ. Va. Med. Sch., Charlottesville, VA 22908.

Constant-flow manometric infusions into the subarachnoid space of the cat were used to assess the effects of Halothane@ and pentobarbital anesthesia on cerebrospinal fluid (CSF) dynamics. Animals were initially anesthetized with pentobarbital (30 mg/kg, i.p.) and atropine (.08 mg/kg, i.p.) and were maintained on either Halothane@ (.7%) or constant pentobarbital infusion (10 mg/kg/hr, i.v.). During each experiment, femoral arterial pressure was monitored, end-tidal CO_2 was maintained at 30 mmHg, and body temperature was regulated at 37.5°C. Following insertion of a 16 gauge needle into the cisterna magna CSF space, resting CSF pressure and steady-state pressures resulting from infusions of artificial CSF at flow rates of 40, 75, 150, and 400 µl/min were recorded and analyzed. CSF formation and resistance to absorption were calculated from the steady-state pressure/flow data.

The manometric data were fit to a logarithmic curve (pressure vs. log (flow)) resulting in correlation coefficients greater than 0.985. An analysis of variance (ANOVA) of the steady-state pressures did not show significant differences between the Halothane@ and pentobarbital groups. However, when the data were fit to a nonlinear model, differences in estimates of CSF production and resistance to CSF absorption at resting pressure were significant ($p<.05$). At resting pressure (30 to 80 mmH_2O), CSF production was calculated to be 20.9 µl/min (Halothane@) and 25.7 µl/min (pentobarbital), and resistance to CSF absorption was found to be 2.45 mmH_2O/µl/min (Halothane@) and 1.84 mmH_2O/µl/min (pentobarbital). At flow rates greater than 40 µl/min, differences in resistance were not significant.

In summary, we have shown that there is a significant difference in estimates of CSF production and resistance to CSF absorption at resting CSF pressure in the cat. At elevated CSF pressures (200 to 600 mmH_2O), however, estimates of resistance to CSF absorption are not significantly different between animals anesthetized with Halothane@ or pentobarbital.

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29.15 BRAINSTEM INTRAPARENCHYMAL VASCULAR CHANGE FOLLOWING EXPERIMENTAL SUBARACHNOID HEMORRHAGE. J.T. Povlishock, D.S. DeWitt*, L.W. Jenkins* and D.P. Becker . Departments of Anatomy and Neurosurgery, Medical College of Virginia, Richmond, Va 23298.

In previous reports we have noted that mechanical brain injury results in widespread brain parenchymal and vascular change reflected in an increased vascular permeability to horseradish peroxidase (HRP) coupled with the generation of profound endothelial alteration. Furthermore, we reported that the post-traumatic hypertensive episode concomitant with an increased prostaglandin synthesis and free radical damage were responsible for the genesis of many of the observed changes. However, in that all the described phenomena were not adequately explained by these traumatically-induced factors, it was questioned whether traumatically-related subarachnoid hemmorhage could also contribute to the genesis of the described alterations. To explore this possibility cats received autologous arterial blood injected into the cisterna magna while the intracranial pressure was maintained within normal limits. Some cats received intravenous peroxidase prior to the cisternal infusion, while other cats received peroxidase delivered into the cisterna magna with the autologous blood. After variable survival periods ranging from 1 to 6 hours the animals were perfused with aldehydes, their brains were serially sectioned with a Vibratome and reacted for the visualization of the HRP reaction product. Alternate serial sections were then prepared for either light microscopy or transmission or scanning electron microscopy. With the infusion of autologous blood mixed with peroxidase, the protein was observed to pass through the glia limitans and reach the interstices of the brain stem parenchyma where its passage was much more pronounced that in the control situation. The luminal surfaces of the pial and superficial intraparenchymal vasculature demonstrated numerous attached leukocytes and some areas of increased vascular permeability to the intravenously injected HRP. Such change appeared consistent with the onset of an aseptic meningitis. Additionally deep within the brain stem parenchyma other microvessels demonstrated an increased vascular permeability to the intravenously injected HRP and the endothelia of such vessles demonstrated numerous microvilli vesicles, and luminal and abluminal pits as well as other forms of endothelial change. In that it was speculated that such intraparenchymal vascular change maybe associated with changes in cerebral blood flow several experiements were designed to assess blood flow via the use of radioactive microspheres. The correlation between the above described morphological changes and blood flow alterations will be presented, and their relation to mechanical brain injury will be discussed.

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29.16 HYPOTHALAMIC NEURONAL NECROSIS IN NEONATAL CATS ADMINISTERED PHARMACOLOGIC DOSES OF MONOSODIUM L-GLUTAMATE. A. E. Applebaum, T. T. Daabees*, L. J. Filer, Jr.*, and L. D. Steginк*. Depts. of Anatomy and Pediatrics, U. of Iowa, Iowa City, IA 52242

Administration of large amounts of the dicarboxylic amino acids (glutamate and aspartate) produce hypothalamic neuronal necrosis in the infants of some animal species. While the neonatal mouse appears to be the most sensitive animal, lesions are not restricted to this species. We have examined the effects of monosodium L-glutamate (MSG) on the hypothalamus of the 8-day-old kitten.

MSG was administered either by gavage (n = 4) or intravenously (n = 4). Control animals received isotonic saline either by gavage (n = 3) or intravenously (n = 2). Blood samples were obtained (arterial catheter) at appropriate time intervals following MSG administration for amino acids, blood gas, and pH analyses. Animals were killed 5 hours after MSG administration by intracardiac infusion of a 2% glutaraldehyde--2% paraformaldehyde solution. Brains were removed, further fixed in the same solution, and embedded in either paraffin or plastic. Either 4 µm paraffin or 2 µm plastic serial sections were made through the hypothalamus and examined by light microscopy.

Neuronal damage was observed in the median eminence and arcuate nucleus of the hypothalamus after an oral dose of MSG at 4 g/kg body weight (20% w/v). However, the observed lesions were much less extensive than those observed in similarly treated infant mice. Damage was characterized by nuclear pyknosis and karyorrhexis. Mean peak plasma glutamate + aspartate concentration was 780 µmoles/dl (normal 9 µmoles/dl) 120 minutes after dosing.

After intravenous infusion of MSG (0.5 g/kg body weight; 0.4 ml/minute of a 1.67% solution, w/v) similar neuronal necrosis was noted. Mean peak plasma glutamate + aspartate concentration in these animals was 510 µmoles/dl 5 minutes after termination of infusion.

Administration of the large oral doses of glutamate produced metabolic alkalosis (pH 7.49 ± 0.03; pCO_2 - 35 ± 1 mm Hg; bicarbonate - 26.2 ± 1.8 mM; normal values 7.36 ± 0.01; 30 ± 1.5 mm Hg, and 16.6 ± 1.6 mM respectively). It is possible that this metabolic alkalosis affects the "blood brain barrier," facilitating dicarboxylic amino acid transfer and formation of CNS lesions.

These data indicate that the hypothalamus of the infant cat, like that of the infant mouse, but unlike that of the infant monkey, is sensitive to pharmacologic amounts of MSG.

29.17 RADIATION CONTROLLED FOCAL PHARMACOLOGY IN THE THERAPY OF EXPERIMENTAL EPILEPSY. M. P. Remler and W. Marcussen.* Department of Neurology, University of California, Davis, Veterans Administration Medical Center, Martinez, CA 94553.

Radiation controlled focal pharmacology is a method using intermediate dose radiation of a portion of the brain to break down the blood-brain barrier (BBB) followed by the administration of a drug which does not cross the normal BBB but which has a desired pharmacologic effect when it does cross the BBB. Therefore, the drug effects only the radiated portion of the brain. Cats with alumina-cobalt chronic epileptic foci were given IV Gama amino butyric acid (GABA) with no suppression of their EEG recorded computor analyzed epileptic spike frequency. Spike fequency ten minutes post infusion was chosen as the best indicator most sensitive of drug efficacy. When the focus received 6,000 rad of Bragg peak proton radiation there was still no significant change in spike frequency. Then, however, when IV GABA was again given, there was dramatic suppression of spike frequency. In every cat tested there was clear suppression of spike activity by the GABA at 9 days post radiation.

When the data from all cats are combined, each cat's GABA post radiation result is normalized against its own GABA before radiation. There is some suppression all during the first ten days post radiation. This suppression is maximum in the 7 to 10 day range. At the end of the week the suppression is so large, 87%, that despite the small number of cats involved the effect is statistically significant. The p value for the 9 day effect against preradiation controls is $<.1$ and for the combined 7 and 9 day effect $<.01$. Also, at 11 days the BBB is "closed" (meaning less than a 10% effect) with a $p<.01$.

29.18 AGE-RELATED ALTERATIONS IN BRAIN UPTAKE OF ANTICONVULSANTS AND DEVELOPMENTAL MODULATIONS OF BLOOD-BRAIN BARRIER PERMEABILITY TO METABOLITES AS AN INDICATOR OF CHANGING NUTRITIONAL REQUIREMENTS IN THE BRAIN. E. M. Cornford, L. D. Braun* and W. H. Oldendorf. Department of Neurology, UCLA School of Medicine, and Southwest Regional V.A. Epilepsy Center & Research Service, V.A. Medical Center, Los Angeles, CA 90073.

The intracarotid injection technique has been utilized to examine blood-brain barrier (BBB) function in studies of newborn (>24 hours), 7, 14, 21 and 28 day-old, as well as adult rabbits. The age-related modulations in BBB transport of adenine, arginine, choline, lactate and tryptophan are defined and demonstrated to be independent of each other. Lactic acid uptake was unusual in that the brain uptake index (BUI) was found to be greatest at 7 days post-partum. Elevated lactate uptake continues until 14 days and is then reduced. As indicated below, for all of the other metabolites examined, a maximal BUI was observed in the newborn brain and BUIs typically showed some sort of inverse relationship to animal age. The BUI of arginine is apparently halved in the first 7 days post-natally, and continues to decrease, reaching the value seen for the adult at an age of 21 days. In contrast, the brain uptake of adenine is unusual in that there appears to be a very gradual reduction in brain uptake occurring throughout the suckling period. A threefold decrease in the BUI of choline was observed during the first two weeks post-partum. Tryptophan uptake undergoes a fourfold reduction in the first four weeks post-natally. The uptake of butanol did not change appreciably over the range of ages examined. In contrast, brain uptake of the anticonvulsant drugs dilantin and phenobarbital was elevated during the first 14 days post partum, then declined to approach rates seen for the adult. The brain uptake rates of the metabolites which gain access by way of specific carrier mediated transport mechanisms are presumed to reflect nutritional requirements of the developing brain. Thus the present study indicates that the inclusion of adenine, arginine, choline, tryptophan and monocarboxylic acids such as lactate would be beneficial in clinical intravenous feeding of the neonate, to meet the apparent needs of the developing central nervous system.

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29.19 EVIDENCE FOR PLATELET-MODULATED PROSTACYCLIN SYNTHESIS IN CEREBRAL MICROVESSELS. M. D. Ferrari*, J. A. Helpern* and K. M. A. Welch. Department of Neurology, Baylor College of Medicine, Houston, Texas 77030.

Several studies have reported that cerebral microvessels (CMV) have the capacity to synthesize prostacyclin (PGI_2) (Maurer, P., Moskowitz, M.A., Levine, L., and Melamed, E., Prostaglandins Med., 4:153-161, 1980; Rosenblum, W.I., and El-Sabban, F., Circ. Res., 43:238-241, 1978). The present paper describes the influence of aggregating platelets on this process.

CMV were isolated from the cerebellum and cortex of female gerbils (50-60 gm, N=15 per isolation) by albumin flotation and glass bead filtration. The isolate was then divided into four aliquots and incubated in Tris (pH=7.4, 100 µl per aliquot, 10-30 min). The supernatants and the CMV themselves were then tested for their influence on adenosine diphosphate (ADP)-induced platelet aggregation, performed in vitro in an aggregometer according to the Born technique. In five experiments, the CMV were preincubated in aspirin (200-400 µg/ml, 45-60 min), washed in Tris and then tested. All results were analyzed using paired t's.

The addition of untreated CMV to the cuvette prior to the addition of ADP (5 µM, final concentration) significantly inhibited platelet aggregation (mean per cent inhibition of control at 3 min [%MIC-3] $\pm$ standard deviation [S.D.]: 49 ± 30, $p<0.01$). In contrast, the addition of aspirin-pretreated CMV and the supernatants from the untreated CMV showed no significant effect (%MIC-3 $\pm$ S.D.: 25 ± 48, $p>0.2$ and 16 ± 23, $p>0.1$, respectively).

These results strongly suggest a triggered platelet-endothelial cell interaction and lend support to the recently described platelet-derived PGI_2 synthesis-stimulating factor, which is thought to be released during platelet degranulation (Coughlin, S., Moskowitz, M., Antoniades, H., and Levine, L., Stroke, 12:117, 1981, and personal communication).

30.1 BIOSYNTHESIS OF MET^5- AND LEU^5-ENKEPHALIN IN BOVINE ADRENAL MEDULLA: DIFFERENTIAL EFFECTS OF TRYPSIN INHIBITORS

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The adrenal medulla has been shown to contain not only met^5- and leu^5-enkephalin (met-enk and leu-enk), but a variety of enkephalin-like peptides. It has been suggested that some of these larger enkephalin-like peptides function as precursors to met-enk and leu-enk. In this study, the formation of met-enk and leu-enk from high molecular weight enkephalin-like peptides was investigated. A soluble protein fraction was prepared from lysed chromaffin granules and rendered free of met-enk, leu-enk, and other low molecular weight enkephalin-like immunoreactivity by ammonium sulfate precipitation and dialysis. This preparation was found to produce low molecular weight enkephalin-immunoreactive peptides when incubated at 37°C. Through the use of HPLC, the production of authentic met-enk and leu-enk as well as of other unidentified low molecular weight enkephalin-like peptides was confirmed. This result suggests that the granule preparation contains both the enzymatic activity and the substrate required for the production of met-enk and leu-enk. The effect of various trypsin inhibitors on the the production of low molecular weight met-enk and leu-enk immunoreactivity was also tested. N-α-ρ-tosyl-lysyl chloromethyl ketone and p-amino benzamidine partially inhibited the production of leu-enk immunoreactivity but were without effect on the production of met-enk immunoreactivity. Conversely, soybean trypsin inhibitor was much more effective in inhibiting the production of met-enk immunoreactivity than of leu-enk immunoreactivity. The effectiveness of these trypsin inhibitors suggests that some of the enzymes involved in the biosynthesis of met-enk and leu-enk may be trypsin-like in nature. An account of the progress made in purifying enzymatic activities involved in the production of met-enk and leu-enk will be presented.

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30.2 METABOLISM OF Tyr-Gly-Gly-Phe-Met-Arg-Phe BY DIPEPTIDYL CARBOXYPEPTIDASE.

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Since the chemical characterization of enkephalins, many different forms of enkephalin like peptides having molecular weight larger than enkephalin have been detected in various tissues. Some of the high molecular weight forms were found to have opioid activity and others were devoid of such activity. Whether the high molecular weight forms are related metabolically to enkephalins is not clear at present. In this study, conversion of Tyr-Gly-Gly-Phe-Met-Arg-Phe (heptapeptide) to [met^5]-enkephalin was studied. The heptapeptide was readily metabolized by a dipeptidyl carboxypeptidase prepared from striatum. The enzymatic products analyzed by HPLC include [met^5]-enkephalin, Tyr-Gly-Gly and Arg-Phe. The conversion of the heptapeptide to [met^5]-enkephalin was inhibited by some dipeptidyl carboxypeptidase inhibitors such as o-phenanthroline, Phe-Ala and SQ 20881. Interestingly, the SQ 20881, an angiotensin converting enzyme inhibitor, was found to have no effect on enkephalin inactivating dipeptidyl carboxypeptidase. Chloride ion, which is known to be essential for angiotensin converting enzyme activity, did not activate the conversion of the heptapeptide to [met^5]-enkephalin. With SQ 20881, it will now be possible to study whether the release of Arg-Phe from the heptapeptide is an activating or an inactivating process. Whether there is a specific dipeptidyl carboxypeptidase for conversion of the heptapeptide to [met^5]-enkephalin still remains to be established.

30.3 BOVINE CHROMAFFIN GRANULES CONTAIN AN ENZYME AND A PRECURSOR WHICH GENERATE METHIONINE ENKEPHALIN IN VITRO.

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Reports that the bovine chromaffin granules contain enkephalins and their precursors indicate that the biosynthetic enzyme(s) could also be present in these secretory granules. An enzyme has been isolated from these granules which cleaves methionine enkephalin from its precursor.

The chromaffin granules of bovine adrenal medulla were isolated by a modification of the method of Smith and Winkler (Biochem J. 103: 480-482, 1967). The enkephalin precursors and their converting enzyme were found in the soluble lysate of the granules. Sephadex G-100 column filtration of the soluble lysate allowed identification of enkephalin precursors by trypsin digestion of fractions, followed by radioreceptor assay (RRA) with neuroblastoma x glioma cells for opiate-like activity. The fraction corresponding to a molecular weight of ~ 22,000 daltons was chosen to monitor the activity of the endogenous enzyme. This precursor was selected because it yielded the largest amount of opiate-like activity upon trypsin digestion. The enzyme was isolated in the void volume of a Sephadex G-200 column separation of the soluble lysate. The activity is not lysosomal in origin: an acid phosphatase profile of the sucrose density gradient used for the final purification of the granules indicated a complete separation of the lysosomes from the chromaffin granules. The enzyme has trypsin-like activity, as determined by the cleavage of the artifical substrates, benzoyl DL-arginine p-nitroanilide hydrochloride (BAPNA) and ^{3}H-N-tosyl-L-arginine methyl ester (^{3}H-TAME). An optimum activity at pH 7.5 was found using ^{3}H-TAME and ^{14}C-methemoglobin as proteolytic substrates. Incubation of this endogenous enzyme with the enkephalin precursor resulted in the production of methionine enkephalin. The incubation was carried out at 37°C, pH 7.5, in the presence of 190 mM Tris Acetate, 20 mM $CaCl_2$, 1 mM dithiothreitol. Opiate-like activity was easily detectable by RRA after four hours incubation, with maximum yield after twenty hours. The reaction was stopped by heat inactivation of the enzyme. The digestion product was identified as methionine enkephalin by high performance liquid chromatography (HPLC) on a Waters reverse phase C_{18} column combined with assay of fractions by RRA. This was confirmed by radioimmunoassay with a specific antibody for methionine enkephalin. All opiate-like activity applied to the HPLC column was recovered as methionine enkephalin. The presence of this converting enzyme with the precursors and products in the chromaffin granules indicate that this enzyme could be important in the biosynthesis of the enkephalins.

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30.4 EVIDENCE FOR MULTIPLE FORMS OF BETA-ENDORPHIN-RELATED SUBSTANCES IN THE PITUITARY OF THE REPTILE, ANOLIS CAROLINENSIS.

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At least four forms of β-endorphin have been isolated from the intermediate lobe of the mammalian pituitary (Smyth and Zakarian, 1980, Nature 288,613-615). These forms differ in the degree of proteolytic cleavage of the C-terminal region and the presence or absence of α,N-acetylation. β-Endorphin(1-31) has full opiate bioactivity; β-endorphin(1-27) has equal or diminished activity, depending on the bioassay system; the α,N-acetylated endorphins are completely inactive. To determine if these post-translational events occur in a lower vertebrate, single intact Anolis carolinensis intermediate pituitaries were incubated for 24 h in complete DMEM containing [^{3}H]tyrosine. The tissue was acid extracted in the presence of protease inhibitors, and an aliquot was immunoprecipitated with affinity purified antibody to the N-terminal region of β-endorphin. The immunoprecipitate was dissociated and fractionated by gel filtration (Sephadex G-75, 10% formic acid). Three peaks of radioactivity were isolated. Sequential immunoprecipitation experiments with affinity purified antisera to the N-terminal region of ACTH and to β-endorphin indicate that the first radioactive peak (K_d=0.12) represents the common precursor for the αMSH-like and β-endorphin-like molecules of this species. The second peak (K_d=0.40) eluted close to β_m-lipotropin. The third peak (K_d=0.68) eluted close to β_m-endorphin. This latter peak was concentrated and fractionated by ion exchange chromatography (sulfopropyl Sephadex, 50% acetic acid). This column was eluted with a linear NaCl gradient (0.05 M to 0.55 M) and at least four peaks of radioactivity were isolated: Peak 1, 0.27 M NaCl; Peak 2 0.33 M NaCl; Peak 3, 0.38 M NaCl; Peak 4, 0.42 M NaCl. The peaks of Anolis β-endorphin did not co-elute with β_c-endorphin(1-31), β_c-endorphin(1-27) or the α,N-acetyl derivatives. An aliquot of each of these peaks was analyzed on a Sephadex G-50 column in 6 M guanidine-HCl; Peak 1 was smaller than β_m-endorphin-(1-31) while Peaks 2,3,4 were about the size of β_m-endorphin (3400 daltons). These reptile β-endorphin-like substance are being further characterized by analyzing proteolytic digests.

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30.5 MULTIPLE FORMS OF B-END IN CNS: REGIONAL DIFFERENCES AND EFFECT OF STRESS. H. Akil, H-L Lin,* Y. Ueda,* S. Lax, M. Walker,* and S. Watson (SPON: H. Akil). Mental Health Research Institute, Univ. of Michigan, Ann Arbor, MI 48109.

Previous work by our group and by others (Smyth and Zakarian, Mains and Eipper) has shown that multiple forms of B-Endorphin exist in mammalian CNS. We have shown that the naturally occuring form B-END (1-27) is 10 times less potent than B-END (1-31) at the opiate receptor. N-acetylation leads to a thousand fold decrease in binding potency. Thus it is critical to determine the ratios of the several forms of B-END in various tissues. We find that rat intermediate lobe synthesizes primarily N-Acetyl-B-END (1-27), while the anterior lobe stores mostly the opiate active B-END (1-31). Recent studies in brain show that B-END (1-31) is predominant in rat hypothalamus, although the other forms are detectable as well. Unidentifiable B-END-like immunoreactivity also exists in hypothalamus (and not in pituitary). Studies of the forms of B-END in terminal regions (such as thalamus and midbrain) are currently in progress. Behavioral manipulation, such as stress, alter both pituitary and brain B-END. The effect of such manipulations on the mulitple forms is being examined. While changes in total immunoreactivity are reliably found, it seems more important to determine changes in ratios of active to inactive forms of B-END, allowing for a more dynamic measure of the activity of the system.

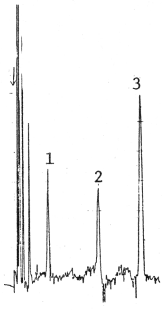
30.6 PARTIAL PURIFICATION AND CHARACTERIZATION OF A NOVEL HUMAN ENDORPHIN FROM CEREBROSPINAL FLUID. B. E. Miller, J.J. Lipman, S. Karkera*, R. Winfield*, W.L. Byrne, K. Mays*, and W.C. North* Depts. of Biochemistry and Anesthesiology, Univ. of Tenn. Ctr. for the Health Sci., Memphis, Tenn. 38163.

For the past three years our laboratory has been engaged in qualitative and quantitative analysis of endorphins contained in human cerebrospinal fluid (CSF). We have identified and routinely measured twelve endorphin components from both chronic pain and pain-free patients. Endorphins were defined as those components capable of displacing ^{3}H-dihydromorphine from homogenized mouse brain in a radioreceptor assay (RRA). One particular endorphin, Peak B, was best correlated with the pain status of the donor chronic pain patient and was also present in larger quantities in the pain-free patients. Peak B, as well as the other CSF endorphins, was initially determined as follows: whole CSF was passed through an Amicon PM-10 filter, the filtrate was lyophilized, this material was resuspended in 0.2 N acetic acid and loaded atop a Sephadex G-10 column and eluted in the same solvent, column fractions were lyophilized and assayed for endorphin activity with the mouse brain homogenate RRA. Based on its G-10 elution position, Peak B does not coelute with any of the structurally identified endorphins. Further characterization was performed using G-10 purified Peak B. This material was found to produce a naloxone-reversible dose-dependent inhibition of the electrically stimulated contractions of both the guinea pig ileum and the mouse vas deferens preparations. Peak B was also shown to produce a potent naloxone-reversible analgesia after intracerebroventricular (i.c.v.) injections into mice assayed by the mouse tail immersion test (48^{o}C). Additionally, HPLC on a reversed phase (Water's μBondapak C-18, eluant: ammonium acetate pH 4/ acetonitrile) column in conjunction with the RRA showed that Peak B's activity was found in a single peak which coeluted with leu enkephalin. However, this endorphin did not coelute with leu enkephalin on G-10. Finally, the biologic activity of Peak B was found to be resistant to trypsin and alpha chymotrypsin (34^{o}C, > 6 hr. incubations),i.e. these enzymes did not change Peak B's activity on the mouse vas deferens assay.

It is likely that Peak B is identical with the major component in Terenius and Wahlstrom's Fraction I endorphin (1975) and possibly also to the humoral endorphin described by Sarne, Azor and Weissman (1978). In conclusion, we believe that Peak B is a potent biologically active endorphin that is chemically different from any hitherto structurally identified endorphin.

30.7 A NEW LC-EC METHOD TO SEPARATE AND QUANTIFY ENDOGENOUS OPIATE PEPTIDES. D. Rodd*, L. H. Fleming*†, E. A. Stein and N. C. Reynolds, Jr.†. Dept. of Biology, Marquette University, and †Sect. of Neurology, Univ. of Wisconsin, Sch. of Med., Milwaukee, WI 53233.

Following the discovery and subsequent neuroanatomic localization of the endogenous opiate peptides (endorphins), research interest has focused on their role in normal physiology as well as pathologic conditions of the brain. To perform these experiments, it is first necessary to be able to measure levels of these peptides in brain with a highly specific yet sensitive procedure. Radioimmunoassay (RIA), while very sensitive, is limited by potential cross-reactivity with other closely related peptides. Liquid chromatography (LC), in contrast, is capable of separating these peptide species but using standard UV detection, lacks the sensitivity necessary to quantify biologically relevant samples. We report an LC technique coupled with electrochemical detection which enables the separation and detection of three principal endorphins, methionine and leucine enkephalin and β-endorphin from small brain samples. Our procedure involves a simple brain extraction technique followed by peptide separation on a reverse phase column with an isocratic buffer system. The peptides can be separated in a relatively short time with a sensitivity in the pmolar range. A typical chromatogram is shown. LC results have been verified from collected separated peptides. This simple procedure allows for the simultaneous measurement of changes in the concentration of these endorphins following various physiologic and pharmacologic manipulations. (Supported in part by NIDA grant #DA02334 and The Scholl Foundation.)



Chromatograph of Endorphin Standards

1) Leucine Enkephalin, 41.6 ng

2) Methionine Enkephalin, 41.6 ng

3) β Endorphin, 5.2 ng

Sensitivity: 2 n A/V full scale
↓ denotes injection

30.8 GAMMA ENDORPHIN-LIKE PEPTIDES IN THE PITUITARY AND BRAIN OF THE RAT. M.B. Chapman* and D.M. Dorsa* (SPON: W. Stahl). GRECC, VA Medical Center, Seattle, WA 98108.

Experiments wherein tissue proteolytic enzyme activity has been inactivated prior to tissue disruption have suggested that alpha-endorphin (αE, BLPH 61-76) may exist only as an artifact of extraction procedures. In the present study we have examined the effect of enzyme inactivation on the content of β and gamma endorphin (γE, BLPH 61-77)-like peptides in brain and pituitary tissue of the rat.

Eight male rats were killed by decapitation and their hypothalami and pituitaries were removed and placed into 300 μl 0.1N acetic acid at room temperature (n=4) or into boiling acetic acid (n=4) for 15 min. The samples were then sonicated, aliquots taken, then centrifuged and aliquots of the supernatant were lyophilized. β and γ endorphin were measured using sensitive (4 and 2 pg/tube) and specific radioimmunoassays for these peptides. The results expressed in ng peptide/mg protein ($\bar{x}\pm$S.D.) were as follows:

	βE-Like Immunoreactivity	γE-like Immunoreactivity
Hypothalamus		
Non Boiled	0.400±0.083	0.18±0.09
Boiled	0.56±0.10	0.056±0.19
Pituitary		
Non Boiled	430±94	34.7±10.2
Boiled	829±105	104.3±26.6

When 500 ng of βE was added to pituitaries prior to boiling and sonication no increase in RIA-γE was noted indicating the effectiveness of boiling in preventing proteolysis. The decrease of γ-E like peptides in brain tissue and concomitant βE increase with boiling suggest the importance of this inactivating procedure for endorphin measurements. However, in contrast to αE, γE-like peptides persist in measureable quantities following this procedure indicating that their presence in brain tissue is not artifactual. In the pituitary both β and γE increased with boiling, suggesting that γE is not only present in pituitary tissue, but may itself be rapidly cleaved by proteases released during the extraction procedure, possibly to αE. Processing of βE appears to differ in the brain and pituitary. In brain the βE to γE cleavage may be a first step to inactivation of opiate-like activity. Alternatively, since γE-like peptides have been reported to possess neuroleptic-like properties, they may be synthesized as modulators of the function of dopamine neuronal systems.

31.1 B-ENDORPHIN AND a-MSH IN THE RAT BRAIN: A COMPARATIVE IMMUNOCYTOCHEMICAL ANALYSIS. H. Khachaturian, K. Tsou,* and S.Watson (SPON: H. Khachaturian). Mental Health Research Institute, Univ. of Michigan, Ann Arbor, MI 48109.

The opioid peptide B-endorphin (B-END) and the neurointermediate lobe hormone a-MSH originate from a common precursor molecule, pro-opiomelanocortin (POMC), synthesized both in the pituitary and hypothalamic arcuate nucleus. Both peptides have been extensively mapped in the CNS by immunocytochemical methods. Recently Watson and Akil (Brain Res., 182:217, 1980) have shown that a-MSH and B-END occur in the same arcuate neurons, and further, have discovered a second a-MSH system (a-2) in the hypothalamus. It appears that the a-2 neurons do not produce, at least in detectable amounts, any other product of the POMC molecule, including B-END. Thus far, it has not been possible to separate the two hypothalamic systems with several antisera against a final common product, a-MSH. Fibers originating from the arcuate B-END/a-MSH neurons project throughout the hypothalamus, amygdala, nucleus accumbens, septal nuclei, periventricular thalamus, periaqueductal gray, and as far caudal as the level of the locus coeruleus and medullary reticular formation. Additionally, fibers originating from the a-2 neurons project to the hippocampus, caudate and cerebral cortex.

Immunocytochemical studies of B-END vs. a-MSH were carried out in male Sprague-Dawley rats, some of which were treated with colchicine to enhance cell-body visualization. B-END cells appeared to be restricted primarily to arcuate nucleus although peri-arcuate cells were also present. In contrast, three a-MSH cell groups could be delineated. One group corresponded to the arcuate B-END cells in serial section analysis. The other two groups (i.e., a-2) were composed of widely scattered neurons starting at mid-hypothalamic level. The more medially located a-MSH neurons were scattered in the periventricular regions and the dorsal part of the ventromedial nucleus. The laterally located a-MSH neurons were scattered in the lateral hypothalamic area and some were medially apposed to the optic tracts. Also many cells surrounded the forniceal columns. More caudally, some neurons appeared to be in the ventral region of the zora incerta. Preliminary cell counts revealed that the total number of a-MSH neurons (a-2) exceeds the total number of B-END neurons. Other studies are under way to determine the comparative distributional patterns of B-END and a-MSH fibers.

31.2 DYNORPHIN IMMUNOCYTOCHEMISTRY: PRELIMINARY DISTRIBUTION AND RELATIONSHIP TO VASOPRESSIN. S. Watson, H. Akil, G. Nilaver, E. Zimmerman, T. van Wimersma Greidanus,* A. Goldstein. Mental Health Research Institute, Univ. of Michigan, Ann Arbor, MI 48109. (SPON: S. Watson).

The opioid peptide dynorphin has been extracted from bovine pituitary preparations and shown to be a potent opiate peptide. The partial sequence of dynorphin (1-13) is different from existing opioid peptides. After the development of antersia against dynorphin, RIA studies indicated a heterogeneous distribution in brain and pituitary. We began the immunocytochemical study of its distribution in rat CNS using antisera primarily against the C terminus of the peptide (i.e., the non-leucine enkephalin region). Some studies were carried out with diluted crude serum and some with affinity purified antiserum. All data were validated with affinity purified sera. The immunohistochemical demonstrations were blockable by dynorphin(1-13) but not by met- or leu-enkephalin, B-endorphin, aMSH, AVP or oxytocin.

Dynorphin immunoreactivity (DYN-ir) was detected in posterior pituitary but not anterior or intermediate lobe. Its pituitary levels were comparable to those for leucine-enkephalin - using antisera and separation techniques which can separate leu-enkephalin from dynorphin (HPLC/RIA). In hypothalamus, dynorphin has been localized in the magnocellular neurosecretory cells of the supraoptic and paraventricular nuclei. Serial section analysis using vasopressin (AVP) and dynorphin antisera clearly demonstrate that these two peptides are in the same cells. No relationship to oxytocin has been detected. Homozygous Brattleboro rats (with no detectable AVP) continue to show DYN-ir in their magnocellular neurons. The DYN-ir cells are the non-oxytocic cells.

It is suggested that dynorphin might be an active neurosecretory substance, vulnerable to the same physiology and pharmacology as AVP. It is further suggested that DYN is under separate biosynthetic control from those seen with AVP. The remainder of the CNS distribution of dynorphin is under study.

31.3 DYNORPHIN IMMUNOREACTIVITY IN TOAD (BUFO MARINUS) SPINAL CORD, BRAIN AND NEUROINTERMEDIATE LOBE, R. I. Cone and A. Goldstein. Addiction Research Foundation, Palo Alto, CA 94304.

Dynorphin is a highly potent opioid peptide, initially purified from porcine pituitary extracts. Dynorphin 1-13 (D1-13; YGGFLRRIRPKLK) contains the leu-enkephalin sequence at the NH_2 terminal, and is as potent as natural dynorphin in the guinea pig ileum bioassay. Using a highly specific antibody (Lucia) raised against thyroglobulin-conjugated D1-13, immunoreactive dynorphin (ir-DYN) has been found in pig, beef and rat pituitary and rat brain and spinal cord. In the rat, ir-DYN is found in highest concentrations within the neurointermediate lobe (NI), spinal cord and hypothalamus (Proc Nat Acad Sci 77: 6207, 1980).

We now report on the presence of ir-DYN in 1 M acetic acid extracts from toad (Bufo Marinus) spinal cord, brain and NI. Tissue concentrations of ir-DYN in spinal cord and brain were 330 and 510 fmol/mg protein, respectively. The highest concentration of ir-DYN was found within NI (50 pmol/mg protein or approximately 5 pmol total). In comparison to values reported for the rat, the tissue concentrations in toad spinal cord, brain and NI were 2 - 3 fold higher.

The size distribution patterns of ir-DYN were also examined in toad spinal cord, brain and NI using Sephadex G-50 gel permeation (0.1 M acetic acid; 0.15 M NaCl; 0.1% triton X 100). There appear to be considerable differences in the size distribution patterns of ir-DYN from region to region. For example, in spinal cord, most of the ir-DYN eluted in the void volume (apparent M_r greater than 30,000) with smaller amounts of ir-DYN eluting either coincident with cytochrome C (apparent M_r of 11 - 12,000) or following elution of ^{125}I-β-endorphin (I-END). In the brain, ir-DYN eluted in two major peaks, one coincident with cytochrome C and a second following elution of I-END (apparent M_r of 2 - 3,000). A third, smaller peak of ir-DYN eluted before I-END (apparent M_r of 4 - 6,000). In the NI, most of the ir-DYN eluted following I-END, with a small amount of ir-DYN eluting coincident with cytochrome C. These regional differences suggest that the toad may prove to be an interesting model for studying different molecular weight peptides containing dynorphin immunoreactivity.

Supported by research grant DA-01199 and NIDA post-doctoral training grant DA-07063-05.

31.4 DEVELOPMENT OF MET-ENKEPHALIN IMMUNOREACTIVITY IN ORGANOTYPIC EXPLANTS OF FETAL MOUSE SPINAL CORD AND ATTACHED DORSAL ROOT GANGLIA. J. Groth*, A. Chalazonitis†, E.R. Simon and S.M. Crain†. Depts. of Psychiat. & Pharmacol., N.Y.U. Med. Sch., N.Y. 10016; †Dept. of Neurosci., Albert Einstein Coll. Med., Bx., N.Y. 10461.

Sensory-evoked synaptic network responses generated in dorsal-horn regions of organized fetal mouse spinal cord explants with attached dorsal root ganglia (DRGs) are selectively depressed by opiates and opioid peptides (Crain et al, Br.Res.'77,'78). Binding assays of cord-DRG explants show high levels of opiate receptors in dorsal cord tissue, and even higher in the neuritic outgrowth of isolated DRG cultures (Hiller et al, Br.Res. '78). Naloxone often enhances the dorsal-horn responses in DRG-cord explants even without prior opiate exposure, suggesting the presence of opioid inhibitory control systems. We have now attempted to study the development of enkephalinergic neurons in these cultures using radioimmunoassays (RIAs).

Cross-sections of fetal mouse cord with pairs of attached DRGs were explanted on collagen-coated coverslips and maintained for 2-4 wks in vitro (i.v.). NGF was added to the medium (Eagle's MEM, with human placental serum and chick embryo extract) during the 1st week i.v. (300 B.U./ml) to insure good survival and maturation of the DRG neurons (Crain and Peterson, Br.Res.'74). Prior to RIAs, the cultures were rinsed for 15 min in Hanks' BSS, and the cord explants were surgically separated from the DRGs with neuritic outgrowth. Groups of 8-15 cord explants or DRGs with outgrowths were pooled in glass microhomogenizers, boiled in 0.2 ml of 0.1N HCl for 15 min to inactivate endogenous peptidases, and stored at -70°C. The tissues were then thawed, homogenized, centrifuged, and the supernatant was incubated for 24 hrs at 4°C with diluted met-enkephalin (ME) antiserum and ^{3}H-ME. Bound ^{3}H-ME was counted after removal of free ME with charcoal (Yang et al, Neuropharm.'77). No ME immunoreactivity (IR) was detected in cord-DRG explants freshly dissected from fetal mice (N=6). During the 3rd wk i.v. moderate levels were detected ranging from 60-200 picrogram/cord explant (N=4), 50-140 pg/pair of DRGs with total outgrowth (N=4), and 85-160 pg/whole cord-DRG culture (N=3). During the 4th wk i.v. higher levels of ME-IR were found, ranging from 140-835 pg/cord explant and 270-800 pg/pr of DRGs with outgrowth (N=7 in each). The levels of ME-IR were much lower when cord and DRG tissues were grown as separate cultures for 3-4 wks i.v. The high levels of ME-IR in the DRGs + outgrowth of DRG-cord cultures are consonant with immunocytochemical evidence of ME in adult rat DRG neurons (Itoga et al, Acta Histochem.'79). The possible roles of opioids in DRG as well as in cord neurons requires further analysis. (Supported by NIDA research grant DA-02031 to S.M.C. and E.J.S.)

31.5 IMMUNOCYTOCHEMICAL LOCALIZATION OF OPIOID PEPTIDES IN NORMAL AND DIFFERENTIATED NEUROBLASTOMA X GLIOMA HYBRID CELLS NG108-15

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The presence of endogenous opioid peptides has been demonstrated in Neuroblastoma x Glioma hybrid NG108-15 cells (Glaser et al Eur. J. Pharm.65:319, 1980). Recently, we described immunocytochemical staining of select cells in these cultures with anti-Met^5-enkephalin (ME) and anti-Leu^5-enkephalin (LE) (Braas et al, J. Histochem. Cytochem. in press, 1981). In order to investigate the possibility that the state of differentiation may account for this variability in staining, we now compare the enkephalin stain between normal cells and cells induced to differentiate with dibutyryl cyclic AMP (dBcAMP) treatment. Cells at an initial plating density of 4000 cells/cm^2 were cultured in Leighton Tubes or 25cm^2 flasks with Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% newborn calf serum, hypoxanthine, aminopterin, and thymidine. Two days after plating, experimental cultures were treated with complete medium containing 1mM dBcAMP for one to five days. Control cells were cultured in complete medium without dBcAMP. The cells were rinsed with DMEM, fixed for 1 hour at room temperature with 2.5% gluraraldehyde, washed, and stained with 1:10,000 anti-ME or anti-LE for 48 hours using the peroxidase-anti-peroxidase immunocytochemical technique (Braas et al, J. Cell Biol. 87:173A, 1980). 25-30% of the volume fraction of control cells stain for enkephalin-like immunoreactivity, localized in rounded or neurite-like cells either singly or in small clusters. After 5 days of treatment with dBcAMP intense stain increased to 60-70% of the cellular volume fraction. The stain is found throughout the cytoplasm, elongated processes, and cytoplasmic droplets adhering to these cells. If these cultures are further treated for 60-96 hours with 10μM arabinosylcytosine (Ara-C), the proliferating undifferentiated cells are killed and a pure culture of differentiated cells is obtained which stains intensely for enkephalin. Specificity tests included solid phase immunoabsorption to remove the antibodies from the antiserum to avoid the potential binding of enkephalin to cellular binding sites. Staining was abolished when cells were incubated with antiserum preabsorbed with enkephalin conjugated to Sepharose 4B beads. No stain was present when primary antiserum was replaced by diluent buffer or 1:10,000 normal rabbit serum. These results indicate that the immunocytochemical staining for enkephalin can be correlated to the state of differentiation induced by dBcAMP treatment. Supported by ACS-IN-112 (K.M.B.), PHS-DA-02763 and NS-15595 (D.C.U.) and RCDA-HD-00935 (G.V.C.).

31.6 DYNORPHIN-LIKE IMMUNOREACTIVITY IN BOVINE ADRENAL MEDULLA.

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Dynorphin-like immunoreactivity was examined in bovine adrenal medulla and isolated chromaffin cells. Using antisera raised against dynorphin-(1-13) (DYN-(1-13)), a sensitive and highly specific radioimmunoassay was developed. Five female rabbits (R31-R35) were immunized with a mixture of DYN-(1-13): methylated bovine serum albumin, (weight ratio: 7 to 1). In this mixture DYN-(1-13) was not covalently linked to methylated bovine serum albumin but rather adsorbed on its ionized surface, thus confering the necessary protection to the peptide for immunogenicity. Several useful sera were obtained by this method, but one with good sensitivity and high titers was chosen (R31D). This antisera bound 50% of ^{3}H-DYN-(1-13) (50 Ci/mmole; NEN) at a dilution of 1:5000. Displacement from the antibody of labelled DYN with unlabelled DYN-(1-13) began at a concentration of 10^{-10} M and was complete at 10^{-7} M. Various fragments: DYN-(1-6), DYN-(1-7), DYN-(1-8), DYN-(1-9), DYN-(1-10), DYN-(6-13), and related peptides: leucine-enkephalin-DYN-(1-5), methionine-enkephalin, β-endorphin, cross-reacted with insignificant percentages, (0.01%-.0003%). Thus a nearly intact antigen was required for recognition by the antiserum. Using this antiserum, acid extracts of bovine adrenal medulla and isolated chromaffin cells were shown to contain 28.5 and 88.0 fmoles of immunoreactive (ir) dynorphin per mg, respectively. The concentration of dynorphin in the chromaffin cells (4.2 pmole/10^6 cells) corresponded to 1/1.4 of the concentration of leucine-enkephalin, as determined in this same extract by leucine-enkephalin radioimmunoassay. Various secretagogues that have been shown to release ir-leucine-enkephalin were also demonstrated to induce the release of ir-dynorphin from the chromaffin cells. These data suggest a neurohormonal function for dynorphin at this level.

31.7 LOCALIZATION OF INCREASED HIPPOCAMPAL LEUCINE ENKEPHALIN-LIKE IMMUNOREACTIVITY FOLLOWING HILAR LESIONS IN THE ADULT RAT.

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It has been reported that a variety of seizure producing treatments (intraventricular kainic acid, ECS, isoniazid) induce an increase in hippocampal enkephalin content (Hong et al, Nature, 128:231, 1980). We report here that hippocampal enkephalin-like immunoreactivity is also dramatically elevated following the placement of small hilar lesions and that in this instance, as well as following kainic acid, the increase in immunoreactivity is primarilly localized within the mossy fiber system.

Small unilateral electrolytic lesions were placed in the hilus of the dentate gyrus of adult rats under ketamine/rompin anesthesia. Two days later the level of leucine enkephalin-like immunoreactivity was assayed and found to be elevated by over 200% within the contralateral hippocampus. Phenobarbital anesthesia reduced but did not eliminate this increase.

Immunocytochemical analysis revealed similar changes in the pattern of hippocampal enkephalin-like immunoreactivity following the hilar lesion and intraventricular kainic acid injection. In both cases no new immunoreactive staining patterns (absent in untreated rats) were seen. Rather, there was a modest bilateral increase in immunoreactivity within the lateral entorhinal cortex and subiculum and a very dramatic increase in immunoreactivity within the mossy fiber system. A higher than normal proportion of the dentate gyrus granule cells were seen to be immunoreactive in the treated rats as well. The lesion induced effects appear not to be directly attributable to deafferentation because 1) the range of the increase (throughout the full septo-temporal extent of both hippocampi) dramatically exceeds the zone of deafferentation and 2) fimbrial transection, creating similar but more extensive deafferentation than the contralateral hilar lesion, does not induce a similar increase in immunoreactivity.

We therefore have a paradigm in which plasticity in the level of enkephalin-like immunoreactivity has been demonstrated within an identified synaptic system. The increase in the number of granule cells seen to be immunoreactive indicates that within a population of neurons the full capacity to express enkephalin-like immunoreactivity may only be seen under special physiological conditions. Experiments are currently in progress to determine what physiological processes induce the observed immunoreactive changes and what consequence the increase has on mossy fiber function.

31.8 ENKEPHALIN-IMMUNOREACTIVE AMACRINE CELLS IN THE RETINAS OF SOME TELEOST FISH.

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Enkephalin-immunoreactive neurons occur widely in the central and peripheral nervous systems and as amacrine cells in the retina (PNAS 76: 3010, 1979; TINS: 292, 1980). Until recently little was known about their role in neural function. Newer studies, however, revealed multiple functional opiate pathways in goldfish retina (Neurosci. Abstr. 6: 613, 1980) and suggested that opiates act upon ON-centre ganglion cells by inhibiting GABA amacrines (Djamgoz, Stell, Chin & Lam, submitted for publication). These studies led us to search for the endogenous opiate neurons in the fish retina.

Goldfish (Carassius auratus), carp (Cyprinus carpio) and catfish (Ictalurus punctatus) were obtained commercially, and the marine teleosts pile perch (Damalichthys vacca), salmon (Onchorhynchus kisutch) poacher (Agonus acipenserinus), and rockfish (Sebastes caurinus) were obtained at Friday Harbor. Fish were decapitated, pithed and enucleated, and retinas were fixed, cryosectioned and FITC-stained by indirect immunofluorescence.

In goldfish and catfish, 6 primary antisera to met^5-enkephalin and 3 to leu^5-enkephalin (dilution 1:200) gave identical, positive results, controlled by preabsorption with synthetic peptide (100 mg/ml). In goldfish, very sparsely distributed neurites were stained throughout the inner plexiform layer (IPL), predominantly in its most distal and proximal levels. In catfish we stained a single class of cells with somata in the amacrine cell layer (ACL), their thick primary neurite descending and branching to form fine fibres in the innermost IPL. In the carp and marine teleosts only one antiserum to leu^5-enkephalin (Chang A-200) was used. The staining in carp was the same as in goldfish. In all four marine species we observed stained somata in the ACL and neurites in the IPL. The stained neurites were unistratified in the distal IPL in pile perch but multistratified or diffuse in the other marine species.

These observations show that enkephalin-immunoreactive retinal neurones, probably amacrine cells, occur widely among teleosts. Our data further support the presence of opiate pathways in teleostean retinas, while indicating that those pathways may differ substantially between species.

(Supported by grants from MRC (Canada) and USPHS).

31.9 ELECTRON MICROSCOPIC IMMUNOCYTOCHEMICAL CHARACTERIZATION OF ENKEPHALINERGIC NEURONS AND SYNAPSES WITHIN THE PREOPTIC NUCLEUS OF THE GOLDFISH. R. Cumming*, T.A. Reaves, Jr. and J.N. Hayward. H. Houston Merritt Electron Microscopy Lab., Dept. Neurology and Neurobiology Program, Univ. North Carolina, Chapel Hill, N.C. 27514.

By light microscopic immunocytochemistry, using antibodies to (met^5)-enkephalin (ENK), we have previously shown that the goldfish preoptic nucleus (NPO) contains perikarya and processes of ENK neurons with anatomical and physiological connections to the neural lobe and with presumed neuroendocrine functions (PNAS 76: 6009 '79). We have sought to characterize the ultrastructure of ENK neurons in the NPO by pre-embedding electron microscopic immunocytochemistry.

Adult goldfish (Carassius auratus) were anesthetized, perfused intra-cardially with a paraformaldehyde-glutaraldehyde fixative, the brain removed and the hypothalamus vibratome-sectioned at 30 microns. We immunostained the sections with the peroxidase-anti-peroxidase technique and subsequently embedded them in Epon for ultramicrotomy.

Electron micrographs were taken throughout the rostro-caudal and dorso-ventral extent of the NPO. ENK immunoreactivity was localized in perikarya, dendrites, axons and axon terminals. We saw low level ENK immunoreactivity in granular (130-170 nm) and agranular form in perikarya and dendrites, while intense reaction product was observed in unmyelinated axons and axon terminals. ENK-labeled axon terminals contained predominantly small clear vesicles (SCVs, 35-45 nm) with scattered large dense-core vesicle (LDVs, 60-85 nm). Some ENK immunoreactive axon terminals possessed mainly LDVs. The LDVs showed labeling of vesicle membrane and core while reaction product was only associated with the periphery of the SCVs. The ENK-labeled vesicles were uniformly distributed throughout the terminal boutons, with occasional concentration of SCVs at synaptic contacts. ENK immunoreactivity was found at discrete regions on the neuronal membrane including points of synaptic contact and rarely at post-synaptic specializations. The external mitochondrial membrane also showed ENK-labeling. ENK-positive axon terminals formed single and multiple contacts with unlabeled dendritic shafts and spines. Axo-somatic ENK boutons were observed on small ($<10\ \mu m$) neurons with large nuclei and minimal cytoplasm located in parvocellular regions of the NPO.

We conclude that in the goldfish NPO the axon terminals of enkephalin-containing neurons form axo-dendritic and axo-somatic synapses on other neurons. These data suggest a neurotransmitter as well as a neuroendocrine function for opioid peptides in the teleost preoptic nucleus. (Supported by NIH Grant NS-13411 and a Neurobiology Fellowship to R.C.)

31.10 DYNORPHIN IMMUNOREACTIVITY IN MAMMALIAN SPINAL CORD AND DORSAL ROOT GANGLION. B. M. Cox, L. J. Botticelli and A. Goldstein. Addiction Res. Fndn. & Dept. Pharmacol., Stanford Univ., Palo Alto, CA 94304.

Dynorphin, a remarkably potent opioid peptide that contains leucine enkephalin as its amino terminus, was isolated recently from porcine pituitary extracts. The first thirteen amino acid residues are YGGFLRRIRPKLK. Dynorphin-(1-13) is as potent as natural dynorphin in the guinea pig ileum bioassay.

The regional distribution pattern of immunoreactive (ir-) dynorphin is different from that of enkephalin or any other known opioid peptide. The concentration is greatest in anterior and posterior aspects of the hypothalamus, with lesser amounts found in midbrain tegmentum. Lower levels are found in striatum, hippocampus, neocortex and cerebellum. In pituitary, ir-dynorphin is found predominantly in pars nervosa (Goldstein & Ghazarossian, 1980. Proc. Natl. Acad. Sci., 77: 6207).

We now report on the distribution and characterization of ir-dynorphin in the mammalian spinal cord and dorsal root ganglion (DRG). Concentrations are highest in the dorsal aspect of the spinal cord of both rabbit and rat (approximately 20, 5 and 1.5 pmol/g in dorsal cord, ventral cord and DRG, respectively, in both species). Levels of ir-dynorphin are relatively uniform over examined segments (C2-4 through S1-3) of rabbit spinal cord and DRG. Multiple unilateral or bilateral dorsal rhizotomy (C5-T1) in the rat did not affect levels of ir-dynorphin in spinal cord. As reported previously (Goldstein & Ghazarossian, 1980), mid-thoracic spinal transection was without effect. Gel permeation column chromatography (Sephadex G-50, 100 x 1.5 cm; 0.1 M acetic acid, 0.15 M NaCl, 0.1% Triton X-100) of rabbit dorsal and ventral spinal cord and DRG extracts revealed three immunoreactive components.

These observations suggest that ir-dynorphin is distributed in a regionally specific fashion in spinal cord and DRG. The neuropeptide appears, most probably, to be contained in short axoned neurons. We surmise this potent opioid peptide may participate in the processing of sensory information in spinal cord and DRG.

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31.11 SCANNING ELECTRON MICROSCOPY OF RAT THIRD VENTRICLE AFTER ACUTE ENDORPHIN INJECTION TO LATERAL VENTRICLE: MACROPHAGE-LIKE CELL RESPONSE. L.C. Saland, E. Ortiz* and A.T. Munger*. Department of Anatomy, University of New Mexico School of Medicine, Albuquerque, NM 87131.

The ventricular system of the brain is a common site for introduction of opiate peptide molecules (endorphins) in many studies of analgesic and behavioral effects in animals and, more recently, via lumbar puncture in man. Acute effects of endorphins may in part be a function of local cellular responses, including ependymal absorption or transport. We have examined the surface of the third ventricle superior to the hypothalamic median eminence (HME) after single acute injection of synthetic ovine β-endorphin, human α-endorphin (courtesy of Dr. N. Ling) or met-enkephalin into the lateral cerebral ventricle (LCV). Young adult male Sprague-Dawley rats received 20ug (in 15-20ul saline) β- or α-endorphin or 100ug met-enkephalin (in 15-20ul saline) to the LCV while awake into previously implanted stainless steel cannulas. Controls received 15-20ul saline alone. 45-60 minutes after injection, animals were lightly anesthetized with ether, then perfused through the heart with saline followed by 1% paraformaldehyde-2.5% glutaraldehyde in 0.075M cacodylate/HCl buffer, pH 7.2, at room temperature. Brain tissue (HME and adjacent hypothalamus) was prepared routinely for scanning EM, and examined with an ETEC autoscan. The ependyma of most control rats exhibited smooth surfaces above the HME, although a few showed occasional neuron-like or macrophage-like supraependymal cells (SEC). β-endorphin-treated rat HME contained numerous free macrophage-like SEC similar to those found in studies of animals with intracerebral infections, or occasional neuron-like cells. Animals treated with α-endorphin or met-enkephalin exhibited occasional macrophages, but appeared instead to have more prominent neuron-like supraependymal cell clusters similar to those observed in rats after peripheral p-chloroamphetamine injections (Saland and Munger, 1981, Brain Res. Bull., in press). Observations suggest that specific opiate peptides as β-endorphin introduced in the cerebrospinal fluid may "attract" macrophages from intracerebral or blood-borne sources. The occasional appearance of SE "neurons" may reflect a cellular response to changes in composition of cerebrospinal fluid. Supported by NIDA grant DA-02269 and NIH RR-08139.

31.12 ENKEPHALINERGIC MECHANISMS IN THE COCHLEA. D.W. Hoffman,* R.A. Altschuler, and J. Fex. LNO, NINCDS, NIH, Bethesda, MD 20205.

Efferent fibers of the olivocochlear bundle terminate under both inner and outer hair cells of the organ of Corti. Although their function is not clear, these efferents may participate in hair cell function, ototoxic mechanisms, and attentional processes. While previous research has supported a cholinergic mechanism at the terminals of these fibers at the bases of hair cells, this laboratory has recently shown the presence of met-enkephalin-like immunoreactivity in the efferent fibers and terminals in the cochlea (Fex and Altschuler, PNAS, Feb., 1981). Further evidence by high performance liquid chromatography (HPLC) and radioimmunoassay (RIA) for the presence of immunoreactive substances in cochlear homogenates and HPLC fractions has also recently been presented (Hoffman et. al., Trans. Am. Soc. Neurochem., March, 1981). In the present study we have further defined the enkephalin-like immunoreactivity in the cochlea and have accumulated evidence supporting receptor mediation of enkephalinergic function in this organ.

Receptor binding was assayed in cochlear homogenates by a filter technique using GF/B filters. ^{3}H-naloxone and ^{3}H-etorphine were each used as ligands, and both were found to bind stereospecifically to cochlear homogenates, as defined by the difference between binding in the presence of 10 µM levorphanol and dextrorphan. The binding of these ligands appears to saturate. The effect of Na^+ on binding of agonists and antagonists was not determined after it became apparent that NaCl (100 mM) enhanced binding of ^{3}H-naloxone to GF/B filters in the absence of tissue.

Identification of immunoreactive substances in the cochlea by HPLC-RIA has been extended by adoption of rapid freezing of whole cochlea in liquid Freon, followed by lyophilization. The freeze-dried organ may then be dry-dissected into constituent tissues. Antisera raised in our laboratory have been carefully characterized as to titer and cross-reactivity, and identification of peptides of interest may be made both by retention time in HPLC and by reactivities to several antibodies of different specificities.

31.13 LIGHT AND ELECTRON MICROSCOPIC FEATURES OF IMMUNOREACTIVE LEU-ENKEPHALIN IN THE MONKEY HYPOTHALAMUS. I:PARAVENTRICULAR NUCLEUS.

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Neurons and terminals containing immunoreactive (IR) leu-enkephalin (leu-enk) were identified in the monkey (M. cynamologous) hypothalamus. Vibratome sections were incubated in leu-enk antiserum (BioFlex) and then processed by the immunoperoxidase technique. Light microscopy revealed labeled cell bodies (with colchicine pre-treatment) in numerous regions of the hypothalamus. Many leu-enk somata (15-30 μm) were present in the dorsal and posterior aspects of the paraventricular nucleus (PVN) and more laterally throughout the perifornical region ventral to the anterior commissure. Positive neurons (15 μm) were occasionally found in the vascular, medial PVN. Fewer immunostained perikarya (10-15 μm) were present in the lateral hypothalamic, periventricular, ventromedial and arcuate regions. IR leu-enk fibers were most numerous in the lateral hypothalamus and were also seen in the areas of cell body labeling, the supraoptic nucleus, and the external layer of the median eminence.

Electron microscopic examination of the dorsal aspect of the PVN revealed that leu-enk somata contained a deeply enfolded nucleus, well-developed endoplasmic reticulum and numerous large granular vesicles (LGV; 80-200 nm) which were heavily labeled with peroxidase reaction product. Leu-enk neurons were contacted by small boutons (0.5-1.0 μm) which contained clear round or pleomorphic vesicles and larger axon terminals (2 μm) with clear pleomorphic vesicles and LGV. Immunostained leu-enk dendrites had spines and contained clear vesicles and LGV filled with reaction product. The dendritic shafts and spines were postsynaptic to the same classes of boutons that contacted the cell body. IR leu-enk axons were unmyelinated, up to 0.3 μm in diameter, present in bundles of unstained axons of similar caliber, and gave rise to boutons (1.0 μm in size) that contained pleomorphic, clear vesicles (40 nm) and many LGV (80-200 nm). Reaction product deposited within the LGV and around the membranes of the smaller vesicles. Labeled boutons synapsed with unlabeled small dendrites, dendritic spines, the shafts of primary dendrites, and somata which were 15-20 μm in size. Most of the synapses appeared to be symmetric. The present results in monkey (1) show a distribution of leu-enk at the light microscopic level which is similar to that found in rat hypothalamus and (2) provide direct evidence at the ultrastructural level to support recent studies in vitro that enkephalins can markedly inhibit PVN neurons.

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32.1 THE ONTOGENY OF CHOLECYSTOKININ IMMUNOREACTIVITY IN VERTEBRATE BRAIN, Steven A. Goldman* and Bruce S. Schneider* (SPON: F. Nottebohm). Rockefeller University, New York City, NY 10021.

Cholecystokinin (CCK) is discretely localized in neurons and terminals of vertebrate brain, and may have a neurotransmittive function. We have previously demonstrated, by the use of radioimmunoassay in combination with regional brain dissection, that the development of CCK in rat brain occurs during the first postnatal month (Schneider et al, J. Clin. Invest. 64: 1348, 1979). To determine whether the appearance of CCK in discrete brain regions is associated with any particular aspects of brain histogenesis, we examined in detail the development of CCK immunoreactivity in the brains of several species. Two precocial species (guinea pig and chicken) were found to attain adult CCK concentrations in all areas (telencephalon, diencephalon, mesencephalon/tectum, and hindbrain) before the perinatal period. In contrast, the altricial zebra finch, like the altricial rat, manifested adult brain CCK concentrations only after several weeks of postnatal development. Each species' final brain CCK level was determined, however, not by its ontogeny, but by its phylogeny: both mammals showed high adult telencephalic CCK concentrations (rat, 550 ng CCK8equiv./g wet weight, guinea pig, 220 ng/g), while both birds showed lower adult telecephalic levels (chicken, 50 ng/g, finch, 60 ng/g).

Within any given species, the time course of development of brain CCK was essentially the same across all brain regions, despite differences in the adult CCK concentration from region to region. However, the patterns of a given species' CCK development were not always monotonically rising curves. Chick brain development in particular is marked by a perinatal rise in CCK concentration to levels far exceeding those of adult chick brain. Within the first month after hatching, CCK concentrations then declined to adult levels. We believe this effect to be due to a neurogenic, perinatal production of a relatively constant brain CCK store, followed by a subsequent dilution of this store by gliogenic and myelinogenic increases in brain weight. Correlation of our comparative ontogenetic data with known patterns of brain histogenesis demonstrate that CCK development comes after most known periods of neuronal proliferation, migration and axonal outgrowth, and occurs during or soon after local synaptogenesis. Sephadex G50 gel filtration disclosed no differences across the species or among different brain areas in the molecular forms of CCK. CCK-8 predominated with minor CCK-33 and void volume (pro-CCK) peaks. Finally, it was determined that duodenal CCK (largely CCK-33) appears much earlier than brain CCK in all species examined, indicating that the gut and brain CCK systems develop independently of each other.

32.2 THE DEVELOPMENT AND DISTRIBUTION OF SOMATOSTATIN-LIKE IMMUNOREACTIVITY IN THE VISUAL CORTEX OF THE RAT. J.K. McDonald, J.G. Parnavelas, N.C. Brecha and J.I. Koenig*. Depts. of Cell Biology and Physiology, The Univ. Tx. Hlth. Sci. Ctr., Dallas, TX 75235 and Dept. of Neurobiology, S.U.N.Y., Stony Brook, NY 11794.

Using immunohistochemistry, we have examined the development of somatostatin-like immunoreactivity (SRIF-IMR) in the rat visual cortex, an area whose morphological development has been studied extensively. Sprague-Dawley albino rats of both sexes at 3,5,7,8,10, and 14 days of age as well as adult animals were used in this study. All animals were perfused with fixative containing 4% paraformaldehyde, 0.1M D,L-lysine and 0.01M Na periodate in 0.1M phosphate buffer. Coronal sections were cut at 30 µm and processed for fluorescence and PAP immunohistochemistry using conventional techniques. SRIF antiserum was raised in rabbits by J. Koenig and preabsorption with 10^{-4}-10^{-6}M synthetic SRIF (Peninsula) revealed no cortical staining.

In the adult, SRIF-IMR cells were observed in layers II-VI but were concentrated primarily in layers II & III and V. Layer I appeared to contain only labeled fibers. Labeled cells were almost exclusively non-pyramidal in morphology although a few pyramids were observed. The non-pyramidal cells were of the multipolar and bitufted varieties and their size and distribution in the cortex were similar to that observed in Golgi preparations (Parnavelas et al., 1977; Feldman and Peters, 1978). Particularly notable were the presence of large multipolar neurons in layer V and cells with horizontally oriented dendrites in layer VI.

At postnatal days 3 and 5, SRIF-IMR was confined to immature bipolar or multipolar neurons in the lower cortical plate. No positive fibers or cell bodies were observed in the upper cortical plate. At day 7, a few positive fibers were observed in layer I, but immunoreactive cell bodies were still present only in the lower cortical layers. At this and subsequent stages of development, non-pyramidal neurons with horizontally oriented dendrites were seen adjacent to the white matter. At day 8, SRIF-IMR cells appeared more differentiated and, for the first time, a few labeled cells were seen in layers II & III. At postnatal day 10, there was an increase in SRIF-IMR fibers in layer I as well as a greater number of non-pyramidal cells in layers II & III although the majority of immunoreactive cells remained in the lower cortical layers. A striking increase in the frequency of SRIF-IMR cells in the upper cortical layers was observed in the latter part of the second postnatal week, and at day 14 the morphology and distribution of the SRIF-IMR neurons were similar to that of adult animals.

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32.3 SOMATOSTATIN (SOM)*, SUBSTANCE P (SP)*, ENKEPHALIN (ENK)*, AND 5 HYDROXYTRYPTAMINE (5HT)* IMMUNOREACTIVE ELEMENTS IN THE NEONATAL RAT SPINAL CORD, R.H. Ho, Department of Anatomy, The Ohio State University, College of Medicine, Columbus, Ohio 43210

The present account describes the distribution of SOM, SP, ENK and 5HT elements in the spinal cord of neonatal (Day 0-1 neonates) Sprague Dawley rats. Animals were perfusion fixed with Zamboni's solution and cryostat sections from representative spinal cord levels were processed by Sternberger's PAP technique. A moderate density of SOM fibers is present in the lateral funiculus, whereas only a small number are located in the dorsal and the ventral funiculi. Neuronal elements containing SOM are most numerous within the superficial laminae (presumptive laminae I, II and possibly III) of the dorsal horn. The outer portion of this region contains SOM varicosities whereas the inner portion also contains immunostained neuronal elements that resemble small perikarya. In addition, there is a widespread distribution of SOM immunoreactivity ventral to the superficial laminae. SOM immunoreactivity is localized within the cytoplasm that either partially or completely surrounds negatively stained nuclei. Thus, we interpret these neuronal elements to be SOM containing cell bodies. The perikarya are of various shapes and sizes and widely dispersed throughout the gray matter ventral to the superficial laminae. The cell density is slightly increased in regions lateral to the central canal and the ventral horn. The distribution of SP elements is generally similar to that described for the adult rat spinal cord. The major difference appears to be the presence of a moderate density of SP fibers in the dorsal funiculus of neonatal cords. The densest distribution of ENK is in the lateral funiculus, although some is present in the dorsal and ventral funiculi. ENK varicosities are present dorsal to the central canal and at thoracic levels, they are present in the presumptive intermediolateral cell column. In other gray regions, ENK varicosities are extremely sparce. 5HT elements are present in all areas of the spinal cord previously described in the adult animal. Control sera consisting of the various antibodies pretreated with the corresponding antigens failed to reveal any of the aforementioned immunoreactivities on adjacent and/or semiadjacent sections. In summary, the distribution of 5HT and SP are quite similar to that described in the adult. However, the localization of SOM and ENK are markedly different when compared to the adult. The functional significance of the abundant SOM cell bodies present during development remains to be determined. (We thank Dr. Robert Elde for the 5HT antibody. Supported by NIH NS-10165 and the Department of Anatomy, The Ohio State University).

* A substance's immunoreactivity is referred to by its name.

32.4 IMMUNOCYTOCHEMICAL LOCALIZATION OF AVIAN PANCREATIC POLYPEPTIDE-LIKE IMMUNOREACTIVITY IN THE HYPOTHALAMUS OF THE RAT. N. Brecha, J.P. Card and R.Y. Moore, Depts. of Neurology and Neurobiology and Behavior, SUNY at Stony Brook, Stony Brook, New York, 11794.

The distribution of avian pancreatic polypeptide-like (APP) immunoreactivity was studied in the rat hypothalamus using the indirect immunoperoxidase method. APP antisera was generously provided by J. Kimmel and cytochemical tests of antibody specificity included preabsorption of antisera with the APP antigen and by substitution of normal serum for the antibody. Some animals received an injection of colchicine into the lateral ventricle two days prior to sacrifice. Perikarya. Immunoreactive perikarya are present in the anterior and tuberal regions of the medial hypothalamus and in the lateral hypothalamic area. None are evident in the mammillary complex or posterior hypothalamic area. The largest numbers of immunoreactive perikarya are observed in selected nuclei of the mediobasal hypothalamus, particularly in the retrochiasmatic area and in the medial portion of the arcuate nucleus. A moderate number of immunoreactive neurons are evident in the ventral anterior hypothalamic area and scattered neurons are present in the medial preoptic area, periventricular nucleus, paraventricular nucleus, dorsal and ventral tuberal areas, dorsomedial nucleus, dorsal hypothalamic nucleus and in the zone surrounding the ventromedial nucleus. In the lateral hypothalamic area immunoreactive neurons are present along its entire rostro-caudal extent with the largest number evident from the level of the suprachiasmatic nucleus to the median eminence. Fibers. Immunoreactive axons are present in the organum vasculosum lamina terminalis, medial and lateral preoptic areas, the anterior and dorsal hypothalamic areas, the dorsomedial nucleus, and in the lateral hypothalamic area. Particularly dense plexuses of axons are demonstrated in the periventricular nucleus, the ventrolateral component of the suprachiasmatic nucleus, retrochiasmatic area, arcuate nucleus, and perifornical area. Scattered fibers are present in the subventricular zone of the median eminence. All of the magnocellular nuclei, supraoptic, paraventricular, circularis and tubermammillary, contain moderate numbers of immunoreactive axons. Few fibers are evident in the ventromedial nucleus or mammillary complex. These observations provide morphological evidence for the presence of systems of APP-immunoreactive neurons with a distribution differing from that of other known peptidergic systems in the rat hypothalamus. (Supported by USPHS Grant NS-16304 to RYM and by USPHS Postdoctoral Fellowship NS-06247 to JPC).

32.5 LOCALIZATION OF VASOACTIVE INTESTINAL POLYPEPTIDE-LIKE IMMUNOREACTIVITY IN IDENTIFIED NEURONS IN THE VISUAL CORTEX OF THE DEVELOPING AND MATURE RAT. J.G. Parnavelas, J.K. McDonald, C.-S. Lin and N.C. Brecha. Dept. Cell Biology, The Univ. Tx. Hlth. Sci. Ctr., Dallas, TX 75235, and Dept. Neurobiology, State Univ. of N.Y., Stony Brook, NY 11794.

Using immunohistochemistry, vasoactive intestinal polypeptide (VIP)-like immunoreactivity was localized in the developing and adult visual cortex of the rat. Sprague-Dawley albino rats of various postnatal ages as well as adult animals were perfused with 4% paraformaldehyde, 0.1M D,L-lysine-HCl and 0.01M Na periodate in 0.1M phosphate buffer. Frozen sections were cut at 30 µm in the coronal plane, incubated in VIP antiserum and processed according to conventional immunohistochemical techniques. Preabsorption of the antiserum with 10^{-5}-10^{-7}M synthetic VIP (Boehringer-Mannheim) revealed no cortical staining.

Neurons which displayed VIP-like immunoreactivity comprised approximately 3% of the neuronal population in the visual cortex (area 17) of the adult rat. Their perikarya were located in layers II-VI but tended to be concentrated in layers II & III. All labeled neurons had the morphological characteristics of cortical non-pyramidal cells (Parnavelas et al., 1977) with the majority being of the bipolar variety as described in Golgi preparations (Feldman and Peters, 1978). Some multipolar non-pyramidal neurons were also present. Intraventricular injections of colchicine (50 µg colchicine in 10 µl saline) intensified the staining but did not reveal any cell types which were not observed in the visual cortex of untreated animals.

VIP-like immunoreactivity appeared to develop for the most part in postnatal life. At day 4, only a few immunoreactive cell bodies could be identified in the lower layers (V and VI) of the cortex. At day 6, immunoreactive cell perikarya were still present predominantly in layers V and VI although a very small number of neurons were seen in the upper cortical layers. The distribution of labeled cell bodies appeared quite different at the beginning of the second postnatal week when the majority were concentrated in the upper cortical layers. Although the distribution of immunoreactive cells at this time resembled that of adult animals, their morphology displayed immature features. It appears that the developmental changes observed in VIP-containing neurons in the visual cortex are reflected in the pattern of the morphological differentiation of these neurons.

We are grateful to Dr. John Walsh for providing the antiserum. The work was supported by NIH grant EY02964.

32.6 DISTRIBUTION OF SOMATOSTATIN IMMUNOREACTIVE NEURONS IN THE HYPOTHALAMUS OF THE RAT. R.Y. Moore, N. Brecha, J.P. Card and T. Yamada. Depts. of Neurology and Neurobiology, SUNY at Stony Brook, Stony Brook, NY 11794, and Dept. of Medicine, UCLA, Los Angeles, Ca.

The distribution of somatostatin-like immunoreactivity in neurons of the rat hypothalamus was studied using the indirect peroxidase-antiperoxidase method. Specificity was determined by pre-adsorption of antiserum with somatostatin. Perikarya. Immunoreactive neuronal cell bodies are found in two principal locations. The first is in the periventricular region, particularly in the anterior hypothalamus. Coextensive with this group cell bodies are present in the suprachiasmatic nucleus. The second is comprised of scattered immunoreactive perikarya present in the caudal anterior hypothalamic area, retrochiasmatic area, ventral tuberal area, perifornical area, caudal periventricular nucleus, ventral premammillary nucleus and in the tuberal area surrounding the ventromedial nucleus with scattered cell bodies present in that nucleus. No positive cells are present in the posterior hypothalamus or mammillary complex. Terminals. In the anterior hypothalamus scattered axons are evident in the organum vasculosum lamina terminalis. Dense axonal plexuses are present in the medial preoptic area, anterior hypothalamic area, and the rostral portion of the suprachiasmatic nucleus. Similarly dense plexuses are present more caudally in the retrochiasmatic area, ventromedial nucleus, arcuate nucleus and the ventral premammillary nucleus. The lateral hypothalamic area shows a scattered to moderately dense axonal plexus. No immunoreactive axons are evident in the supraoptic or paraventricular nuclei. Caudally, no immunoreactive axons are evident in the mammillary complex. Scattered axons are evident in the posterior hypothalamic area whereas the tuberomammillary and supramammillary nuclei show a more dense innervation. The pattern of immunoreactive somatostatin neurons differs from that of other peptidergic neuron systems in rat hypothalamus. (Supported by USPHS Grant NS-16304 to RYM and USPHS Postdoctoral Fellowship NS-06247 to JPC).

32.7 HIPPOCAMPAL NEUROPEPTIDES: DISTRIBUTION ACROSS SPECIES. G.E. Hoffman and S. Wray*. Dept. of Anat., Univ. of Rochester Sch. of Med. & Den., Rochester, NY 14642.

Recent evidence suggests an increasing role for neuropeptides in limbic system function. In certain instances the patterns of localization of the peptides has substantiated their functional role. Most investigations of neuropeptide localization in the hippocampus have been restricted to the rat or one of the other rodent species. However, striking differences in both hippocampal anatomy and function among species have been noted. Thus, a comparative investigation of the organization of somatostatin (SS), vasoactive intestinal polypeptide (VIP), LHRH and substance P (SP) in mouse, rat, rabbit and cat was conducted. The animals were perfused with neutral picric acid formalin and sectioned at 50 or 75 um on a vibrating microtome. The sections were processed using the unlabeled antibody enzyme method. SS neurons in the mouse were found mainly in the stratum oriens of the hippocampus proper and in the hilus of the dentate gyrus. The former cells gave rise to axons that coursed through many of the layers of the hippocampus to the stratum lacunosum. In general this pattern was consistent throughout the species examined. Likewise, the distribution of VIP neurons was consistent in the rat and mouse. Some of these neurons sent fine axons to the pyramidal layer much like basket cells. A few LHRH neurons were consistently observed in the hippocampus of the mouse but not the other species. Their random distribution suggested that these were stray cells of the septal LHRH populations. Nonetheless, in the mouse and in all the other species examined, sparse LHRH axons were observed scattered throughout the hippocampus. SP axons were rarely observed in the hippocampus of the rat and mouse. However, in the rabbit a moderate density of such fibers concentrated adjacent to the cellular layers of the hippocampus and dentate gyrus was observed. In the cat, these fibers formed a dense plexus. These results suggest that peptides such as substance P may underlie species differences in hippocampal function and further support a role for a variety of neuropeptides in limbic function. Supported by NS13725 and RCDA NS00321.

32.8 PRODUCTION OF MONOCLONAL ANTIBODIES AGAINST VASOACTIVE INTESTINAL POLYPEPTIDE. Illana Gozes, Diane Barry*, Robert Benoit*, Fu-Tong Liu*†, David H. Katz*†, Robert J. Milner*, and Floyd E. Bloom. The Salk Institute and Scripps Clinic and Research Foundation†, San Diego, CA 92037.

Vasoactive intestinal polypeptide (VIP) was originally isolated from the intestine but has been subsequently shown to be widely distributed throughout the body, with a high concentration in the hypothalamus and cerebral cortex. In addition, this 28 amino acid polypeptide has been shown to be localized in and released from nerve terminals in the brain, suggesting that VIP may function as a neurotransmitter.

In order to integrate knowledge on the synthesis, localization, and the structure-function relationships of VIP we have prepared monoclonal antibodies against VIP. (Balb/c x A/J) F1 mice were injected intraperitoneally and subcutaneously with synthetic porcine VIP electrostatically complexed with methylated bovine serum albumin and emulsified in complete Freund's adjuvant. Mice were boosted intraperitoneally at 2 week intervals with the complexed antigen in aluminum hydroxide. The mouse with the highest antibody titer against VIP was boosted with antigen intravenously and intraperitoneally and sacrified 4 days later. Immune spleen cells were fused with SP2/0 tumor cells in the presence of polyethylene glycol and plated in 2500 wells. At 3 weeks 500 wells contained growing hybrid cells and of these 130 showed immunoreactivity against VIP as measured by a radioimmunoassay using ^{125}I labelled VIP. 32 hybridomas were stable after 8 weeks in culture.

We are presently using these antibodies to identify putative precursors for VIP. Preliminary results suggest that some of the anti-VIP positive hybridomas secrete antibodies that also recognize high molecular weight proteins which may represent VIP precursors. Fractionation of rat brain proteins by gel exclusion chromatography showed the presence of high molecular weight material having VIP-immunoreactivity detectable by radioimmunoassay. This finding has been corroborated by experiments using two conventional rabbit antisera against VIP. We are also using these antibodies to identify presumptive VIP precursors by in vitro translation of brain mRNA and molecular cloning experiments.

These experiments were supported by grants from the Sun Oil Company, Mobil Foundation, Inc., McNeil Pharmaceuticals, and USPHS grant AI-13874.

32.9 CEREBRAL CORTICAL GLYCOGEN METABOLISM REGULATION BY VASOACTIVE INTESTINAL POLYPEPTIDE (VIP).

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The regulatory role of putative neurotransmitters on CNS energy metabolism has attracted increasing attention. To study such synaptic functions, we used a recently described, highly sensitive method (1), by which the effects of putative neurotransmitters on ^{3}H-glycogen levels newly synthesized from ^{3}H-glucose can be monitored. Mouse cortical slices (250 µm thick,) resuspended in a modified Krebs-Ringer bicarbonate buffer and preincubated for 15 min at 37°, were then incubated in the presence of ^{3}H-glucose (0.5Ci/mmol) for 30 min. At the end of that period, the synthesis of ^{3}H-glycogen reached a plateau, and drugs or vehicle were added for 15 min. The incubation was ended by rapid centrifugation. After sonication and recentrifugation, the ^{3}H-glycogen in the supernatant was separated from ^{3}H-glucose and other contaminants by ethanol precipitation on filter paper. Using this separation technique, the contamination by ^{3}H-glucose is < 0.5%. Filters were then counted in a liquid scintillation counter. Enzymatic hydrolysis of newly synthesized ^{3}H-glycogen was induced by VIP in a concentration-dependent manner. The EC_{50} for this effect was 30 nM. Consistent with a previous report (1), norepinephrine (NE) displayed a similar action, with an EC_{50} of 300 nM. The VIP effect was not mediated by a release of NE, since it was not blocked by noradrenergic antagonists and was still present in mice in which an 85% depletion of NE was induced by intracisternal 6-OHDA injections (2x25 ug). Interestingly, preliminary results show a decrease in ^{3}H-glycogen levels in these lesioned mice. Another cortical putative neurotransmitter, somatostatin, had no glycogenolytic effect; the possible action of other neuropeptides present in cerebral cortex on ^{3}H-glycogen metabolism is currently being investigated. This effect of VIP on cortical carbohydrate metabolism should increase the energy substrates available at the cellular level. Given the radial pattern of arborization of the intracortical VIP neuron, (see Morrison, J.H., et al., this volume) and the tangential intracortical trajectory of the noradrenergic fibers, a role of VIP in the regulation of energy metabolism within individual columnar modules in cerebral cortex may be envisaged, while that of NE may span adjacent columns.

(1) T. T. Quach, et al., (1978), J. Neurochem., 30, 1335-1341.

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32.10 THE IMMUNOHISTOCHEMICAL CHARACTERIZATION OF SOMATOSTATIN (SS) AND VASOACTIVE INTESTINAL POLYPEPTIDE (VIP) NEURONS WITHIN THE CEREBRAL CORTEX.

J.H. Morrison, P.J. Magistretti, R. Benoit, and F.E. Bloom. A.V. Davis Center and Laboratory of Neuroendocrinology, The Salk Inst., San Diego, CA 92138.

Several laboratories have demonstrated biochemically that SS and VIP are present in the cerebral cortex. We have utilized specific antibodies to each of these peptides to visualize the VIP and SS-containing neurons within the cerebral cortex.

SS-positive cells are present throughout the cerebral cortex and hippocampus (principally CA1) and are particularly numerous in the pyriform cortex. In the neocortex, they are distributed in two major laminar bands, a supragranular band in layers II and III and an infragranular band in layers VI and deep V. Labelled cells are relatively rare in layers IV and superficial V. The cell bodies are variable in size and shape, but tend to be multi-polar and 12-20 µm in diameter. The dendrites are relatively short, and rarely extend more than 50 µm from the cell body in the plane of section (40 µm thick). Terminals are present in all layers, but are particularly dense in layer I.

VIP-containing cells are most numerous in layers II and III, but are present also in layers IV, V and VI. A fine plexus of terminal boutons is present in layers II-V. The cell bodies are generally ovoid and measure approximately 8-10 µm across and 15 µm in the radial axis. The VIP-positive neurons are radlally bi-polar, with a few major dendrites emanating from the superficial and deep poles of the soma. These major dendrites are generally beaded and can be followed for extremely long distances (up to 1 mm in a 40 µm thick section) and invariably are oriented perpendicular to the pial surface. The processes do not branch profusely and rarely extend for any appreciable distance in the tangential plane. Thus, an individual VIP-positive neuron may span the entire vertical thickness of the cerebral cortex, receiving synaptic input in all cortical laminae, and impinging upon other cortical elements in a narrowly defined, radial column. Given the effect of VIP on cortical carbohydrate metabolism (see Magistretti, et al., this volume), the VIP neuron may be an important component of the cortical column, modulating the availability of energy substrates as neuronal activity increases locally. The radial VIP system stands in striking contrast to the tangential noradrenergic system, which may also be involved in metabolic regulation, but has the capacity to intersect a longitudinal array of columns across a vast expanse of neocortex. Supported by USPHS Grants AA 07273 and AA 03504. PJM supported by Swiss NSF Fellowship.

32.11 IMMUNOCYTOCHEMICAL CHARACTERIZATION OF VENTRAL LATERAL GENICULATE NUCLEUS EFFERENTS TO THE RAT SUPRACHIASMATIC NUCLEUS.

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Autoradiographic analysis of the efferent projections of the rat ventral lateral geniculate nucleus (vLGN) have demonstrated that the ventral aspect of the suprachiasmatic nuclei (SCN) receive a bilateral innervation which is most dense in the ipsilateral SCN (Swanson et al., 1974; Ribak and Peters, 1975). Moore et al. (1979) have demonstrated that this projection arises from a distinct lamina of cells at the dorsal limit of the vLGN. The present study demonstrates that the vLGN-SCN projection can be defined immunocytochemically following staining with antibody directed against avian pancreatic polypeptide (APP; generously provided by J. Kimmel). APP immunoreactivity was localized in brains of adult male and female rats using the indirect peroxidase antiperoxidase technique. Histochemical tests of antibody specificity included absorption of the antiserum with APP antigen and substitution of normal serum for APP antiserum. Within the rat SCN, APP immunoreactivity is restricted to varicose axons in the ventral and lateral aspects of the nucleus; the dorsomedial segment of the nucleus is totally devoid of immunoreactivity and immunoreactive perikarya are not present in any portion of the SCN. The immunoreactive axons in the ventrolateral component of the nucleus form an extensive plexus which is distributed in a pattern closely corresponding to the distribution of retinal and vLGN afferents to the SCN. Because of this similarity, both the retina and vLGN were examined as possible sites of origin for APP axons in the SCN. No immunoreactive perikarya are observed in the retina following immunoperoxidase staining for APP and neither unilateral nor bilateral enucleation causes an observable alteration in the pattern of APP axon distribution within the SCN indicating that the fiber plexus is not of retinal origin. In contrast, APP immunoreactive neurons are present in the same area of the vLGN in which retrogradely filled neurons are found after iontophoretic injection of HRP into the SCN. In addition, bilateral electrolytic lesions of the vLGN result in a total loss of immunoreactive axonal staining in both SCN while unilateral vLGN lesions cause a loss of immunoreactive fibers that is approximately twice as great in the ipsilateral SCN. These observations provide further information on the histochemical organization of the rat SCN and demonstrate that the vLGN projection to the SCN is chemically distinct from other afferents to the SCN. (Supported by USPHS Grant NS-16304 to RYM and by USPHS Postdoctoral Fellowship NS-06247 to JPC.

32.12 MOTILIN RELATED IMMUNOREACTIVITY IN PURKINJE CELLS OF THE MAMMALIAN CEREBELLUM.

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Motilin, a 2,700 dalton peptide initially isolated from porcine gut, has recently been demonstrated in the brain and anterior pituitary of several mammalian species, including rat, dog, monkey and man, by radioimmunoassay and immunocytochemistry. Reports of relatively high concentrations of this peptide in the cerebellum were of special interest since other peptides are known to be present in negligible concentrations in this part of the brain. We have localized motilin in the cerebellum using a rabbit antiserum generated against a synthetic porcine motilin-thyroglobulin conjugate. Immunoperoxidase labeling of 6 µm deparaffinized sections of rat, monkey and human cerebelli with the antiserum at a dilution of 1:1000 revealed intense "Golgi-like" reactivity in the cell bodies and processes of many, but not all, Purkinje neurons. Dendritic trees could be traced in their entirety, including terminal spines. Axons were traced through the granular layer and white matter to numerous terminals innervating cell bodies and proximal dendrites of the deep cerebellar nuclei. Other neurons and their processes in the cerebellum were not reactive. A few reactive neuronal perikarya were seen in the rat hypothalamus, as well as many fiber terminals in the median eminence. Immunoreactivity was also noted in anterior pituitary cells. Preabsorption of 1 ml of the final dilution of antiserum with 10 µg of synthetic porcine motilin completely abolished immunoreactivity in the hypothalamus and pituitary, whereas 100 µg of the antigen was required to significantly block staining in the Purkinje neurons. Furthermore, preabsorption of the antiserum with rat cerebellar extract completely blocked the staining in both hypothalamus and pituitary. Chromatography of the cerebellar and other brain extracts on Sephadex G50 revealed two immunoreactive peaks. One eluted soon after the void volume. The other eluted similarly to synthetic motilin. Reverse phase high pressure liquid chromatographic data as well as immunological data using region-specific antibodies, however, indicated that the material in cerebellum is distinct from synthetic porcine motilin. These results suggest that the Purkinje system of the cerebellum contains a substance immunologically related to, but not identical to synthetic porcine motilin. (Supported by the Parkinsons Disease Foundation, Columbia University.)

32.13 ULTRASTRUCTURE OF SUBSTANCE P AND METHIONINE-ENKEPHALIN IMMUNOREACTIVE NEURONS IN THE GLOMERULAR LAYER OF THE HAMSTER OLFACTORY BULB. Gail D. Burd, Barry J. Davis, and Foteos Macrides. Worcester Foundation for Experimental Biology, Shrewsbury, MA.

We have previously reported the presence of the methionine-enkephalin (MET) and substance P (SP) immunoreactive neurons in the hamster olfactory bulb (Soc. Neurosci. Abstr., 6: 244, 1980). The present investigation examined the ultrastructural features of the immunoreactive neurons located in the glomerular layer (GL).

Hamsters were anesthetized with Nembutal and perfused with a 2% paraformaldehyde and 0.08% picric acid fixative. The olfactory bulbs were cut at 20 µm on a Vibratome. The sections were incubated in primary antisera and processed with the unlabelled antibody enzyme immunocytochemical procedure. The sections were then placed in 1% paraformaldehyde and 3% glutaraldehyde, osmicated, dehydrated, infiltrated with Epon-Araldite, and flat-embedded.

MET-immunoreactive neuronal somata were numerous in the periglomerular region (PGR) of the GL. They were generally ovoid in shape and, from our light microscopic measurements, had major and minor diameters of 10 µm and 7 µm, respectively. A thin rim of perikaryal cytoplasm surrounded the nucleus which was generally spherical in shape and contained dense chromatin material and one or more nucleoli. MET-immunoreactive processes were also present in the PGR and within glomeruli. Based on the size and ultrastructural features of the somata and the distribution of the neuronal processes, the MET-immunoreactive neurons in the GL were identified as periglomerular cells.

SP-immunoreactive neuronal somata were also present in the PGR. They were larger and less numerous than the MET-positive somata. SP-positive somata were spherical or ovoid in shape and, from our light microscopic measurements, had major and minor diameters of 13 µm and 11 µm, respectively. Depending on the plane of section, the nucleus appeared to be ovoid or spherical in shape and was surrounded by a large or moderate perikaryal cytoplasmic area. No nuclear invaginations were observed. The chromatin material was generally evenly distributed throughout the nucleus and a nucleolus was occasionally observed. SP-positive processes were present in the PGR and within glomeruli. Synaptic contacts were present on SP-immunoreactive dendrites in both of these regions. Based on the size and ultrastructural features of the somata and the distribution of the neuronal processes, SP-immunoreactive neurons were identified as external tufted cells. However, the size and location of the SP-positive neurons are consistent with the interpretation that some short-axon cells might also contain SP.

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32.14 ULTRASTRUCTURAL EXAMINATION OF SUBSTANCE P AND MET-ENKEPHALIN IMMUNOREACTIVE ELEMENTS IN THE RAT SPINAL CORD. J.C. Bresnahan, R.H. Ho, and M.S. Beattie, Dept. Anat., Ohio State Univ. Sch. Med., Columbus, OH. 43210

Following perfusion with 4% paraformaldehyde, sections from the spinal cord (8 rats) were cut on a vibratome. The PAP technique Sternberger was used to demonstrate met-enkephalin (ENK)* and substance P (SP)* on semi-adjacent sections. Sections were then osmicated, dehydrated, and flat embedded in Maraglas between sheets of smooth plastic for light microscopic observation prior to sectioning for the electron microscope.

LM observations of the distribution of SP and ENK in the osmicated sections corroborated published reports. Particularly noteworthy was the prominent staining of SP and ENK elements in the lateral spinal nucleus (LSN). Certain cells in this region were studded along their dendrites and somata with terminals that stained for either SP or ENK. Also of interest was the cytoplasmic labelling for SP or ENK of scattered cells, the laminar locations of which corresponded to previously published descriptions of labelled cells following colchicine treatment.

EM samples were taken primarily from cervical levels and from laminae I and II. Sections were systematically scanned and all labelled profiles were photographed. SP axons were small and unmyelinated. SP terminals had a mean diameter of 1.03 nm, and contained round, clear vesicles and dense cored vesicles (DCVs, mean no./term. = 2.2). The mean diameter of SP DCVs was 83.2 nm. The post-synaptic elements were small diameter dendrites and dendritic spines. Synaptic junctions were primarily assymetrical and often displayed subjunctional dense bodies. Lightly labelled somata and dendrites were also observed and on one occassion a labelled SP terminal was seen to synapse with a labelled dendrite.

ENK elements were small unmyelinated axons and terminals (mean diameter = .87 µm) with clear synaptic vesicles and DCVs (mean no./term. = 4.7). The mean diameter of ENK DCVs was 89.0nm. The post-synaptic elements were small diameter dendrites and synaptic junctions were symmetrical.

Comparing the SP and ENK terminals, the ENK population did not exhibit as many clearly identifiable synaptic junctions and contained more DCVs (U-test, $p < .01$), and these DCVs were larger than those present in SP terminals (U test, $p < .05$). Currently these studies are being expanded to include ultrastructural analysis of the LSN and laminae IX and X, as well as somatostatin elements. (Supported by Grants NS-14457 and -10165.)

*(A Substance's immunoreactivity is referred to by its name.)

32.15 IMMUNOCYTOCHEMICAL DOUBLE-LABELING FOR CHOLECYSTOKININ AND SOMATOSTATIN IN THE RAT HIPPOCAMPUS. R. S. Greenwood, K.K. Winstead*, Dept. Neurology and Neurobiology Program, University of North Carolina School of Medicine, Chapel Hill, N.C. 27514

Cholecystokinin-like (CCK-L) and somatostatin-like (SRIF-L) immunoreactivity has been found in rat hippocampal cells and fibers. Using the indirect immunofluorescent technique we elected to compare cellular localization of these peptides in the same hippocampal section. We incubated each septal hippocampal section with a primary antiserum to the COOH-terminal peptides of cholecystokinin and gastrin (Ab 4562**) and to a primary antiserum of somatostatin. Rhodamine-conjugated secondary antibody localized immunoreactivity with the first primary antiserum while fluorescein-conjugated secondary antibody localized immunoreactivity with the secondary primary antiserum. In an adjacent section we reversed the sequence of the primary antisera. This technique allows identification of those cells immunoreactive to the second primary antiserum alone. It does not identify cells immunoreactive to both antisera. We are exploring double-labeling techniques to identify these latter cells.

As in previous studies, CCK-L immunoreactive neurons were found in all layers of Ammon's horn and in the polymorphic zone of the dentate gyrus (Soc. Neurosci. Absts. 6:620, 1980). SRIF-L immunoreactive neurons were most numerous in the stratum oriens of Ammon's horn and the polymorphic zone of the dentate gyrus. Stratum oriens neurons with only SRIF-L immunoreactivity frequently appeared as small round cells with few processes. This cell type was generally located in the deepest part of the stratum oriens. The stratum oriens neurons with only CCK-L immunoreactivity varied in size and location but were usually larger and more superficial than SRIF-L immunoreactive neurons.

In the dentate few cells immunoreactive to only one antibody could be identified. Generally neurons with SRIF-L immunoreactivity alone were morphologically indistinguishable from those with CCK-L immunoreactivity alone. Usually neurons with only CCK-L immunoreactivity were found just beneath the granule cell layer. SRIF-L immunoreactive neurons were more evenly distributed in the polymorphic zone of the dentate.

We conclude that the distributions of CCK-L and SRIF-L immunoreactive hippocampal neurons are so distinct that even in those areas of the hippocampus where both types of immunoreactive neurons are found the two populations generally include dissimilar cells.

(**CCK-L antiserum a generous gift from Prof. Jens Rehfeld)

33.1 TRACING OF SUBSTANCE P AND ENKEPHALIN IMMUNOREACTIVE NEURONS PROJECTING TO THE GUINEA PIG INFERIOR MESENTERIC GANGLION: IMMUNOHISTOCHEMISTRY COMBINED WITH FLUORESCENT RETROGRADE LABELLING. C.-J. Dalsgaard*, T. Hökfelt*, L.-G. Elfvin*, L. Terenius* and P. Emson* (SPON: S. Vincent). Depts. of Anatomy and Histology, Karolinska Institutet, Stockholm, Dept. of Pharmacology, Uppsala University, Uppsala, Sweden and MRC Neurochemical Pharmacology Unit, Dept. of Pharmacology, Cambridge, United Kingdom.

The inferior mesenteric ganglion (IMG) of the guinea pig is rich in fibers immunoreactive to different neuropeptides such as substance P (SP), enkephalin (ENK), vasoactive intestinal polypeptide (VIP) and a cholecystokinin/gastrin-like peptide. To determine the origin of these peptidergic fibers a combination of fluorescent retrograde tracing and indirect immunohistochemistry was used, initially using SP and ENK antisera.

"True blue" (5 µl, 5%) or propidium iodide (5 µl, 3%) was applied to the IMG of adult guinea pigs. After four days the animals were perfused with ice-cold 10% formalin. Dorsal root ganglia (DRG) at levels L2 and L3 were dissected out and cut on a cryostat and the sections were examined in a Zeiss fluorescence microscope equipped with appropriate filter combinations. The same sections were then processed for indirect immunohistochemistry with antisera against SP and reexamined in the fluorescence microscope.

When photographs of the ganglia were compared some neurons containing the fluorescent tracer were SP immunoreactive. In addition there were some cell bodies labelled by the tracer only, as well as some substance P-positive perikarya containing no retrograde label.

A similar study was performed on the spinal cord aiming at identifying ENK-containing neurons projecting to the IMG. Application of fluorescent tracer in the IMG resulted in a labelling of cell bodies in the sympathetic preganglionic nuclei mainly in the L2-L3 segments. In order to achieve a sufficiently strong ENK immunoreaction in cell bodies of this region, the animals were treated intrathecally with colchicine 24 h before sacrifice. Following this procedure a large number of perikarya of the sympathetic preganglionic nuclei contained ENK immunoreactivity.

These findings indicate that the SP fibers in the IMG, at least in part, belong to primary sensory neurons at the lumbar level. The ENK-positive fibers may have their origin in the preganglionic nuclei of the spinal cord at the same level.

33.2 IMMUNOHISTOCHEMICAL STUDIES OF THE DISTRIBUTION OF SUBSTANCE P, SOMATOSTATIN AND CHOLECYSTOKININ IN RELATION TO THE SACRAL PARASYMPATHETIC NUCLEUS OF CAT. I. Lowe*, D. Blais*, O. Ronnekleiv, C. Morgan, I. Nadelhaft and W. de Groat. Dept. of Pharmacology, School of Medicine, Univ. of Pittsburgh and V. A. Medical Center, Pittsburgh, PA 15261.

Recent studies in our laboratory using retrograde and transganglionically transported HRP have demonstrated the projection of pelvic nerve visceral afferents into the vicinity of preganglionic neurons of the sacral parasympathetic nucleus (SPN). In the spinal cord the primary afferent axons were distributed first within Lissauer's tract (LT) from which collaterals extended through Lamina I around both sides of the dorsal horn to reach the intermediate gray matter. The lateral collateral projection (LCP) around the dorsal horn (DH) was very prominent and entered the dorsal band (DB) of the SPN (lamina V) as periodically arranged bundles of collaterals spaced at approximately 200 µm in the rostrocaudal direction. Relatively few collaterals entered the lateral band (LB) of the nucleus in lamina VII. Various peptides including substance P (SP) cholecystokinin (CCK) and somatostatin (ST) have been proposed as potential transmitters in primary afferent neurons. In the present investigation we have used immunohistochemical methods to study the distribution of these peptides within the sacral spinal cord and the relationship of the peptides to visceral afferent and efferent pathways as defined by the HRP technique. Cats were anesthetized and perfused with 4% paraformaldehyde. The spinal cord was removed and sectioned on a cryostat. Sections were processed according to standard techniques using the PAP method or the indirect immunofluorescence technique.

The distribution of SP and CCK in LT, the LCP and the SPN was in general similar to the distribution of pelvic nerve afferents with the exception that SP occurred also in the deeper layers of the DH (lamina II). Horizontal sections showed that in laminae I and V, SP and CCK terminals had a periodic distribution (interperiod distance approximately 200 µm). ST terminals exhibited a markedly different distribution with large numbers occurring in the LB of the SPN and in laminae II and outer III and fewer in the LCP (lamina I). Commonly, the region along the border between lamina I and II contained a low density of ST. All three peptides were present in the dorsal grey commissure and surrounding the central canal.

In summary, the localization in the sacral spinal cord of SP and CCK is very different from that of ST. The former correspond more closely with the distribution of pelvic nerve afferent projections. However, all three peptides were present in the region of the SPN and therefore should be considered as potential transmitters in the sacral autonomic pathways.

33.3 NON ADRENERGIC, NON CHOLINERGIC SECRETOMOTOR NERVES OF THE GUINEA PIG PANCREAS. J.S. Davison+*, G.T. Pearson*, J. Singh*, R.C. Collins* and O.H. Petersen*. Dept of Physiology The University, Dundee, U.K. (+ - Dept. Physiology, The University, Calgary, Canada). (SPON: G.E. Lucier)

Electrical field stimulation (FS) of isolated guinea-pig pancreatic segments evokes a tetrodotoxin sensitive release of amylase that is resistant to guanethidine and atropine, demonstrating the existence of non adrenergic, non cholinergic (NANC) secretomotor nerves. Stimulation of these NANC nerves is not associated with pancreatic acinar cell depolarization as is the case following cholinergic nerve stimulation. Moreover, FS in the presence of atropine has no effect on ^{45}Ca efflux from preloaded pancreatic segments. The NANC transmitter, therefore, appears to operate through an intracellular coupling mechanism other than calcium, since secretagogues such as Ach, which activate secretion via calcium, evoke ^{45}Ca efflux and membrane depolarization.

F.S. induces complex triphasic changes in endogenous cAMP and cGMP levels consisting of an initial rapid rise then fall in intracellular concentration followed by a slower rise to a secondary peak which then declines slowly. This triphasic response is also evoked by exogenous Vasoactive Intestinal Peptide (VIP - $10^{-7}M$). Since VIP also can induce enzyme secretion and V.I.P. nerves are present in the exocrine pancreas of many species, it should be regarded as a prime candidate for the unknown NANC transmitter released by F.S.

The time course of the change in cAMP levels is rather more rapid than that of the cGMP such that there appears to be a reciprocal relationship between the two nucleotides. This relationship might indicate that levels of one nucleotide might regulate the metabolism of the other.

33.4 CAPSAICIN-INDUCED SALIVATION IN RATS. P. J. K. Dobry and N. Masiques*. CNS Research, The Upjohn Company, Kalamazoo, MI 49001.

Capsaicin, a releaser of substance P, was tested in a sialogogic assay adapted from S.E.Leeman and R.Hammerschlag (1967, Endocrinology 81:803-810). Male Sprague-Dawley rats (85-110 g) were anesthetized with 50 mg/kg sodium pentobarbital i.p. Control salivary rates were ≤2.0 mg/3 minute collection (measured as the increased weight of cotton balls placed into the rat's mouth immediately after saline injection). Test compounds in 0.9% saline were injected i.v. Statistical significance for sialogogia was determined by the Mann-Whitney U test, and for antagonism of sialogogia by Student's t test.

Over a 100-fold range of doses (0.1-10 mg/kg) capsaicin produced small but significant amounts of saliva (usually 4-9 mg/3 min., occasionally >20 mg/3 min.), much lower than the maximum produced by substance P (SP) (250 mg/3 min.). Salivary rate may have been limited by capsaicin's low solubility. Salivary rate in the second 3-min. period was less than half that in the first 3 min. and reached control rates by 9 min. (This was also true for salivation induced by small doses of SP.)

Capsaicin-induced salivation is antagonized by atropine sulfate, cyproheptadine HCl, and phenoxybenzamine HCl, but not by morphine sulfate, naloxone HCl, or propranolol HCl. SP-induced salivation is antagonized by none of these. Capsaicin (0.3-0.6 mg/kg) partially antagonized salivation induced by SP, but not by pilocarpine.

When administered in doses 6 min. apart, neither capsaicin nor SP showed tachyphylaxis in the salivary response.

Capsaicin produced a variety of overt reactions in the anesthetized rat. All rats given 0.01-10.0 mg/kg capsaicin flushed during or immediately after the injection. Almost all had irregular twitching of the tail and caudal body as if responding to a noxious stimulus or an analeptic. With increasing doses, an increasing proportion of rats had cyanosis, labored breathing, apnea, and a resultant need for resuscitation. Thirty-one percent of capsaicin-treated rats died during a saliva collection; data from these rats were not included in the analysis. Rats treated with high doses of SP showed flushing, cyanosis, deep and labored breathing, and apnea, but not twitching of the body.

In summary, capsaicin- and SP-induced salivation share some characteristics, but they respond differently to antagonists.

33.5 REGULATION OF AIIR IN THE RAT BRAIN AND UTERUS DURING THE ESTROUS CYCLE. F.M. Chen*, R. Hawkins*, and M.P. Printz* Dept. of Medicine Div. of Pharmacology, Univ. Calif. San Diego, La Jolla, CA 92093.

Angiotensin II (AII) is postulated to be involved in the CNS and peripheral regulation of blood pressure and water homeostasis. Reports indicate that estrus rats have decreased spontaneous and AII-induced drinking.[a] However, AII-induced uterine contractility displays increased sensitivity during estrus.[b] The present study was therefore designed to determine whether the AII receptor is regulated during the estrous cycle and to correlate any changes with the reported drinking variation and uterine response during the estrous cycle. Stages of the estrous cycle were determined by vaginal smear and organ size. AII receptor (R) determination was monitored by high affinity, saturable, specific AII binding. We found that AII binding in the uterus is highest on the day of estrus as compared to diestrus days. In contrast, in the brain, the olfactory bulb has significantly higher binding in diestrus than estrus. Other brain regions also show lowest binding during estrus and highest in diestrus, except the preoptic area in which the change is similar to that found in the uterus. In conclusion we found: 1) Rats drink less and have least AII binding in the brain during estrus; 2) Uterine AII binding is highest in estrus when the uterus is most sensitive to AII; 3) These data imply that brain AIIR and uterus AIIR are regulated separately. Furthermore, the reduced spontaneous and induced drinking, and augmented uterus contractility to AII can be explained by alterations of AIIR.
(Supported by HL 25457)

[a]Dekanski, J. Brit. J. Pharmacol. 9:187, 1954; Schwarz, H. et.al. J. Pharmacol. Exp. Therap. 114:418, 1955.

[b]Findlay, A.L.R. et.al., J. Endocr. 82:215, 1979

33.6 BOMBESIN-LIKE PEPTIDES: OXIDATION RESULTS IN LOSS OF BIOLOGICAL ACTIVITY. Terry W. Moody[1], Jacqueline B. Crawley[2] and Thomas L. O'Donohue[3]. [1]Dept Biochemistry, George Washington Univ. Sch. Med., Washington, D.C. 20037, [2]Clinical Psychology Branch and [3]Lab. Clin. Sci., NIMH, Bethesda MD 20205.

Bombesin (BN) represents one class of peptides which may function as a central nervous system (CNS) regulatory agent as it fulfills many criteria required for CNS transmitters. In particular, immunoreactive BN is present in discrete regions of rat brain, BN-like peptides are localized to synaptosomes and released from nerve terminals by depolarizing stimuli, high affinity binding sites for (Tyr^4)BN, a potent BN analogue, are present in rat brain and upon central injection, BN causes various biological responses such as hypothermia, hyperglycemia and analgesia with a similar structure activity relationship to that required for receptor binding.

Here endogenous rat brain peptides were extracted with acetic acid, fractionated by high pressure liquid chromatography and characterized by radioimmunoassay. Two major BN-like components were detected, fraction A which co-chromatographed with BN-sulfoxide and fraction B which coeluted with authentic BN. If the acetic acid used during the extraction process was deaerated extensively and purged with nitrogen before use, only fraction B was present. Therefore, endogenous BN-like peptides may be oxidized by air in the extraction solution. In an in vitro bioassay for BN, fraction A had no detectable biological activity, whereas fraction B was equipotent to BN. Also, synthetic BN-sulfoxide was characterized. The oxidized peptide was 60% as immunoreactive as BN; however, BN-sulfoxide was not a potent hypothermic agent upon central administration and was 30-fold less potent than BN in a brain radioreceptor assay. These data indicate that when BN-like peptides are oxidized, immunological activity is retained but receptor binding, as well as biological activity, is reduced dramatically. Therefore, oxidation of BN-like peptides may inactivate their function at the CNS synaptic level.

33.7

WITHDRAWN

33.8 A COMPLETE RENIN-ANGIOTENSIN SYSTEM IN NEUROBLASTOMA X GLIOMA HYBRID CELLS. M.C. Fishman, E. Zimmerman, and E. Slater*. Laboratory of Developmental Neurobiology, N.I.C.H.D., N.I.H., Bethesda, MD. 20205, Neurology Dept, College of Physicians and Surgeons, Columbia University, N.Y., N.Y. 10032, and Medical Services, Mass. General Hospital, Boston, Mass. 02114

All components of the renin-angiotensin system have been identified by enzymatic and immunocytochemical methods in a widespread distribution in brain (Hirose et al., Brain Res. 191:489, 1980) but it is not known whether they are neural in origin, nor whether the cascade is completed within the circulation or the cells. We have shown that mouse neuroblastoma X rat glioma (NG108-15) cells contain immunoreactive renin (34 pg/mg protein) and immunoreactive angiotensin II (AII) (62 fM/mg protein) as measured by radioimmunoassays, and converting enzyme and an angiotensinogen as measured by enzymatic assays. Immunocytochemistry by the peroxidase-antiperoxidase methods reveals renin and AII within essentially all of the cells, and both were distributed throughout the cell body and processes of each cell. Two methods to enhance differentiation--serum withdrawal (using a defined medium) and 1mM dibutyryl cAMP--cause an increase in renin concentration. The renin is in an inactive form, and becomes enzymatically active (generates AI) after exposure to trypsin. Trypsin activated plasma renin has been equated with a prorenin. We suggest that activation of intracellular renin may generate AII within nerve cells.
(Supported in part by grants HL-21247 and HL-24105).

33.9 ENKEPHALIN-LIKE IMMUNOREACTIVITY (Enk-IR) IN RAT ADRENAL IS INCREASED BY NICOTINIC RECEPTOR BLOCKADE. Bohn, M.C., Kessler, J.A., Golightly, L.* and Black, I.B. Div. Develop. Neurology, Cornell Univ. Med. Coll., New York, N. Y. 10021

Recent studies have demonstrated that adrenal chromaffin cells contain both catecholamines and small peptides with opiate activity. While factors regulating catecholamine metabolism are well characterized, little is known about regulation of neuronal peptides. Employing an immunocytochemical approach, we have investigated various factors known to be important in the regulation of adrenal catecholamine metabolism for possible effects on Enk-IR.

In normal rats, only rare chromaffin cells were stained for Enk-IR. However, a dramatic increase in Enk-IR occurred following splanchnic nerve lesion as reported previously (Schultzberg et al., Neurosc.3, 1169, 1978). To determine whether this effect was mediated by nicotinic receptors, rats were treated with nicotinic-blocking agents. Both chlorisondamine and pempidine reproduced the effect of adrenal denervation, resulting in an increase in Enk-IR.

To ascertain the relationship of catecholamine (CA) metabolism to Enk-IR, we examined the effects of catecholaminergic agents. Enk-IR was increased 4 days after treatment with reserpine, a drug which depletes chromaffin vesicles of CA, and causes a reflex increase in presynaptic nerve activity. This effect was apparently not mediated solely by increased presynaptic nerve activity, since treatment with phenoxybenzamine or 6-hydroxydopamine, which also increase impulses to the adrenal, did not increase Enk-IR.

These studies suggest that levels of enkephalins and/or enkephalin-like peptides are normally depressed by presynaptic nerves acting via nicotinic receptor mechanisms. In addition, catecholamine depletion of chromaffin vesicles may influence enkephalin metabolism in the adrenal.

(This work was supported by NIH grants NS06400, NS10259, HD12108 and NS06801 and the Dysautonomia Foundation).

33.10 SECRETAGOGUES THAT ACT BY DIFFERENT MECHANISMS INDUCE SECRETION OF OPIOID PEPTIDES AND CATECHOLAMINES FROM CHROMAFFIN AND PHEOCHROMOCYTOMA CELLS. S.P. Wilson, R. Slepetis*, K.-J. Chang, N. Kirshner* and O.H. Viveros. Wellcome Research Laboratories, Research Triangle Park, NC 27709 and Department of Pharmacology, Duke University, Durham, NC 27710.

Opioid peptides (OP), including Met- and Leu-enkephalin, are stored with catecholamines (CA) in adrenomedullary chromaffin vesicles (Mol. Pharmacol. 16:1101, 1979). Consequently, OP and CA are co-secreted from the adrenal medulla (Adv. Biochem. Psychopharmacol. 22:191, 1980). We have used cultured bovine adrenal chromaffin cells to further explore the relationship between OP and CA secretion. Chromaffin cells, in culture for 4-10 days, secreted both OP and CA in response to stimulation with nicotine. The extent of both OP and CA secretion was concentration dependent with half-maximal and maximal secretion observed at 3 and 10 μM nicotine, respectively. Nicotine-evoked secretion of both OP and CA was dependent on the presence of Ca^{++} and was blocked by d-tubocurarine. Veratridine, K^{+} or Ba^{++} also stimulated the proportional secretion of OP and CA. Although the chromaffin cells preferentially secreted norepinephrine as compared to epinephrine, OP secretion was proportional to total CA secretion and not to that of either amine alone. OP secretion was also proportional to cellular OP content, i.e. reserpine-treated cells with twice the OP content of untreated cells secreted twice as much OP when stimulated with 20 μM nicotine. Hence, newly synthesized OP in chromaffin cells are stored in functional chromaffin vesicles.

Dissociated cells from a human pheochromocytoma tumor were also cultured (7-9 days) and the secretion of OP, endogenous CA and previously taken up [^{3}H]norepinephrine from these cells was examined. Nicotine, veratridine or Ba^{++} stimulated the secretion of OP, total CA and ^{3}H from the pheochromocytoma cells. Secretion of ^{3}H closely paralleled the secretion of OP, but secretion of endogenous CA was lower. Secretion of OP was more sensitive to stimulation by the calcium ionophore ionomycin than was secretion of CA. In the presence of 10 μM nicotine, the extracellular concentration of Ca^{++} required for maximum secretion was 0.2 mM for OP and 1.5 mM for CA and ^{3}H. As compared to the bovine chromaffin cells, veratridine was more potent, ionomycin less potent and nicotine equipotent in evoking CA secretion from the human pheochromocytoma cells. The results show that human pheochromocytomas secrete OP as well as CA and that there may be heterogeneous storage pools of CA and OP in cultured pheochromocytoma cells.

34.1 IS GABA-ERGIC MECHANISM RESPONSIBLE FOR ANTICONVULSANT PROPERTIES OF SODIUM VALPROATE? Michael S. Myslobodsky & Meir Morag* Psychobiology Research Unit, Department of Psychology, Tel-Aviv University.

A single dose of GABA-transaminase inhibitor, γ-vinyl GABA (GVG), administered to Wistar rats causes sedation and EEG synchronization reaching proportions of wave-spike activity (Myslobodsky et al., Pharmacol. Biochem. Behav. 11:483, 1979). Given that photically-induced sensory after-discharges (SAD) and the slow secondary negativity (SN) of the VEP faithfully reflect the degree of pro- and anti-convulsive action of various drugs, they were anticipated to undergo a facilitation after GVG treatment. Time-related two to three-fold enhancement of the SN and SAD area were obtained after 500 mg/kg (i.p.) of GVG. The changes seemed to parallel brain GABA increase. This effect was detectable at 1 hr, reached plateau at 3-4 hr and remained at this level 7 hr after GVG. Even about 24 hr after GVG, VEP components remained clearly hypersynchronized.

Sodium Valproate (VPA, di-n-propylacetic acid) which is also believed to block GABA-transaminase and succinic semialdehyde dehydrogenase causing GABA increase mostly in the nerve terminal compartment (Gale, K. & Iadarola, J.M. Science, 208:288, 1980) reduced SN amplitude by 70% when administered in a dose of 200 mg/kg (i.p.). It also totally eliminated SAD, when they were present, within 15-30 min. The recovery of VEP amplitude began at 3 hr. Given this finding it was anticipated that VPA would antagonise GVG-induced wave-spike discharges and paroxismally-enhanced SN and SAD. Indeed, VPA (200 mg/kg) administered 3 hr after GVG caused SN and SAD suppression within 15-30 min in all animals, this effect lasting about 2 hr. A typical GVG-hypersynchronized pattern of VEP recovered within the next 2-3 hr.

SN and SAD are believed to be organised by recurrent inhibition mediated by a pathway through axon collaterals and interneurons feeding back synaptic inhibition to the output and/or adjacent cells. VPA effects may be attributed to a disinhibitory action of a system located presynaptically on recurrent collaterals of the output neurons, or nerve terminals of inhibitory interneurons or both. Convulsant benzodiazepine, Ro 5-3663 (2 mg/kg, i.p.) which attenuates presynaptic inhibition (Schlosser, W. & Franco, S. J. Pharmacol. Exp. Ther., 211:290, 1980) potentiated SN-SAD within 1-3 min (the effect lasting 10-20 min) and partially antagonized VPA-induced SN-SAD suppression. These findings are interpreted as suggesting that VPA acts via a mechanism using a transmitter intimately connected to but not identical with GABA.

These findings also indicate that "GABA weakness" concept in epilepsy cannot be generalized to petit mal seizures.

34.2 FACILITATION OF THE NEUROCHEMICAL RESPONSE TO ANTIDEPRESSANTS BY ACTH AND YOHIMBINE. R. Duman*, J. Slopis*, D. Kendall* and S.J. Enna (SPON: B.T.Ho).

Numerous studies have suggested that the clinical response to antidepressants may be related to the drug-induced decrease in β-adrenergic and serotonin$_2$ (5-HT$_2$) receptor binding and function in cerebral cortex. This theory is partially based on the finding that the time (1-3 wks) necessary for observing a maximal reduction in receptor binding is quite similar to the delay observed in the clinical response. Recently it has been shown that the lag time for the receptor response can be significantly reduced if the antidepressant is co-administered with yohimbine, an α_2-adrenergic receptor blocker. In the present investigation, this drug interaction was examined further. For the study, male Sprague-Dawley rats (100-500 g) were treated chronically with yohimbine (2 mg/kg, b.i.d., s.c.) in combination with one of a number of antidepressant drugs (10 mg/kg, once daily, i.p.) and β-adrenergic and 5-HT$_2$ receptors in frontal cerebral cortex were quantified using receptor binding assays. As reported by others, this combination caused a more rapid decline in β-adrenergic receptors (3 days), though this was observed only with imipramine and DMI. However, yohimbine in combination with amitriptyline, iprindole and mianserin caused a more rapid (3 days) reduction in 5-HT$_2$ receptor binding, though no significant decrease in β-adrenergic binding was noted with these agents at this time. Since α-receptor antagonists are known to increase ACTH release and because this hormone has been shown to affect receptor site concentrations, the influence of ACTH on the drug-induced receptor changes was examined. Indeed ACTH (8 units/animal, once daily, s.c.) in combination with iprindole and mianserin reduced the number of 5-HT$_2$ receptors in 3 days. Like with yohimbine, only imipramine and DMI were able to reduce the number of β-receptors in this time period when administered with ACTH. These findings suggest that the more rapid onset in the antidepressant induced decrease in β-adrenergic and 5-HT$_2$ receptor binding observed with yohimbine may be mediated, at least in part, by an increase in the circulating levels of ACTH. If the receptor changes brought on by antidepressants are necessary for their clinical efficacy, then these results imply that the speed of onset in response may be a function of the hormonal state of the patient. (Supported in part by USPHS grants NS-13803 and NS-00335).

34.3 CHLORPROMAZINE BINDS TO AND INHIBITS CALMODULIN IN RAT BRAIN. T.L. WALLACE,* C.Q. EARL,* J.F. HABAS* AND B. WEISS, DEPT. OF PHARMACOLOGY, MEDICAL COLLEGE OF PA., PHILADELPHIA, PA 19129

Chlorpromazine (CPZ) and other phenothiazine antipsychotics inhibit the activity of calmodulin (CM) by binding to it in a Ca^{2+}-dependent, reversible manner. This binding can be made irreversible by irradiating CPZ and CM with ultraviolet (UV) light. When CPZ is irreversibly bound to CM, the activity of CM is irreversibly inhibited. These properties allowed us to investigate whether CPZ binds to and inhibits CM in crude preparations of brain where other naturally occurring brain constituents might offer competing binding sites for the antipsychotic. Rat caudate nuclei were sliced into 20 μ sections with a McIlwain tissue chopper. CPZ was added and irradiated with UV light (366 nm at 4°C). Tissues were then homogenized, boiled and dialyzed to remove unbound CPZ. CM activity in each sample was then determined from its ability to activate a CM-dependent form of phosphodiesterase. UV irradiation of tissues in the presence of CPZ resulted in an irreversible inhibition of CM activity. The concentration of CPZ required to produce 50% inhibition of CM was 10 μM. Complete inhibition was seen by 15 minutes. In the absence of CPZ, there was little or no effect on CM activity in samples irradiated for 15 minutes, although at longer times of irradiation there was significant inhibition of CM. Similar results were found using crude homogenates of brain or the 100,000 x g supernatant fraction. To demonstrate directly that the phenothiazine was bound to CM and to identify other possible binding sites, the 100,000 x g supernatant fraction of rat caudate nuclei and cerebral cortices were incubated with 20 μM CPZ (1 Ci/mmole) and irradiated with UV light for 30 minutes. The samples were dialyzed to remove CPZ and placed on SP-Sephadex columns (0.9 x 7 cm). Two-ml fractions were eluted sequentially using a discontinuous pH gradient, and each fraction was assayed for radioactivity and CM activity. Several peaks of irreversibly-bound ^{3}H-CPZ were found. In some of these peaks, the binding of CPZ was Ca^{2+}-dependent, whereas in others it was Ca^{2+}-independent. The Ca^{2+}-dependent peaks were associated with CM activity but peaks that showed Ca^{2+}-independent binding of CPZ were devoid of CM activity. These findings showed that photoactivated CPZ binds irreversibly to CM in the presence of other competing brain constituents, suggesting that this activator protein may be an endogenous phenothiazine binding site. These studies also showed that CPZ binds in a Ca^{2+}-independent manner to other materials in brain that do not possess CM activity.

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34.4 LACK OF CORRELATION BETWEEN OPIATE-INDUCED BEHAVIORAL HYPERSENSITIVITY AND DOPAMINE RECEPTOR DENSITY. K.R. Carlson and T.F. Seeger*. Department of Pharmacology, University of Massachusetts Medical School, Worcester, MA 01605.

We examined the hypothesis that opiates, when administered chronically, increase dopamine receptor density in striatum and mesolimbic areas, thereby accounting for enhanced stereotypy and locomotion in response to dopamine agonists following chronic treatment. Each rat's striatum and mesolimbic area (n. accumbens and olfactory tubercle) was assayed separately with the object of correlating receptor density with the extent of behavioral change. Stereotypy (chewing) and locomotion (number of crossings of quadrants) in response to 0.5 mg/kg apomorphine were measured before and 2 weeks after a 3-week treatment with saline or methadone (10 increasing to 20 mg/kg/day s.c.). Each drug group was subdivided into those Ss which increased in the behavioral measures and those which decreased or showed no change, relative to pre-chronic drug behavior. Rats were sacrificed 24 hr. after the second apomorphine test, and specific binding of ^{3}H-spiroperidol to homogenates of each brain area was measured. Half the methadone group showed increases in chewing, as did a few saline Ss; the difference among the subgroups in duration of chewing was significant ($F=10.49$, $df=3,19$, $p < .001$). However, there was no difference among the subgroups in striatal receptor density ($F < 1.0$). Similarly, half the methadone Ss increased in number of crossings and none of the saline Ss did ($F=7.90$, $df=2,19$, $p < .005$), but there was no difference in mesolimbic receptor density ($F < 1.0$). The negative binding results were not attributable to inadequate specificity or sensitivity of the assay, since a 3-week treatment with 1.0 mg/kg haloperidol produced a significant 26% elevation in striatal receptor density, a value completely in line with other reports. We conclude that changes in dopamine receptor density do not account for opiate-induced behavioral hypersensitivity.

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34.5 DEPRESSANT EFFECT OF TIMOLOL, A BETA-ADRENERGIC BLOCKER, ON NEUROMUSCULAR TRANSMISSION IN NORMAL AND MYASTHENIC MUSCLES. James F. Howard, Jr. and Bruce R. Johnson*. Department of Neurology, University of North Carolina, Chapel Hill, NC 27514

Timolol maleate, (Timoptic), used topically for the treatment of glaucoma, has been reported to acutely worsen the strength of a patient with myasthenia gravis (MG). The depressant action of this beta-adrenergic blocker on neuromuscular transmission was investigated in vitro in forelimb flexor digitorum longus muscles from normal rats and in intercostal muscle from normal patients and those with MG.

In rat muscles, Timolol in concentrations found in human plasma after ocular instillation and oral ingestion (5uM to 100uM) reversibly reduced the amplitude of miniature end-plate potentials (MEPP) and end-plate potentials (EPP) in a dose dependent manner. Rise times and half-decay times of these potentials were shortened following bath application of the higher concentrations. Similar results were found in normal human muscle.

In intercostal muscle from patients with MG, Timolol in concentrations of 10uM to 100uM also reversibly depressed MEPP and EPP amplitudes in a dose dependent manner.

These results demonstrate that Timolol maleate has a direct depressant effect on neuromuscular transmission at concentrations which are normally found in human plasma. This effect may account for the clinical worsening of strength which has been reported by a myasthenic patient after receiving this drug.

34.6 RDS-127: A POTENT NONHYDROXYLATED DOPAMINE AGONIST. S.P. Arneric*, D.B. Goodale, J.P. Long*, G.F. Gebhart, J.M. Lakoski, J. Mott*, C.F. Barfknecht*, (Spon: W. Steele). Dept. of Pharmacology, College of Medicine, and Div. Chem., Col. Pharm., University of Iowa, Iowa City, IA 52242

Responses indicative of dopamine (DA) receptor activation were studied in male Sprague Dawley rats following the administration of RDS-127 (2-N,N-di-n-propylamino-4,7-dimethoxyindane). To differentiate the site(s) of action where RDS-127 interact(s) with DA receptors, the potencies of RDS-127 were calculated in four models which are relatively selective for assessing pre- or postsynaptic receptor activity. Using the presynaptic model of Walters and Roth (1976, N-S Arch. Pharmacol., 296, 5), RDS-127 (0.05-12.8 μmol/kg, s.c.) dose-dependently inhibited the accumulation of DOPA concentrations in the caudate nucleus (ED_{50} = 0.15 μmol/kg) and the olfactory tubercle (ED_{50} = 0.93 μmol/kg). Pretreatment with haloperidol blocked the inhibition by RDS-127. In an electrophysiological model, single unit extracellular action potentials were recorded from neurons in the substantia nigra (SN). Recording from the SN permits the sampling of presynaptic elements in the pars compacta nigrostriatal units as well as postsynaptic elements of the pars reticulata units which are part of the striatonigral feedback system. RDS-127 decreased the firing of neurons in the pars compacta of SN (ED_{100} = 40 ± 10 nmol/kg, i.v., N=6); haloperidol reversed this suppression of unit activity by RDS-127. In contrast, the firing of units in pars reticulata of SN were either not altered, or increased in response to RDS-127 (40-400 nmol/kg, i.v.). The work of Strombom (1976, N-S Arch. Pharmacol., 292, 167) suggested that low doses of DA agonists activate presynaptic DA receptors to decrease locomotor activity (LA) and high doses activate postsynaptic receptor to increase LA. Low doses (0.05-0.2 μmol/kg) of RDS-127 produced a decrease and high doses (0.8-12.8 μmol/kg) produced a long-lasting (> 5h) increase in LA. Pretreatment with pimozide, but not metergoline, blocked the hyperactivity produced by high doses of RDS-127; α-methyl-p-tyrosine did not attenuate this response. In another postsynaptic model (Ungerstedt, 1971, Acta physiol. scand. 82, Suppl. 367: 69) RDS-127 produced contralateral rotational behavior in unilateral 6-hydroxydopamine produced lesions of SN (potency ratio to apomorphine = 0.13). Thus, the consistent findings between these different models indicate that RDS-127 is a direct acting dopamine agonist which in low doses preferentially activates presynaptic DA receptors. The absence of hydroxyl substituents on RDS-127 may account for its long duration of action. (supported by GM-22365 and NS-12114).

34.7 ERYTHROSIN B AND NA,K-ATPase: RECEPTOR-LIKE INTERACTIONS WITH BRAIN SYNAPTIC MEMBRANES. E.K. Silbergeld, S.J. Morris, and S.M. Anderson. Neurotoxicology, NINCDS, NIH, Bethesda MD 20205.

FD&C Red 3, tetraiodofluorescein, or erythrosin B (EB), interacts specifically and potently with Na,K-ATPase, inhibiting specific glycoside binding, ATP catalysis, and ^{86}Rb uptake (Silbergeld (1981) Neuropharm. 20: 87). In addition, EB acts on ATPase in brain, and not in red cell, myenteric plexus, or kidney cortex. Several reports suggest the existence of two enzymes in brain tissue, one of which is unique to the brain. Our results, using kinetic analyses of [^{3}H]-ouabain (OUA) binding and association-dissociation parameters, reveal two sites with Kd's of 1-2 and 80-140 nM. EB, in concentrations as high as 100 μM, only inhibits OUA binding to its high affinity site. [^{14}C]-EB binds saturably to crude synaptic membranes with an apparent Kd of 40-100 nM. It reaches equilibrium at 37° within 30 min. Like OUA, its binding is predominantly localized in the membrane fraction of purified synaptosomes: there are 217 fmol/mg protein EB binding sites; 714 fmol/mg protein OUA binding sites; and 10 μmol Pi liberated/20 min/mg protein of Na,K-ATPase activity. EB binding was compared to OUA binding: specific binding of both ligands is ATP-dependent. Both are displaced by unlabelled EB, rose bengal, diiodofluorescein, and eosin Y, while fluorescein and other derivatives do not displace either ligand. Neurotransmitters, such as GABA, dopamine, 5-HT, norepinephrine, and glycine, and neuroactive drugs, such as atropine, LSD, QNB, butaclamol, apomorphine, valium, and naloxone, also fail to displace either EB or OUA. However, although EB potently displaces OUA binding, none of the glycosides (ouabain, digitoxin, or strophanthidin) displaces EB. In further distinction, EB binding is not temperature sensitive, destroyed by boiling or by freezing and thawing of tissue. EB binding is enhanced by Rb and Cs, as compared to OUA binding, which is enhanced by Na and Li. Compounds which affect aspects of ATPase function -- ethanol, dithiothreitol, n-ethyl maleimide, oligomycin, tetrodotoxin, chlorpromazine, lead, ethacrynic acid -- do not displace EB. Vanadate, a known inhibitor of ATPase, weakly displaces EB binding (28 percent inhibition at 100 μM). These interactions of EB with brain tissue suggest the existence of a specific "receptor-like" recognition site which is associated with, but distinct from, such defined components of the enzyme as glycoside binding and ATP catalysis. The nature and function of this site is not presently known. Preliminary experiments with solubilized brain tissue ATPase provide evidence for separation of EB and OUA receptor sites, as well as further confirmation that EB binding is associated with ATPase activity.

34.8 BENZODIAZEPINE INHIBITION AND SELECTIVE BENZODIAZEPINE ANTAGONIST EXCITATION OF CA1 CELLS MEASURED INTRACELLULARLY IN THE HIPPOCAMPAL SLICE. P.L. Carlen, N. Gurevich, and P. Polc. Depts. of Medicine & Physiology, University of Toronto, Addiction Research Foundation and Playfair Neuroscience Unit, Toronto Western Hospital, Ontario, Canada; Pharma Research Dept., F. Hoffmann-La Roche & Co. Ltd., Basle, Switzerland.

Midazolam, a water soluble benzodiazepine, was applied by pressure ejection onto the somatic area of CA1 cells recorded intracellularly in constantly perfused guinea pig hippocampal slices. Threshold effects were noted at 10^{-10}M. Recordings were obtained from 21 cells with similar results for 10^{-9}M (10 cells) and 5 x 10^{-9}M (11 cells) midazolam doses. Within 1 to 2 minutes following drop application, hyperpolarization of 1 to 5 mV and decreased input resistance (10 to 50 %) occurred in 75% and 67% of cells respectively. Spontaneous activity, when present, decreased in 11/12 cells. IPSPs were increased in 14/18 cells. EPSPs were unchanged in 8 cells and slightly increased (15 to 30 %) in 4 cells. The afterhyperpolarization (AHP) following a train of 3 to 4 spikes elicited by a 100 msec constant current depolarizing pulse was augmented in 9/13 cells. The above effects lasted 10 to 20 minutes after drop application and were unaffected by the prior intracellular injection of chloride ions (3 cells). These preliminary results suggest that midazolam augments calcium mediated potassium conductance in CA1 cells.

RO14-7437 is a selective benzodiazepine antagonist which demonstrates potent inhibition of the specific high affinity binding of ^{3}H-diazepam to brain synaptosomal fractions. This compound, when applied by ejection onto the CA1 somatic layer using 10^{-9} and 10^{-8}M concentrations caused a depolarization (7/8 cells), increased input resistance (3/6 cells), increased spontaneous activity (2/3 cells), decreased IPSPs (4/7 cells) and decreased AHP (3/3 cells). EPSPs were unaffected. The above occurred in cells during midazolam-induced inhibition (4 cells). These excitatory affects also occurred in 4 cells not previously exposed to midazolam suggesting the possible existence of endogenous benzodiazepine ligands in the guinea pig hippocampus.

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34.9 ^{3}H-CL 218,872 BINDING TO RAT BRAIN. A. Lippa*, B.J. Bradford*, D. Benson* and B. Beer. CVR-CNS Research, Med. Res. Div., American Cyanamid Co., Lederle Labs., Pearl River, NY 10965.

The development of novel triazolopyridazines (TPZ) which competitively inhibit ^{3}H-benzodiazepine (^{3}H-BDZ) binding has led us to investigate the binding of ^{3}H-CL 218,872, a representative TPZ. The binding of ^{3}H-CL 218,872 (specific activity 15 Ci/mole) was determined by a centrifugation method in both cerebral cortex and cerebellum. Clonazepan (1-10 µm) was used to determine non-specific binding which represented 60-85% of the total. In equilibrium binding studies, specific ^{3}H-CL 218,872 binding was saturable and temperature dependent. One component ($Kd_1 \approx 10$ nM) was observed in cerebellum while two components ($Kd_1 \approx 10$ nM and $Kd_2 \approx 200$ nM) were observed in cerebral cortex. BDZ inhibited ^{3}H CL 218,872 binding with potencies which paralleled their potencies in inhibiting ^{3}H-flunitrazepan binding. BDZ inhibitions conformed with the law of mass action. These findings suggest that ^{3}H-CL 218,872 specifically binds to and discriminates between BDZ receptor subtypes. This compound may be a useful probe for studying BDZ receptor heterogenicity.

34.10 BENZODIAZEPINES ANTAGONIZE THE EXCITATORY ACTION OF KAINIC ACID ON CA_1 HIPPOCAMPAL NEURONS: A MICROIONTOPHORETIC STUDY IN THE RAT. G. de Bonnel* and C. de Montigny, Institut P. Pinel and Département de psychiatrie, Univ. de Montréal, Montréal, Québec, H3C 3J7.

Diazepam (DZP) pretreatment reduces the neurotoxicity of kainic acid (KA); in the hippocampus, the CA_1 region is more effectively protected by DZP than the CA_3 region (Fuller and Olney, Neurosci. Abst., 5: 1880, 1979). The presents experiments were undertaken to determine if benzodiazepines antagonize the KA-induced excitation of pyramidal neurons in CA_1 and CA_3.

All experiments were conducted in urethane-anesthetized male Sprague-Dawley rats (250-350 g). Five barelled micropipettes were used for unitary recording and iontophoresis of the following solutions: KA (1 mM in NaCl 0,4 M; pH: 8), glutamate (GLU) (50 mM in NaCl 50 mM; pH: 8), acetylcholine (ACH) (20 mM in NaCl 0,2 M; pH: 4), chlordiazepoxide (CDP) (10 mM in NaCl 0,2 M; pH: 3), flurazepam (FLU) (10 mM; pH: 3). In a first series of experiments DZP or lorazepam (LOR) were injected intravenously during the activation of CA_1 or CA_3 pyramidal neurons by iontophoretic application of KA, ACH or GLU. In a second series, CDP or FLU were applied iontophoretically on CA_1 or CA_3 units activated with KA, ACH or GLU.

The intravenous administration of low doses of DZP or LOR (0.5-1 mg/kg) drastically reduced or abolished the KA-induced activation of CA_1 neurons but did not modify or only slightly reduced the excitatory effect of ACH and GLU. In contrast, the same doses of DZP or LOR had little or no effect on KA induced activation of CA_3 pyramidal cells.

Congruent results were obtained with iontophoretic application of CDP and FLU. Both drugs applied on CA_1 pyramidal neurons excited by KA produced a marked reduction of their firing rate, whereas applications of these drugs with the same currents had little or no effect on the same cells when activated by ACH or GLU. In CA_3, CDP and FLU were much less potent in reducing the excitatory effect of KA.

In view of the protective effect of DZP against the neurotoxicity of KA in CA_1, the antagonism of the neuroexcitatory action of this amino acid on the same neurons by DZP provides further evidence for the excitotoxic hypothesis. Accordingly, DZP has a lesser effect on KA-induced excitation in CA_3 where it does not afford protection against KA neurotoxicity. The differential effect of benzodiazepines on the action of KA in CA_1 and CA_3 suggests that this amino acid activates different types of receptors in these two regions. The blockade of the benzodiazepine-sensitive type of "KA-activated" receptors might contribute to the anxiolytic and/or anticonvulsant effects of these drugs. (Supported by MRC grant MA-6444 and a C.R.S.Q. fellowship to C. de M.).

34.11 PHENOBARBITONE BINDING SIES IN RAT BRAIN MEMBRANES, M. Willow,* G.A.R. Johnston and I.G. Morgan. Department of Pharmacology, University of Sydney, NSW 2006 and Department of Behavioural Biology, Australian National University, Canberra, ACT, 2601, Australia.

Radioligand binding studies have established that pentobarbitone and related barbiturates enhance GABA binding to synaptosomal membranes by increasing the affinity of GABA for its high-affinity recognition sites (Willow, M. and Johnston, G.A.R., Neurosci. Lett., 18:323, 1980). The present study has examined the characteristics of phenobarbitone binding to crude synaptosomal membranes isolated from rat brain in an attempt to elucidate molecular mechanisms underlying the interaction between barbiturates and GABA receptor/ionophore complexes.

Crude synaptosomal membranes were prepared according to methods previously described (Willow, M. and Johnston, G.A.R., Neurosci. Lett., 18:323, 1980). Phenobarbitone binding was determined by incubating the resuspended membrane material (1.0 mg protein/2 ml Tris-citrate buffer, 50 mM, pH 7.1) for 15 min at 4°C with 0.5 µM tritiated phenobarbitone (8.1 Ci/mmol) in the presence of varying concentrations of phenobarbitone. This was followed by centrifugation for 15 min at 48,000 x g av, removal of the supernatant and determination of the pelleted radioactivity.

Under the conditions employed, total and non-specific (in presence of 10 mM phenobarbitone) binding averaged 14,000 and 9000 c.p.m./mg respectively. Scatchard analysis suggests that there is a single class of binding sites of relatively low affinity (Kd 99.7 ± 8.1 µM, n=3) and high density (Bmax 810 ± 65 pmole/mg, n=3). Phenobarbitone binding is displaced by a number of substituted barbiturates with the following order of potency: methohexitone>thiopentone>butobarbitone>secobarbitone>amylobarbitone>pentobarbitone>phenobarbitone>barbitone>barbituric acid. For those barbiturates which possess anaesthetic/anticonvulsant properties, there is an excellent correlation ($P<0.001$) between the ability to displace phenobarbitone and the ability to enhance GABA binding. GABA, bicuculline methochloride, picrotoxinin, and diazepam were all without effect on phenobarbitone binding at 100 µM, suggesting that barbiturate binding sites are distinct from GABA recognition sites and ionophores.

34.12 CHRONIC TRICYCLIC ANTIDEPRESSANTS ALTER α_1- AND α_2-ADRENOCEPTOR RESPONSIVITY AS MEASURED BY THE EFFECTS OF SELECTIVE AGONISTS ON THE ACOUSTIC STARTLE REFLEX. M. Davis, D.B. Menkes, J.H. Kehne*, D.W. Gallager, and G.K. Aghajanian, Depts. Psychiat. and Pharmacol Yale Univ. Sch. Med., New Haven, CT 06508.

Biochemical, electrophysiological, and behavioral experiments suggest that chronic tricyclic antidepressant (TCA) treatment may depress central responsiveness to α_2-adrenergic agonists but enhance responsiveness to α_1-adrenergic agonists. However, this conclusion can only be inferred by comparison across studies that employ different drugs, doses, and methods of evaluation. This experiment evaluated how chronic TCAs would alter adrenergic sensitivity using a design where the effects of both types of agonists were measured on the same behavior in the same animal. The acoustic startle response was chosen since this reflex is depressed by systemic administration of the α_2-agonist clonidine and enhanced by intrathecal administration of the α_1-agonist phenylephrine. The action of both agonists can be blocked selectively by their respective antagonists (e.g., yohimbine or WB-4101).

Male albino rats were injected i.p. once a day with either saline, 10 mg/kg desipramine, 10 mg/kg amitriptyline, or 5 mg/kg iprindole. Two weeks later (24 hrs after the last saline or TCA injection) rats were given saline or 20 or 40 µg/kg clonidine and tested for acoustic startle over the next 40 min. Clonidine markedly depressed startle in rats pretreated with saline but had much less of an effect in rats pretreated with desipramine. However, this was peculiar to desipramine, since clonidine still markedly depressed startle in rats pretreated with amitriptyline or iprindole.

Following these treatments all rats were injected for another week with their respective TCA drugs. They were then implanted with intrathecal catheters and one day later infused with either 12.5 or 25 µg of phenylephrine directly onto the spinal cord. Phenylephrine increased startle in each of the groups over the 40-min test session. However, the magnitude of these effects were greater in rats pretreated with each of TCAs than those treated with saline. No correlation was found between the enhanced behavioral response to phenylephrine and receptor binding of the α_1-antagonist ^{3}H-prazosin in the spinal cord. In contrast, following denervation via intrathecal 6-OHDA a supersensitive behavioral response to phenylephrine did correlate with increases in ^{3}H-prazosin binding in the cord.

Taken together, the data suggest that different TCAs share in their ability to enhance responsiveness to the α_1-agonist phenylephrine but not in their ability to blunt the effect of the α_2-agonist clonidine.

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34.13 SPECIFIC H^3-PENTYLENETETRAZOL BINDING IN THE RAT BRAIN. H. Lal, W. C. Davis* and M. K. Ticku. Department of Pharmacology, The Texas College of Osteopathic Medicine, Fort Worth, Texas 76107 and Department of Pharmacology, University of Texas Health Science Center, San Antonio, Texas 78284.

Although pentylenetetrazol (PTZ) produces pronounced neuropharmacological effects that include an induction of anxiety (for review see Lal and Shearman, Ann. Rep. Med. Chem. 15:51), convulsions, amnesia, and stimulation of brain cyclic AMP, attempts to demonstrate specific effects on brain neurotransmitters have met with only limited success. We, therefore, examined the possible ligand properties of PTZ in the rat brain fractions. PTZ was labeled by tritium gas exchange method and purified for the present study. When incubated with the brain membrane fraction, specific H^3-PTZ binding was only 10% of the total binding. However, solubilization of the mitocondrial plus microsomal ($P_2 + P_3$) fractions with Lubrol (1%) increased the specific binding of H^3-PTZ with signal to noise ratio of over 50%. Further fractionation of Lubrol extract through gel-filtration revealed that H^3-PTZ was bound specifically and in saturable fashion to a protein with approximate M. W. of 185,000 daltons. H^3-PTZ binding to Lubrol soluble protein was displaced by PTZ (IC_{50},10 μM), heptamethylenetetrazol (IC_{50},1 μM), 7-methyl-9-isopropyl-pentamethylenetetrazol (IC_{50},6 μM), 1-methyl-5-cyclohexyltetrazol (IC_{50},10 μM), 1,3-dihydro-5-methyl-2H-1,4-benzodiazepin-2 one or RO5-3663 (IC_{50},2 μM), ethosuximide (IC_{50},20 μM) and trimethadione (IC_{50},100 μM). Diazepam, flurazepam, GABA or (+) bicuculline failed to inhibit H^3-PTZ binding to the Lubrol soluble fraction at 10 μM concentration. There was no H^3-PTZ binding to 61,000 dalton fraction to which benzodiazepines specifically bind with high affinity.

34.14 MULTIPLE CENTRAL BENZODIAZEPINE RECEPTORS AND THEIR REGULATION BY GABA AND PYRAZOLOPYRIDINES. F.J. Ehlert*, P. Ragan*, W.R. Roeske* and H.I. Yamamura (SPON: N.W. Pedigo). Dept. of Pharm., Univ. of Ariz., Health Sci. Center, Tucson, AZ 85724.

Recent studies of the interaction of some triazolopyridazine and alkyl β-carboline-3-carboxylate derivatives with benzodiazepine (BDZ) receptors have provided evidence for multiple central BDZ receptors. In the present study, we have used propyl β-carboline-3-carboxylate (PCC) as a probe for investigating the heterogeneity of the BDZ receptor and its regulation by GABA and pyrazolopyridines.

The results of PCC/[^{3}H]PCC competition experiments on homogenates of the cerebral cortex of the rat brain revealed a small population of superhigh affinity BDZ receptors having a dissociation constant of 30 pM and a relative abundance of 3%. When measured by competitive inhibition of [^{3}H]flunitrazepam ([^{3}H]FLU) binding, the binding of PCC was generally consistent with that determined by direct measurements of [^{3}H]PCC binding and suggested the existence of two major populations of high and low affinity binding sites having dissociation constants of 0.54 and 10 nM and relative abundances of 52 and 45%, respectively. The effects of GABA on the binding of FLU at 37°C to the multiple BDZ receptors was determined by FLU/[^{3}H]FLU and FLU/[^{3}H]PCC competition experiments and by direct measurements of [^{3}H]FLU binding. In all experiments, a large GABA enhancement of FLU binding was demonstrable that was readily potentiated by chloride (100 mM NaCl). In contrast, no siginificant effect of GABA on the binding of PCC was detected by PCC/[^{3}H]FLU competition experiments or by direct measurements of [^{3}H]PCC binding when assays were carried out at 37°C in the presence of 100 mM NaCl. Studies of the effects of the pyrazolopyridine, tracazolate (ICI 136,753), on BDZ receptor binding at 0°C revealed that this compound enhanced the binding of [^{3}H]FLU in a chloride dependant manner whereas no such interaction was demonstrable for [^{3}H]PCC binding. The maximum enhancement of [^{3}H]FLU binding by tracazolate was approximately 50% with the ED_{50} for this effect being 0.3 μM. In summary, our results demonstrate that the binding of FLU to BDZ receptors is regulated by GABA and tracazolate in a chloride dependant fashion whereas the binding of PCC is not markedly altered under the same conditions (37°C, 100 mM NaCl). The differential effects of GABA and tracazolate on the binding of FLU and PCC may represent an important biochemical correlate for pharmacologically antagonistic effects of BDZ's and PCC.

35.1 DISTINCT POPULATIONS OF EXCITATORY AMINO ACID RECEPTORS REVEALED BY THE SELECTIVE DEPRESSANT EFFECT OF BARBITURATES.
V.I. Teichberg, N. Tal*, O. Goldberg*≠ and A. Luini*. Depts. of Neurobiology and Organic Chemistry≠, Weizmann Inst. of Science, Rehovot, Israel.

The use of selective antagonists of excitatory amino acids have allowed us to observe the existence in the rat striatum of at least four pharmacologically distinct populations of excitatory amino acid receptors, namely those for N-methyl-D-aspartate, (NMDA receptor), for quisqualate (Quis receptor), for L-glutamate and L-aspartate (Glu-Asp receptor) and for kainate (KA receptor). This finding has prompted us to investigate whether some of these receptors might be the site of action of some anticonvulsant, anaesthetic or antiepileptic drugs. Tested in a Na^+ efflux assay that we have recently developed (Luini, A., Goldberg, O., and Teichberg, V.I., PNAS, 78, May (1981)), at various concentrations in the 0.1 to 1mM range, barbiturates were observed to inhibit the increase in the specific $^{22}Na^+$ efflux rate produced in $^{22}Na^+$-preloaded striatal slices by Quis and KA but did not affect the responses to NMDA, Glu or Asp. At saturating conditions, the barbiturates behaved as partial blockers of the Quis and KA receptors and the following maximal extents of inhibition were observed: Thiopentone: 80%; Secobarbital: 75%; Pentobarbital: 55%; Amobarbital: 45%; Phenobarbital: 40%; Barbital: 10%. Chloroform at 10mM was also found to inhibit the Quis and KA receptors but it did not affect the responses to NMDA, Asp or Glu. The fact that the NMDA and Glu-Asp receptors are totally unaffected by barbiturates and chloroform suggest the existence of differences between the nature or the membrane environment of the ionophores coupled to the receptors of NMDA and of Glu-Asp, and those of the KA and Quis receptors. One may speculate that the barbiturates and chloroform act via the lipid bilayer to prevent an effective coupling between the receptor and the ionophore. The excitatory amino acid receptors were found to be unaffected by the two antiepileptic drugs, ethosuximide (used at 1mM) and diphenylhydantoin (used at 100μM) or the centrally acting muscle relaxant, mephenesin (used at 1mM) but the latter two drugs as well as the barbiturates were found to inhibit the increase in $^{22}Na^+$ efflux rate from striatum slices produced by 100μM veratridine. The data suggest that the action potential Na^+ channel is one of the targets of these drugs.

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35.2 EFFECTS OF ACIDIC EXCITATORY AMINO ACIDS ON ^{45}CALCIUM ACCUMULATION BY RAT STRIATAL SYNAPTOSOMES.
Konrad C. Retz, Alice C. Young and Joseph T. Coyle, Dept. of Neuroscience, Johns Hopkins Univer sity School of Medicine, Balto, MD 21205

The mechanisms whereby glutamic acid (GLU) and its conformationally restricted analogue, kainic acid (KA), produce neurotoxicity remain unknown. Recent studies have implicated influx of Ca^{++} ion as the mediator of agonist-induced myopathy of the cholinergic neuromuscular junction (J. Cell Biol. 82:811, 1979). Accordingly, we have examined the effects of GLU and KA on the uptake/accumulation of $^{45}Ca^{++}$ into synaptosomes prepared from the rat striatum. Unpurified synaptosomes in P_2 membrane fractions were prepared as previously described (J. Neurosci. Res. 4: 383, 1979) and resuspended in a Krebs-HEPES buffer (J. Biol. Chem. 252:2764, 1977) and preincubated for 5 min at 37°C under O_2. Drugs and the $^{45}CaCl_2$ (0.5-0.8 mCi/mmole) were added simultaneously; the reaction was terminated by adding 2.5-5.0 vol of ice-cold Ca-free buffer followed by rapid centrifugation. After a rapid wash of the pellet, it was solubilized in Protosol and ^{45}Ca was measured by liquid scintillation spectrometry. Unless specified all studies were conducted wih 5 mM K^+ ion present.

The uptake of Ca^{++} was biphasic in the presence of both 5 mM and 60 mM K^+ and consisted of an initial rapid phase within the first 30 sec. followed by a slower phase, apparently linear for 10 min. Incubation of the synaptosomes for 30 sec. in 60 mM K^+ stimulated Ca^{++} uptake by 40-50% and was not reversed by 5 μM tetrodotoxin (TTX). Veratridine, 100 μM, caused a lesser stimulation that was reversed by TTX. Calcium uptake in the presence of both 5 mM and 60 mM K^+ was inhibited by 20-30% when either 0.5 mM La^{+++} or 30 mM Mg^{++} were present; both ions are known to inhibit Ca^{++} transport (Phil. Trans. R. Soc. London B. 265:57, 1973). Glutamate, 0.1-10 mM, caused stimulation of Ca^{++} uptake with an apparent EC_{50} of 0.2 mM, and a maximal stimulation of 25-35% that was not reversible by TTX, in contrast to the stimu- lation produced by veratridine. An aspartate congener, 1 mM N-methyl-D,L-aspartate (NMDLA), also stimulated Ca^{++} uptake but to a lesser degree than did GLU. In contrast, KA usually produced no change or inhibition of Ca^{++} uptake at concentrations of 10-1000 μM reaching maximal values of 20% inhibition at 1000 μM.

Our preliminary studies indicate that two excitatory amino acids, GLU and NMDLA, stimulate the uptake of Ca^{++} by rat striatal synaptosomes. Although KA did not stimulate uptake under the present experimental conditions, it is possible that KA may mobilize intracellular Ca^{++} stores.

35.3 INTERACTION BETWEEN N-METHYL-D-ASPARTATE AND KAINATE RECEPTORS IN THE STIMULATION OF cGMP FORMATION IN MOUSE CEREBELLAR SLICES INCUBATED IN VITRO.
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Neurophysiologic and ligand binding studies suggest that the potent excitants kainic acid (KA), glutamic acid (Glu) and N-methyl-D-aspartic acid (NMDA) act at different neuronal receptors. The toxic effects of KA involve a coopertive interaction with endogenous excitatory neurotransmitters whereas its neuroexcitatory effects appear to be direct. In the present study, we have examined the effects of KA and Glu on the formation of cGMP in mouse cerebellar slices in the presence of an NMDA antagonist as a means of determining receptor interactions.

Adult male albino mice were decapitated, the cerebellum removed and sliced at 0.3 mm perpendicular to the pial surface. After 1 hr preincubation at 37°C in Krebs-HCO_3:-5% CO_2-95% O_2 buffer, the slices were transferred to fresh medium containing drugs and incubated for 15 min. The reaction was terminated by boiling the tissue in 50 mM Tris-HCl, pH 7.5 containing 0.5 M EDTA and cyclic GMP was measured in the extract by RIA.

Under these conditions basal levels of cGMP were 6 ± 1 pm/mgp; 60 mM potassium or 0.3 mM ouabain produce a 5-fold stimulation with maximal effects at 15 min. Kainic acid (EC_{50} = 180 μM) was ten-fold more potent than Glu (EC_{50} = 1.5 mM) but Glu (Δ_{max} = (+) 8.0 pm cGMP/-mgp) was more efficacious than KA (Δ_{max} = (+) 5.6 pm cGMP/mngp). Ibotenic acid failed to stimulate cGMP between 10 and 1000 μM.

When the putative NMDA antagonist, D-α-amino-adipate (DAA), (250 μM) was included with KA (500 μM), a significant increase in cGMP levels was observed with respect to KA only ($N = 11$; $p < 0.02$; ANOVA). The antagonist alone did not alter basal cGMP levels. This effect was specific to KA since DAA failed to alter the cGMP response of either Glu or Ibo.

To better define the interaction between KA and DAA, the effects of these drugs on the release of endogenous amino acids into the medium was assessed by an HPLC method. KA produced greater than a 200% increase in the release of both aspartic and glutamic acids ($p < .05$) but did not affect the basal release of glycine, glutamine or alanine. DAA alone did not significantly reduce the basal or KA induced amino acid release.

In summary, DAA markedly enhances KA induced stimulation of cGMP in adult mouse cerebellar slices. The results are consistent with the hypothesis that the direct action of KA on cGMP stimulation is adumbrated by neurotoxic effects produced by the release of an endogenous excitatory agent whose effects are blocked by DAA. The results support the existence of two separate receptors which are capable of neurotoxic interactions.

35.4 ALTERATIONS IN SYNAPTIC TRANSMISSION IN THE HIPPOCAMPAL SLICE PRODUCED BY THE IONTOPHORETIC ADMINISTRATION OF EXCITATORY AMINO ACIDS.
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We have recently observed that brief (eg. 10 sec) iontophoretic administration of kainic acid to the CA1 cell body region of the hippocampal slice preparation can produce a massive and prolonged (usually 15 min or more) potentiation of the population spike evoked by Schaffer collateral stimulation. In the present study the effects of various excitatory amino acids and other substances have been examined. Drugs were administered iontophoretically (0-100 nA, 10-120 sec) from 7 barelled microelectrodes, the centre barrel of which was used to record extracellular activity from the CA1 cell body region of rat transverse hippocampal slices. The Schaffer collateral pathway was stimulated with glass microelectrodes (0.1 or 0.2 msec pulses, 3-85 μA at 0.1 Hz).

During its administration, kainate produced an initial increase in the amplitude of the population spike and in the neuronal firing rate, followed by a decrease in the population spike and an increase in the late negative wave as the cells overdepolarized. The ensuing potentiation started immediately or up to 5 min after the end of the iontophoretic application, but in slices which did not potentiate (approx. 25%) recovery of the population spike from the depression could take up to 10 min. L-glutamate, L-aspartate and their more potent analogs N-methyl-DL-aspartate (NMA), quisqualate, D-homocysteate and (±)-ibotenate all produced qualitatively similar effects to kainate during their administration. On termination of iontophoresis it usually took 1-5 min for the population spike to recover to its initial level and this recovery appeared to correlate with the recovery of units from their overdepolarized state. With the analogs recovery could be followed by an increase in the population response, although this potentiation was considerably less than that produced by kainate.

Acetylcholine was a weaker neuronal excitant but often produced large increases in the population spike during administration with low doses but depressions with higher doses; whereas GABA depressed the response. No long lasting changes in the synaptic response were observed following the application of these agents.

These results demonstrate that kainate relatively selectively produces a long lasting potentiation of synaptic excitation in the CA1 region of the hippocampal slice. Preliminary studies have suggested that kainate also selectively potentiates for long periods the population spike recorded in the dentate gyrus in response to perforant path stimulation. Possibly these actions of kainate may be correlated with its neurotoxic effect and might explain the necessity for intact excitatory hippocampal afferents for the full development of this neurotoxicity.

35.5 IS γ-HYDROXYBUTYRATE AN INDEX OF GABAERGIC ACTIVITY? F. Cattabeni+, M. Eli+ and E. Coen+ (SPON: L. Beani), Inst. of Pharmacology, Univ. of Urbino and Inst. of Pharmacology and Pharmacognosy, Univ. of Milan.

The presence of γ-Hydroxybutyrate (GHB) in mammalian brain is now well established and it is presumed to be formed from GABA via succinic semialdehyde. Since GHB possesses pharmacological activities, much effort has been made in order to prove its physiological role within the CNS. However, no indications exist about the possibility to utilize GHB as an index of GABA-ergic functional activity. The experiments here described were performed in order to prove this hypothesis. Blockers of glutamate decarboxylase (isoniazide, 450 mg/kg i.p., 45 min.) reduce GABA levels in brain areas by 70%. Concomitantly, GHB is significantly reduced, although to a lesser extent (25%). Blockers of GABA-Transaminase (γ-acetylenic GABA, 100 mg/kg i.p., 2 hours) double GABA levels and decrease those of GHB. Sodium valproate (200 mg/kg i.p., 30 min and 1 hour), a compound which seems to block not only GABA transaminase but also the enzymes responsible for the degradation of succinic semialdehyde, induces an increase in both GABA and GHB levels. These findings clearly indicate therefore that GHB is formed by a great extent from GABA in the CNS and that drugs interfering with GABA metabolism alter GHB levels accordingly. Moreover, Pentamethylenetetrazole increases GABA levels in cortex but not in cerebellum of animals during convulsions (1 min and 30 sec after 100 mg/kg i.p.), whereas GHB is slightly but significantly decreased. After the convulsions have ceased, cortical GABA levels are returned to control values, whereas GHB levels are by 50% higher than controls. These data therefore indicate that during convulsions GABAergic activity is decreased, as indicated by lower GHB levels, whereas increased GABAergic activity, as indicated by higher GHB levels, could account for the disappearance of convulsions 20 min after Pentamethylenetetrazole treatement. These results further support the hypothesis that GHB could be considered an index of GABAergic activity and that Pentamethylenetetrazole acts through the GABAergic system.

35.6 GABA PRODUCES A BIPHASIC RESPONSE, AN INITIAL DEPOLARIZATION FOLLOWED BY A HYPERPOLARIZATION, WHEN APPLIED TO CAT VESICAL PELVIC GANGLIA. M.L. Mayer,* J.P. Gallagher, H. Higashi,* and P. Shinnick-Gallagher. Dept. of Pharmacology and Toxicology, Univ. Texas Med. Br., Galveston, Texas 77550, USA.

The action of GABA on parasympathetic neurons has been studied with intracellular techniques. When recordings were made with electrodes filled with potassium salts of impermeant anions bath applied GABA evoked a monophasic depolarizing response associated with a conductance increase. When electrodes were filled with permeant anion salts the GABA response became biphasic: initially the membrane depolarized by 10-20mV before rapidly repolarizing to a plateau value associated with a maintained conductance increase, presumably reflecting anion redistribution. The membrane then hyperpolarized and the conductance decreased to 26%-86% of resting values. On the rising phase of the GABA-induced depolarization, the cell fired action potentials, while during the subsequent hyperpolarization the electrical threshold required to fire the neuron increased. In cells that were spontaneously active, action potential generation ceased during the hyperpolarization. Muscimol and 3-APS were less effective in evoking the conductance decrease. Ion substitution experiments revealed no reduction of the hyperpolarizing response in calcium or sodium free media, while chloride reduction augmented both phases of the GABA response. Iontophoretically applied GABA did not evoke biphasic responses. Interaction between bath and iontophoretically applied GABA revealed no apparent reduction in the driving force for the iontophoretic response during the latter phase of the biphasic response to bath applied GABA. The physiological significance of this unusual GABA response is unclear. Supported by NIH Grant NS 16228 to P.S.-G. and a Harkness Fellowship to M.L.M.

35.7 ETHYLENEDIAMINE AS A GABA-MIMETIC. T.W. Stone, H.G.E. Lloyd*, M.N. Perkins*, M. Parker*, N.G. Bowery*, and D.R. Hill*, Dept. of Physiology St. George's Medical School, London SW17 and Dept. of Pharmacology, St. Thomas's Medical School, London, SW1, UK.

The simple diamine ethylenediamine, $NH_2.(CH_2)2.NH_2$, is often regarded as a pharmacologically inert carrier for theophylline, in the form of aminophylline. Having noticed that aminophylline induced the release of (^{14}C)-GABA from brain slices, we now present the following evidence that ethylenediamine (EDA) may act upon GABA recognition sites in the central and peripheral nervous system.

1. EDA causes an apparent release of (^{14}C)-GABA from preloaded slices of mouse cerebral cortex.
2. EDA inhibits significantly the uptake of (^{14}C)-GABA into cortical slices (23% inhibition at 1mM).
3. Applied by microiontophoresis, EDA is a powerful depressant of neuronal firing in vivo. This action is blocked by bicuculline in doses which also block GABA but not glycine. Strychnine blocks glycine but not EDA.
4. EDA depolarises sympathetic ganglia with a potency of about 0.05 relative to GABA. EDA and GABA dose response curves are parallel and both compounds are blocked by bicuculline.
5. EDA displaces (^{3}H)-GABA from $GABA_A$ sites on rat cortex synaptosomal membranes, with an IC_{50} of 5μM.

The neuronal depressant properties of EDA seen in electrophysiological experiments are shared by related compounds such as diaminopropane and N-methyl EDA, though these are less effective.

It is suggested that in view of its simple structure, lacking either a hydroxyl or carboxyl grouping normally considered essential for actions at GABA sites, EDA may prove a useful tool for probing GABA or bicuculline receptor mechanisms.

35.8 EFFECTS OF GABA ON HYPOTHALAMIC NEURONS: A NEUROPHARMACOLOGICAL STUDY. J.-T. Cheng*, F. C. Barone and M. J. Wayner. (SPON: R. T. Bartus). Brain Research Lab., Syracuse Univ., 601 University Ave., Syracuse, NY 13210.

Male rats were anesthesized with urethane and prepared for recording from neurons in the lateral hypothalamus (LH). Seven barrel electrodes were utilized to record simultaneously from and apply chemicals to single neurons. The center barrel and one outer barrel were filled with 2 M NaCl and used for neuronal recording and current balancing, respectively. The other outer barrels were filled with 2 M L-Glutamic acid, 0.5 M γ-Aminobutyric acid (GABA), 5 mM Picrotoxin, 5 mM Bicuculline, and 0.1 M Glycine HCl. After a stable discharge frequency was established chemicals were ejected microiontophoretically from the multibarrel electrodes. Dose effect relations were determined and control procedures were utilized to eliminate current and pH effects (Barone et al., Brain Res. Bull. 5: 325-332, 1980). Also, those regions which project to the LH and are known to contain GABA neurons (Barone et al., Brain Res. Bull. 7, in press), such as the reticular thalamic nucleus (Houser et al., Brain Res. 200: 341-354, 1980) and reticular zone of the substantia nigra (Fonnum et al., J. Neurochem. 29: 221-230, 1977), were stimulated, 0-500 μA 0.5 msec single pulses, in conjunction with neuropharmacological testing. Lateral hypothalamic neurons were very sensitive to GABA and relatively small ejection currents (0-5 nA) were required to eliminate completely neuronal discharges. Picrotoxin and Bicuculline when applied alone usually did not alter or slightly elevated neuronal discharge frequency. When these antagonists were administered prior to and during GABA ejection, the decreases due to GABA were attenuated. Glycine also decreased the discharge frequency of hypothalamic neurons but only at higher ejection currents and the effects could not be altered by Picrotoxin or Bicuculline. In some cases, the antagonists could partially block the decrease in hypothalamic neuronal activity induced by electrical stimulation of known GABA inputs. These data indicate the significance of GABA receptors in the LH and are consistent with the high GABA metabolic activity found in the LH (Fonnum et al., J. Neurochem. 29: 221-230, 1977) associated with GABA synapses in the medial forebrain bundle. (Supported by NIH Grant NINCDS USPHS No. 13543.)

35.9 CARRIER-MEDIATED RELEASE OF GABA FROM RETINAL HORIZONTAL CELLS. S. Yazulla and J. Kleinschmidt. Dept. Neurobiology and Behavior, SUNY StonyBrook, NY 11794.

GABA is very likely the transmitter of H1 horizontal cells in the goldfish retina. L-Glutamate (Glu) and L-aspartate (Asp) are candidates for the photoreceptor transmitter in goldfish, and as such would be expected to influence directly the synaptic release of GABA by H1 cells. The mechanism by which transmitter is released from H1 cells is unknown. We found by LM autoradiography that Glu, Asp and ouabain cause release of ^{3}H-GABA from H1 cells in a manner suggesting efflux through carrier-mediated transport.

Isolated goldfish retinas were preincubated in 200 µl of 0.72µM ^{3}H-GABA for 15 min, postincubated in 200µl of a variety of experimental media for 15 min, fixed and embedded in EPON. When preincubated in the light, goldfish retinas took up ^{3}H-GABA heavily into H1 cell somata and dendrites (HSD) as well as axon terminals (HAT). Postincubation of retinas, preloaded with ^{3}H-GABA, in 0.5 to 10mM Glu or 0.1 to 10mM Asp caused a dose-dependent loss of label from HSD and little loss from HAT. We found that this loss was not due to metabolic breakdown of ^{3}H-GABA nor to calcium dependent vesicular release. 0.1mM ouabain also released ^{3}H-GABA selectively from HSD. Further, 1mM nipecotic acid, a selective blocker of neuronal high-affinity GABA uptake, as well as lithium substitution for extracellular sodium, a condition which would fail to activate neuronal GABA uptake, both blocked ^{3}H-GABA uptake into H1 cells as well as Glu- or ouabain-induced release of ^{3}H-GABA from HSD preloaded with ^{3}H-GABA. These results suggest that ^{3}H-GABA is released from HSD via a sodium-dependent membrane carrier mechanism which can be activated by an increase in intracellular sodium concentration brought about by the opening of cation selective membrane channels by Glu or Asp or by blocking the sodium pump with ouabain. Furthermore, this carrier may be identical with the carrier responsible for uptake of ^{3}H-GABA into horizontal cells, and the direction of net transport of GABA may depend on the physiological state of the cell.

The differential release of ^{3}H-GABA from HSD and not from HAT implies that Glu and/or Asp receptors and sodium pump sites are restricted to HSD, i.e. the only part of the H1 cell known to release transmitter synaptically. Thus, carrier-mediated transport of GABA may be the mechanism by which H1 cells effect their localized synaptic actions.

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35.10 GAMMA-VINYL GABA: PROLONGED EFFECT ON PICROTOXIN-INDUCED SEIZURES IN MICE. F.P. MILLER* AND J.I. ALLEN* (SPON:R.P. MAICKEL). Merrell Research Center, Cincinnati, OH 45215.

Gamma-vinyl GABA (GVG), a specific irreversible inhibitor of GABA transaminase (GABA-T), elevates whole brain GABA levels in mice (Jung, M.J. et al., J. Neurochem., 29:797, 1977). This effect persists up to 5 days after a single dose and has been shown to correlate with the degree of protection against audiogenic seizures in mice (Schechter, P.J. et al., Eur. J. Pharmacol. 45:319, 1977). The present study investigated the effect of GVG on seizures induced in mice with the specific GABA antagonist, picrotoxin (PIC) as a function of time after injection. Groups of 10 male, CD-1 mice (Charles River) were administered GVG at 1000 mg/kg i.p.. At 2, 4, 16, 24, 48, 65, 72, and 96 hours after GVG, PIC was administered at a dose of 2.5 mg/kg i.v. (CD98). In a group of 110 control mice, this dose of PIC caused seizures with a mean latency of 4.47 min. ± 1.85 S.D. Seizure reduction was considered significant if an individual mouse did not exhibit seizures for more than 8 minutes after PIC. At 2 and 4 hrs post-GVG, nearly all of the mice exhibited seizure latencies in excess of 8 min, in agreement with the results of Schechter et al. (Psychopharmacol. 54:149, 1977). This effect on seizure latency persisted, with significant increases in seizure latency being exhibited at 16 hrs (80%), 24 hrs (80%), 48 hrs (60%), 65 hrs (25%) and 72 hrs (11%) after GVG. No effect was seen at 96 hrs after GVG administration. These results illustrate a long-lasting effect of GVG on PIC-induced seizures, an effect which appears to mirror the long-lasting elevation of whole brain GABA concentration as reported by Jung, M.J. et al. (J. Neurochem., 29:797, 1977) following administration of a single dose of GVG.

35.11 EVIDENCE FOR MUSCLE RELAXANT ACTIVITY OF γ-VINYL GABA IN MICE. Gary D. Novack and Kathleen M. Owenburg*. Merrell Research Center, Cincinnati, OH 45215

A role for GABAergic drugs in amelioration of disorders of increased muscle tension has been suggested by Davidoff (Neurology 28:46, 1978). In addition, intracerebroventricular administration of GABA or baclofen, a GABA analogue, was shown to block morphine-induced Straub tail (ST) in mice (Ukai et al., Jap. J. Pharmacol. 26(S) 1189, 1976; Kameyama et al., Jap. J. Pharmacol. 28:249, 1978). ST has been shown to be blocked by a number of clinically effective muscle relaxants at doses which do not produce a loss of righting reflex (Ellis and Carpenter, Neuropharmacol. 13:211, 1974). Since γ-vinyl GABA (GVG) is an irreversible inhibitor of GABA-transaminase and raises CNS GABA levels in mice 5 to 8-fold after doses of 800 to 1600 mg/kg, i.p. (Jung et al., J. Neurochem. 29:797, 1977), we were interested in evaluating GVG as a possible muscle relaxant by assessing its ability to inhibit ST.

Groups of 10 mice were treated with either water or GVG, 200, 400, 800 or 1600 mg/kg i.p., at various intervals (1, 2, 4, 16, 24, 48 or 60 hr) prior to morphine sulfate, 60 mg/kg s.c. Mice were evaluated for ST in a quantal fashion 15 min after morphine. GVG inhibited ST at 2, 4, and 16 hr after dosing. The ED50's for this response were 1268, 796 and 1289 mg/kg, respectively. At the highest dose used, inhibition at 1 and 24 hrs was 30% and 10%, respectively. No inhibition was seen at 48 and 60 hrs.

The observed inhibition of ST was not due to direct narcotic antagonism, as GVG does not have naloxone-like activity in analgetic models. In addition, ST was inhibited at doses which did not elicit a loss of righting reflex. Therefore, the inhibition of ST by GVG seen in the present study is interpreted to be a muscle relaxant effect. The time to onset and peak effect of the ST blockade by GVG corresponds to that reported for elevation of whole brain GABA levels (Jung et al.). This suggests that the muscle relaxant effect of GVG may be related to its effects on the GABA system. Thus, GVG may be useful in the treatment of human muscle spasms.

35.12 FOMINOBEN: A NOVEL AMINO ACID ANTAGONIST. Herbert M. Geller and Frank Baldino, Jr. CMDNJ-Rutgers Medical School, Dept. of Pharmacology, Piscataway, NJ 08854.

Fominoben hydrochloride is a novel agent with the unusual composition of antitussive and respiratory stimulant properties. A recent report has emphasized the ability of fominoben to reduce the specific binding of benzodiazepines (BDZ) to central nervous system BDZ receptors (Antoniadis et al., 1980). In this series of experiments we examined the physiological actions of fominoben on neuronal activity and possible interactions of fominoben with inhibitory amino acids and BDZ on rat hypothalamic neurons grown in tissue culture.

The activity of single spontaneously active (or glutamate-driven) neurons was recorded extracellularly. Depressions of activity were elicited by local application of amino acids (GABA or glycine) or flurazepam, a water-soluble BDZ, as well as by focal electrical stimulation of the culture, which elicits a GABA-mediated inhibition. Reductions in activity were quantitated on line through the use of peri-event histograms. Fominoben was then perfused through the recording chamber at concentrations between 1-100 µM. Further histograms were computed to assess the efficacy of the locally applied agents during fominoben perfusion.

The major action of fominoben was to reduce or antagonize the actions of both amino acids and flurazepam. At 50 and 100 µM concentrations, fominoben attenuated the actions of GABA on all 14 cells on which it was tested, while 5 of 7 cells displayed a concomitant and smaller reduction in the efficacy of glycine. Electrically-induced inhibitions were also reduced at these concentrations. At 10 µM, fominoben reduced the efficacy of GABA in only 3 of 7 cells, and of glycine in 2 of 4 cells, and was able to antagonize the depressant effects of flurazepam in each of 3 cells. These changes were observed without any consistent actions on the rate or pattern of activity in the cultures.

In summary, fominoben antagonized depressions of firing elicited by both GABA and glycine, though it displayed a greater potency in antagonizing GABA. Flurazepam-induced depressions were also reduced. These actions thus bear significant resemblance to the actions of picrotoxin. A major difference is that fominoben is without effect on the rate or pattern of neuronal activity as is characteristic of high doses of amino acid antagonists. We hypothesize that this difference may be related to the absence of a convulsant action of fominoben. Thus, these interesting properties of fominoben hydrochloride may prove to be of use in further physiological and biochemical studies of the GABA-benzodiazepine-chloride ionophore complex. (Supported by NSF grant BNS 77-09241 and NIH grant NS 15468.)

35.13 THIP, GABA-TRANSAMINASE INHIBITORS AND BENZODIAZEPINES BLOCK A RESPONSE OF MUSCIMOL IN MICE. M. Krishna Menon. Psychopharmacol. Res. Lab., V.A. Med. Ctr., Sepulveda, CA 91343 and Dept. of Psychiatry, UCLA School of Medicine, Los Angeles, CA 90024.

In a particular strain of mice, relatively high doses of muscimol (2-3 mg/kg, i.p.) caused myoclonic jerks of high frequency (peak effect 73 jerks/min). This response of muscimol was blocked in a dose-dependent manner by a GABA agonist 4,5,6,7-tetrahydroisoxazolo (5,4-c) pyridin-3-ol-hydrate (THIP). It was also observed that both γ-acetylenic GABA (100 mg/kg, i.p.) and γ-vinyl GABA (1500 mg/kg, i.p.) blocked the muscimol-induced myoclonic jerks, the intensity of which paralleled the elevation of brain GABA level. It seems that, in this particular strain of mice, muscimol or one of its metabolites possibly acts on certain specific binding sites in the CNS eliciting myoclonic jerks and that these receptor sites are different from those to which GABA or THIP binds with high affinity.

The benzodiazepines (i.p. 10 min prior) also blocked the muscimol-induced jerks in a dose-dependent manner. When compared with diazepam, their relative potencies to cause a 50 percent blockade of muscimol response were: diazepam = 1, medazepam = 0.24, oxazepam = 1.27, flurazepam = 1.90, lorazepam = 3.01, nitrazepam = 3.93, clonazepam = 33.14 and flunitrazepam = 116.00. If our earlier assumption that the muscimol-induced myoclonic jerks originate at the level of the spinal cord is valid, the present method seems to offer a convenient means to evaluate the inhibitory effects of benzodiazepines on the spinal cord. If so, the present results indicate the possible value of flunitrazepam in the management of neurologic disorders in which preferential action on the spinal cord is desired. Supported by the Veterans Administration.

35.14 BACLOFEN: A SELECTIVE DEPRESSANT AT SYNAPSES MADE BY CA3 PYRAMIDAL CELLS IN THE HIPPOCAMPAL SLICE. Brian Ault* and J. Victor Nadler. Dept. Pharmacology, Duke Univ. Med. Ctr., Durham, NC 27710.

The anti-spastic drug, baclofen, has been proposed to act by interfering with excitatory transmission mediated by acidic amino acids. We have investigated the actions of this compound on transmission in slices of the rat hippocampal formation, where glutamate and aspartate are probable transmitters of several excitatory pathways.

(±)Baclofen (20 μM) strongly depressed extracellularly recorded synaptic responses of CA1 and CA3 pyramidal cells to stimulation of stratum radiatum (Schaffer collateral-commissural fibers) and responses of CA1 pyramidal cells to stimulation of stratum oriens. In contrast, responses of dentate granule cells to stimulation of perforant path or associational-commissural projections, responses of CA3 pyramidal cells to mossy fiber stimulation and responses of CA1 pyramidal cells to stimulation of temporo-ammonic fibers were little affected. Thus baclofen preferentially inhibited transmission at synapses made by axons of CA3 pyramidal cells. Although glutamate probably mediates transmission at these synapses, baclofen cannot be regarded as a blocker of glutamatergic/aspartergic transmission in general, since it failed to inhibit transmission at other such hippocampal sites.

The depressant action of baclofen was stereospecific. A threshold effect of (-)baclofen was obtained at 0.5 μM and 2.5 μM reduced the amplitude of the Schaffer collateral-commissural extracellular EPSP by about 50%. (+)Baclofen was two orders of magnitude less potent. The depressant action of baclofen on these synapses closely resembled, in potency and stereospecificity, its depression of spinal cord reflexes. Synapses made by axons of CA3 pyramidal cells may therefore serve as models for studying the mechanism of action of baclofen.

Baclofen has been proposed to act at bicuculline(BIC)-insensitive GABA receptors. We have therefore compared the effects of baclofen, GABA and the GABA agonists, 3-aminopropanesulfonic acid (3-APS) and imidazoleacetic acid (IAA), on the Schaffer collateral-commissural extracellular EPSP. GABA (3 mM), 3-APS (1 mM) and (±)baclofen (5 μM) similarly reduced the amplitude of this response. BIC (50-500 μM) only partially reversed the actions of GABA and 3-APS, whilst the response to baclofen was unaffected. IAA, which is thought to interact only with BIC-sensitive receptors, had relatively little effect on the extracellular EPSP, but depressed pyramidal cell firing. BIC (100 μM) reversed this action. These results are consistent with the hypothesis that baclofen inhibits excitatory transmission by interacting with a BIC-insensitive GABA receptor. (Supported by NIH grant NS 16064.)

35.15 NEUROPHARMACOLOGICAL CHARACTERIZATION OF A SELECTIVE TAURINE ANTAGONIST. G. G. Yarbrough, D. K. Singh* and D. A. Taylor. Merck Institute for Therapeutic Research, West Point, PA 19486.

6-Aminomethyl-3-methyl-4H-1,2,4-benzothiadiazine 1,1-dioxide hydrochloride (TAG), applied by microiontophoresis, caused a prompt and readily reversible antagonism of the inhibitory effects of taurine and β-alanine on rat somatosensory cerebral cortical neurons and cerebellar Purkinje neurons whilst not affecting the actions of γ-aminobutyric acid (GABA). TAG also consistently reduced, but did not abolish the synaptically evoked inhibitions of Purkinje cells produced by electrical stimulation of the cerebellar surface. On the isolated amphibian spinal cord (ASC), TAG caused a dose-related enhancement of the dorsal root-ventral root potential while not substantially affecting the dorsal root-dorsal root potential. Qualitative studies on the ASC showed that the depolarizing actions of taurine and β-alanine on the dorsal roots, but not those of GABA or glycine, were reduced by TAG. Somewhat surprisingly, TAG also offset the inhibitory effects on cortical cells and the dorsal root depolarizing actions of muscimol, 3-aminopropane sulfonic acid and piperidine-4-sulfonic acid, all of which have heretofore been considered as selective GABA agonists. Systemically administered TAG does not appear to penetrate the blood-brain barrier but intracisternal (i.cis.) injections in mice produced a dose-related behavioral excitation leading to marked clonus and death. Additionally, i.cis. TAG reduced taurine but not glycine-induced lethality in mice. These findings lend a substantial degree of credence to the repeated suggestions that taurine may subserve a neurotransmitter type function in the mammalian central nervous system. Furthermore, the availability of a relatively specific antagonist might reasonably be expected to facilitate efforts to elucidate the physiological functions of taurine not only in the CNS but in other organ systems in the body as well.

35.16 THE EFFECT OF HOMOCYSTEINE IN BRAIN. S.M. Wuerthele, W.J. Freed, B.J. Hoffer. Dept. of Pharmacology, Univ. Colorado Medical School, Denver, CO 80262, and Lab of Clinical Psychopharmacology, St. Elizabeth's Hospital, NIMH, Washington, D.C. 20032.

Homocysteine, a monocarboxylic sulfur-containing amino acid, produces convulsions in rats and mice when administered systemically (Sprince et al., Ann. N.Y. Acad. Sci. 166: 323, 1969). In order to evaluate its effect on the central nervous system directly, extracellular recordings were made from neurons in rat cerebral cortex, cerebellum and midbrain during local application of homocysteine by iontophoresis or pressure ejection. Homocysteine produced dose-dependent increases in the activity of 17 cells, while the activity of 5 cells was decreased. There was no regional specificity to these responses. When homocysteine and glutamate were ejected from different barrels of the same pipette, homocysteine was equipotent with glutamate in increasing neuronal activity. 3 of 4 cells inhibited by homocysteine were also inhibited by glutamate. Neither glutamate- nor homocysteine-induced excitations were affected by simultaneous application of magnesium, suggesting that these responses were the result of a direct action of the drugs.

Homocysteine can be methylated in brain ($^{5}NFH_4$homocysteine methylytransferase) and liver (betaine homocysteine methyltransferase, BHM) to form the nonconvulsive amino acid methionine. When applied locally, methionine had no effect on spontaneous activity. Betaine, an endogenous methyl donor derived from choline, blocks convulsions produced in mice by a number of systemically administered agents, including homocysteine (Freed et al., Epilepsia, 20: 209, 1979). The enzyme using betaine as a methyl donor (BHM) is not present in brain (Finkelstein et al., Arch. Biochem. Biophys. 146: 84, 1971); however, to determine if betaine had a direct effect, it was applied locally on central neurons by pressure ejection. When applied in this fashion, betaine reversed both homocysteine- and glutamate-induced increases in neuronal activity, while having no effect on spontaneous discharge. These results suggest that betaine's anticonvulsant properties are not the result of its action as a peripheral methyl donor. The fact that betaine derivatives are used as membrane solubilizers (Goenne and Ernst, Anal. Biochem. 87: 28, 1978) and that betaine antagonizes a number of different convulsants could suggest that betain's anticonvulsant effects are related to a direct action on membranes. Alternatively, since betaine blocks the excitatory response to glutamate, it is possible that betaine acts at the level of the glutamate receptor.

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35.17 RETROGRADE TRANSPORT OF L-[^{3}H]PROLINE BY CORTICAL LAYER V PYRAMIDAL CELLS. Simon LeVay and Helen Sherk*. Dept. Neurobiology, Harvard Medical School, Boston, MA 02115.

L-[^{3}H]proline has been used for many years as an anterogradely-transported neuroanatomical tracer. We were surprised to observe selective retrograde transport of this compound by giant layer V pyramidal cells in the visual cortex of the cat.

50μCi of L-[2,3-^{3}H]proline (S.A. 20-40 Ci/mmole), sometimes in combination with horseradish peroxidase (HRP), was injected into the pulvinar, superior colliculus (SC), a pontine visual area, four visual cortical areas, lateral geniculate nucleus (LGN) and claustrum. After a 1-3 day survival the animals were perfused with paraformaldehyde or glutaraldehyde and the brains frozen-sectioned and processed for autoradiography. Following injections in the pulvinar, SC and pons, giant pyramidal cells were labeled in layer V of several visual cortical areas including 17, 18, 19 and PMLS. The labeling extended into the apical and basal dendrites. Except after the pulvinar injection, there was no diffuse labeling of the cortical neuropil such as might have been produced by anterograde or transneuronal transport. Furthermore, in those cases where HRP was injected together with the [^{3}H]proline, the autoradiographically-labeled cells also contained HRP reaction product. It thus seems likely that their labeling resulted from terminal uptake followed by retrograde axonal transport. It may be that the same population of cells projects to all three targets.

The labeling was selective in three senses: 1) Other neurons projecting to the same injection sites (e.g. retinal ganglion cells and parabigeminal neurons after the SC injections) were unlabeled. 2) Other amino acids injected into the SC (L-[^{3}H]-glutamate, L-[^{3}H]leucine) were not transported to the cortex. 3) Cortical cells in other layers were not labeled when proline was injected into their appropriate targets (e.g. LGN, claustrum, associational and contralateral cortex).

The selective uptake and retrograde transport of tritiated amino acids (glycine, GABA, glutamate, aspartate) by different populations of CNS neurons has been taken as supportive evidence that these or similar compounds are used as neurotransmitters (Hunt et al., '77; Streit, '80; Baughman and Gilbert, '81). While there is no other evidence to implicate proline as the corticotectal neurotransmitter, the present finding suggests the possibility that it or a related compound may be involved in neurotransmission in this pathway.

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35.18 UTILIZATION RATES OF PUTATIVE AMINO ACID NEUROTRANSMITTERS IN RAT CEREBELLUM. M.E. Freeman, J.D. Lane and J.E. Smith. Psychiatry Research Unit, Department of Psychiatry, Louisiana State University Medical Center, Shreveport, LA 71130.

Glutamate (Glu) and gamma-aminobutyric acid (GABA) are thought to have a neurotransmitter role in the cerebellum. This study was initiated to determine the utilization rates of these and other amino acids in five regions of the rat cerebellum. Eight male F-344 rats were implanted with chronic jugular catheters and allowed to recover from surgery for 3-4 weeks, receiving periodic injections of saline through the catheter. 0.2mCi D-[U-^{14}C]-glucose was injected through the catheter either 60 (N=4) or 90 (N=4) minutes prior to sacrifice by total immersion in liquid nitrogen. The heads were removed and stored at -70°C. The cerebellum was removed at -20°C, cut into 0.5 mm saggital sections, and dissected into five regions: lateral cortex, deep nuclei, molecular layer, granular layer, and white matter. The tissue was homogenized in ice-cold 7 percent trichloroacetic acid. Content and specific activity of Glu, aspartate (Asp), GABA, glutamine (Gln), serine (Ser), glycine (Gly) and alanine (Ala) were determined by a previously described procedure (Freeman et. al., Anal. Biochem. 106:191-194, 1980). Levels of Glu, Asp, and GABA in the lateral cortex, deep nuclei, and molecular layer generally agree with previously published values (Rea et. al., Neurochem. Res. 6:31-37, 1981). Glu and Asp levels in the granular layer were significantly lower ($P < .001$, Student's t-test) than in the molecular layer; white matter levels were lower still ($P < .001$). GABA levels were significantly higher ($P < .01$) only in deep nuclei. The utilization rates were calculated from the fractional rate constants (K) which were determined from a semilogarithmic plot of the specific radioactivity at 60 and 90 min prior to sacrifice. $\text{Utilization}_A = K(\text{content}_A)$, where $K = \ln 2/t_{\frac{1}{2}}$. Asp and Glu rates of utilization were 2- to 8-fold higher than the control amino acid Ala. Glu utilization was significantly higher in the lateral cortex ($P < .01$) than in the other areas tested, supporting its proposed neurotransmitter role in this region. However, there were no significant differences in Glu utilization between the molecular and granular layers, suggesting possible involvement in both granular cells and parallel fibers. Asp utilization in the various regions was relatively similar to that of Glu, suggesting either a neurotransmitter role or a metabolic interrelationship with Glu. GABA utilization was significantly higher only in the deep nuclei ($P < .001$). These data support the proposed action of amino acids as neurotransmitters in the cerebellum. (Supported in part by USPHS Research Grant DA-01999-04).

35.19 NET CONSUMPTION OF AMINO ACIDS IN SELECTIVE REGIONS OF THE CNS IN INSULIN HYPOGLYCAEMIA. R. F. Butterworth, F. Landreville* and A. Merkel*. Lab. of Neurochemistry, Clinical Res. Inst. of Montreal, Montreal, Quebec H2W 1R7, Canada.

The neurological symptoms of hypoglycaemia have been suggested to reflect a depression of neural function in progressively descending levels of the CNS. Preliminary studies in our laboratory have indicated that certain modifications of glutamic acid, aspartic acid and GABA, amino acids essential to neuronal integrity, may be causally related to the neurological impairment associated with insulin hypoglycaemia since these changes were found to *precede* the appearance of symptoms (R. F. Butterworth and A. D. Merkel, Neurosci. Lett. 5: 446, 1980). The present study was undertaken to measure the net consumption of amino acids in specific regions of the CNS at various times during the development of insulin hypoglycaemia. Adult male Sprague-Dawley rats (180-200 g) were fasted 24 h., then administered insulin (NPH) 100 IU/kg, i.p. As blood glucose fell below 40 mg/100 ml, neurological symptoms (hindlimb weakness, mild catalepsy) appeared. More pronounced hypoglycaemia led to loss of righting reflex and convulsions. Amino acids were measured in discrete regions of the brain by the double isotope dansyl microassay previously described (Analyt. Biochem. 64: 389, 1975, J. Neurochem. 33: 575, 1979). No detectable consumption of *amino acids was detected in whole brain at times up to the onset of convulsive activity. However, the amino acid pool of cerebral* cortex and striatum decreased significantly by 1.7 μmole/g and 3.5 μmole/g respectively *prior to the onset of hypoglycaemic convulsions*. No changes were found at corresponding times in any other brain regions studied. These findings suggest that the neurological symptoms of insulin hypoglycaemia may be due, at least in part, to consumption of amino acids essential to neuronal function as alternative energy sources in selective regions of the CNS. That the amino acid pools in cerebral cortex and striatum are *selectively* decreased prior to hypoglycaemic convulsions is intriguing; it is precisely these regions of brain that have been shown to display the greatest signs of neuronal damage in insulin-hypoglycaemic rats and of patients dying in hypoglycaemia. (This work was supported by grants from the Banting Foundation, Toronto, and the United Cerebral Palsy Research and Educational Foundation).

36.1 PHYSIOLOGICAL AND ULTRASTRUCTURAL ALTERATIONS IN TRANSMITTER OUTPUT WITH DENERVATION AND OUABAIN AT LOBSTER NEUROMUSCULAR SYNAPSES. R.G. Chiang* and C.K. Govind. Scarborough College, University of Toronto, 1265 Military Trail, West Hill, Ont., M1C 1A4, Canada.

The single excitor motoneuron to the distal accessory flexor muscle on the walking legs of the lobster (Homarus americanus) forms high-output synapses amongst the distal fibers and low-output synapses amongst the proximal fibers (Meiss D.E. & C.K. Govind, 1979. J. exp. Biol. 79: 99-114). The transmitter release characteristics of these different synapses can be experimentally altered. Thus seven days after transecting the motor axon, the high-output synapses from the denervated muscle resemble low-output synapses from the control muscle on the contralateral leg. Conversely 10 Hz stimulation of the motor axon for 2 hr. in 10^{-4} M ouabain causes a significant increase in quantal release amongst low-output synapses as compared to their counter-parts on the contralateral side. Thus quantal release of transmitter is decreased by denervation and increased in the presence of ouabain.

Serial section electron microscopy of the physiologically identified synaptic regions in the denervated muscle showed a reduction in surface area of the terminals, synapses and presynaptic dense bars compared to the control contralateral muscle. In addition, there was a significant reduction in the size of dense bars per synapse. Comparison of the ouabain - treated synapses with their untreated counter-parts on the contralateral side showed no differences in the surface area of terminals and synapses, but an increase in the size of dense bars. Since the change in dense bar size is directly related to quantal output in both denervated and ouabain-treated muscles, the dense bars appear to be intimately associated with the release of transmitter. Whether they assist in the release of transmitter or are a by-product of transmission is unknown. They do however reliably measure activity at these lobster neuromuscular synapses. Supported by NSERC and MDA of Canada.

36.2 COMPARISON OF THE EFFECTS OF CYCLIC AMP AND NH_4^+ ON THE ELECTROPHYSIOLOGY OF THE VENTRAL WHITE CELL OF PLEUROBRANCHAEA. Rhanor Gillette. (SPON: L. Barr). Dept. of Physiol. & Biophys. and Neural & Behav. Biol. Program, Univ. of Illinois, Urbana, IL 61801.

The ventral white cell of Pleurobranchaea may be stimulated into prolonged, recurrent and endogenous burst episodes by food stimuli applied to the mouth of the animal (M. Gillette, this volume); these burst episodes drive multiple cycles of coordinated ouput in the feeding motor network (Gillette et al., 1980, J. Neurophysiol. 43:669). We have found that similar recurrent burst episodes may be stimulated both by cyclic AMP (Gillette et al., 1979, Neurosci. Abs.: 4) and by NH_4^+. A salient characteristic of stimulation by relatively low concentrations of cyclic AMP analogs and IBMX (10^{-5}-10^{-4} M) and NH_4^+ (15 mM, pH 7.5) is that the initially enhanced excitability of the cell progressively wanes in the continued presence of the agents. This is reflected in progressive increases in the interburst intervals. Agent washout is followed by pronounced decrease in excitability, which slowly recovers over 20-60 min.

In voltage clamp, triangular voltage ramps (-80-0 mv) reveal a negative slope region (NSR) between -35 to -25 mv in the IV curve induced by the agents, whose peak amplitude is greater on the down ramp than the up ramp, resulting in unconventional hysteresis. The NSR of the up ramp is reduced, and that of the down ramp abolished in nominally O-Ca^{++} saline. Analysis of current tails near V_K after depolarizing pulses shows relatively large slow inward tail currents with activation and decay times of seconds which are greatly reduced in O-Ca^{++} and Co^{++} salines. The amplitudes of such tails are markedly enhanced by both the drugs and NH_4^+; however while cyclic AMP-stimulated tail amplitudes decline slowly over 20-30 minutes after washout, the NH_4^+-stimulated tail amplitudes fall rapidly to control levels. The IV curves for cyclic AMP-stimulated cells also lose amplitude of NSRs only slowly. In contrast, the NSRs of NH_4-stimulated cells quickly decay after washout. It seems likely that the initial stimulation, slow waning, and post-washout depression of the cell excitability by NH_4^+ parallels rapid increase, slow recovery and post-washout undershoot of intracellular pH (Boron and DeWeer, 1976, J. Gen. Physiol. 67:91) and is in part due to accompanying changes in the slow inward current characteristics. It is hypothesized that increases in the Ca^{++}-activated K^+ conductance caused by intracellular Ca^{++} accumulation also contribute to the slow waning of excitability and post-washout depression for both cyclic nucleotide stimulation and NH_4^+. Supported by NSF-BNS-79-18329.

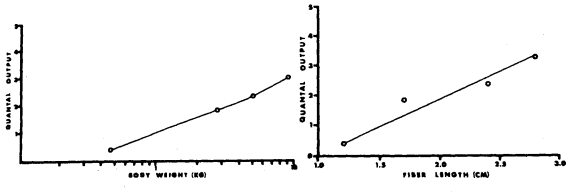
36.3 GROWTH OF LOBSTER NEUROMUSCULAR SYNAPSES: PHYSIOLOGY AND ULTRASTRUCTURE. D. E. Meiss, Dept. of Biology, Clark Univ., Worcester, MA 01610 and C. K. Govind, Division of Life Sci., Scarborough College, Univ. of Toronto, West Hill, Ontario, Canada M1C 1A4.

Neuromuscular synapses of the distal accessory flexor muscle (DAFM) of the lobster, Homarus americanus, form discrete populations which vary in transmitter release properties. The extremes are represented by low- quantal release synapses on proximally located muscle fibers and high-quantal release ones on the distally located fibers (Meiss and Govind, J. Exp. Biol. 79:99-114, 1979).

Growth of comparable low-output synapses was studied in lobsters of varying sizes in which there was an approximate 18-fold increase in body weight and a two-fold increase in muscle fiber length. Physiological properties of synaptic transmitter release were determined from focal microelectrode recordings at discrete synaptic regions. These analyses showed that transmitter quantal output increased proportionally with the logarithm of the body weight (Fig. 1A) and linearly with muscle fiber length (Fig. 1B).

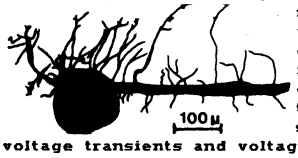
Serial thin section E.M. analysis (Govind and Chiang, Brain Res. 161:377-388, 1979; Govind and Meiss, Cell Tiss. Res. 198: 455-463, 1979) was done on physiologically identified synapses from the 0.5 kg and 5.0 kg animals. Synapses were characterized by a doubling in the mean size and the number of presynaptic membrane dense bars. Since these densities are regarded as active sites of transmitter release, their increase in size and number in the present study provides a morphological basis for the observed physiological increases in transmitter quantal output.

Fig. 1A Fig. 1B



36.4 **Aplysia BUCCAL GANGLION MOTORNEURONS: MORPHOLOGY AND ELECTROTONIC PROPERTIES.** **Katherine Graubard and William H. Calvin, Departments of Zoology and Neurological Surgery, University of Washington, Seattle 98195**

Many buccal motorneurons of Aplysia have large somata (150-250 mu), thick axons (15-20 mu), and a large number of thin to medium (1-10mu) processes leaving the axon to terminate in the neuropile within 350 mu. In addition, similar branches are often seen originating from the soma, a finding atypical of invertebrate neurons. Using two microelectrodes, current steps were applied and the voltage transients and voltage-current curve measured. The cells were filled with HRP or Lucifer and studied in whole mount. Electrotonic reconstructions (Rall 1959) yield estimates for membrane resistivities which are 10-100 times lower than the 500,000 ohm cm observed in abdominal ganglion (Graubard 1975). Retrograde voltage spread is 90-95%; indeed, the reversal for somatic ACh is within 90% of the chloride IPSP reversal potential (Gardner 1977).



A synapse at the tip of a thin process is nearly as effective in delivering current to the axon as is centrally-located synaptic input (Graubard and Calvin, The Neurosciences, Fourth Study Program, 1979). Whatever the membrane leakiness along the thin branch, the large central load will provide an easy path for the synaptic current; in this sense, the cell's geometry is the prime determinant of synaptic effectiveness, as seen from the axon, e.g., from the spike trigger zone. For synaptic output from a dendritic tip, however, neighboring inputs will be at least 20-40 times more effective than axonal inputs.

Voltage attenuation from tip to soma or axon was severe and could not be reduced by activating many inputs simultaneously (Turner and Calvin, Cell. Molecul. Neurobiol., 1981). The central load upon a dendrite is comprised of an irreduceable load (nonsynaptic membrane, such as the axon) and a load which can potentially be made invisible (simultaneous inputs to other branches preventing current flow into them). In lobster stretch receptors, most of the central load could be removed by activating other dendrites symmetrically. By reducing voltage attenuation, this greatly enhances the dynamic range compared to the one-input case, which has a limited current range between rheobase and saturation. In our buccal ganglion neurons, most of the central load is irreduceable, as it is dominated by the large axon. (NIH grants NS 04053 and NS 15697).

36.5 OPTICAL MEASUREMENT OF POTENTIAL CHANGES IN AXONS AND PROCESSES OF NEURONS OF A BARNACLE GANGLION. V. Krauthamer* and W.N. Ross* (SPON: S. Fraley) Dept. of Physiology, New York Medical College, Valhalla, N.Y. 10595.

Optical recordings of transmembrane voltages at multiple sites on single cells, combined with standard intracellular recording and staining methods, allows for an analysis of the regional properties of neurons. We have used this combination of techniques to examine cells of the supraesophageal ganglion of the giant barnacle, Balanus nubilus.

Ganglia were stained with one of several voltage-sensitive dyes, WW375, WW401, or NK2367 (see L.B. Cohen and B.M. Salzberg, Rev. Physiol. Biochem. Pharmacol. (1978) 83. p. 35. for review). The preparation was mounted on the stage of a Zeiss Universal Microscope and imaged with a x40 water-immersion lens onto a 6 x 6 square array of photodiodes, each element corresponding to 40 x 40 μm^2 in the object plane (A. Grinvald, W.N. Ross, and I. Farber, P.N.A.S. (1981) in press). Cells were impaled with a microelectrode and action potentials were evoked repetitively. The resulting changes in absorption, detected on each element at 750 ± 25 nm, were averaged on a laboratory computer. Optically detected action potentials could be seen without averaging from the cell bodies (typically 60 μm in diameter). Signals were also detected from antennular nerves and the cross-commissural connective. Extracellular recordings, Lucifer Yellow injections, and appropriate conduction times confirmed that the signals corresponded to action potentials propagating along single axons or large processes.

Similarly, when square hyperpolarizing current pulses were injected into the soma, optical signals corresponding to the electrotonic spread of these pulses were detected in axons over 500 μm away and in the commissural process. After correcting for distortions due to electronic filtering the optical records were compared with those predicted from a passive membrane model based on cellular geometry determined from Lucifer Yellow dye injections.

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36.6 SURVEY OF APLYSIA BUCCAL GANGLIA NEURONS FOR NEUROTRANSMITTERS. J.K. Ono, Division of Neurosciences, City of Hope Research Institute, Duarte, CA 91010.

The bilateral organization of the buccal ganglion of the marine mollusc, Aplysia californica, and the information available about synaptic circuits of the ganglia make it an ideal preparation for extensive surveys of neurons containing specific neurotransmitters. The buccal ganglia consists of symmetrically paired hemi-ganglia containing neurons which have anatomical and functional counterparts in each hemi-ganglia. Some of these neurons have been identified by other investigators as motoneurons (B15, B16) or as interneurons (B4, B5, S1's) mediating a variety of synaptic responses. Acetylcholine (ACh) has previously been identified as the neurotransmitter used by certain motoneurons and interneurons (B15, B16, B4 and B5) in this preparation.

Techniques for isolating individual neurons and sensitive assays for ACh, histamine, octopamine, serotonin, and dopamine permit measurement of these amines in individual neurons or pooled groups of small neurons. Preliminary data indicate that aside from the previously identified cholinergic neurons, many other buccal neurons contain significant amounts of ACh. Maps indicating the location of various buccal neurons which contain significant quantities of transmitter substances will be presented. (Supported by N.I.H. grant #NS-15583).

36.7 PHYSOSTIGMINE PRODUCES PROLONGED CALCIUM DEPENDENT ACTION POTENTIALS IN LEECH NEURONS. J.Yang* and W.M.King* (SPON: C.M.Lent). Neurosciences Section, Brown Univ., Providence, R.I. 02912.

Physostigmine (eserine), known primarily for its reversible inhibition of acetylcholine esterase, was found to prolong action potentials in the Retzius cells of the segmental ganglia of the American leech, Macrobdella decora. This effect occurred over a period of 10-15 minutes at bath concentrations of 1-10mM; these concentrations are several orders of magnitude greater than that required for acetylcholine esterase inhibition. Exogenously applied ACH, 10^{-5}M, sufficient to cause membrane depolarization had little effect on action potential duration. A short duration stimulus evoked bursts of action potentials and trains of subthreshold responses and eventually a 30-50mV, 200-400ms duration prolonged action potential. The prolonged action potentials showed a early depolarization followed by a late plateau phase. Ionic substitution of Na with choline in the perfusion medium showed Na dependence of the fast phase. The longer slow plateau persisted in Na free solution when Ca was present at a concentration of 2mM. Raising the Ca concentration increased the threshold of the prolonged action potentials. At 10mM Ca, it was not possible to elicit a prolonged action potential; however, in preparations which showed spontaneous activity, long duration plateaus were observed despite the inability to induce such a response electrically.

Intrasomatic iontophoresis of physostigmine was not effective in prolonging the action potential.

Preliminary results indicate that physostigmine produces prolonged action potentials in leech Retzius cells and this effect resembles that of tetraethylammonium in the same preparation and occurs at similar doses.

36.8 THE ACTIVE ZONE AT APLYSIA SYNAPSES: INTRAMEMBRANOUS ORGANIZATION. C. H. Bailey* and M. Chen. Center for Neurobiol. and Behavior, Depts. Anat., Physiol., and Psychiatry, Columbia Univ., P & S, and the N.Y.S. Psychiatric Institute, New York, N.Y. 10032.

The vertebrate active zone is characterized by differentiated paramembranous appostions that are coextensive with regions of specialized membrane and vesicle accumulation. An inability to visualize this full complement of morphological specializations in the molluscan nervous system has resulted in confusion over the most basic aspects of synaptic architecture and has raised the possiblity of fundamental differences in synaptic structure and perhaps function between Aplysia and the vertebrates.

Recently we have used selective cytochemical techniques to demonstrate the presence of discrete active zones at Aplysia central synapses and to describe the organization of the presynaptic area (Bailey, Kandel and Chen, J. Neurophysiol., 1981). En face views at the level of the presynaptic membrane reveal oval-shaped discs containing dense projections that can exist in regular hexagonal networks remarkably similar to the precise grid described in vertebrates. In the present study we have extended this analysis by exploiting freeze-fracture techniques to examine the intramembranous architecture of specialized vesicle-release zones at Aplysia synapses.

Our preliminary results correlate well with the appreciation of the presynaptic area obtained by selective staining. In thin sections, Aplysia active zones are often characterized by apposed membranes that curve gently inwards towards the presynaptic element. Consistent with this configuration, freeze-fracture replicas reveal presumptive active zones as shallow indentations (P-face) or slight elevations (E-face) of the presynaptic membrane. These sites often appear restricted to varicose expansions. Within these regions the cytoplasmic leaflet of the presynaptic membrane displays varying numbers of intramembranous particles and small plasmalemmal pockets thought to represent points of vesicle fusion. We are currently examining synapses under conditions of maximal stimulation to determine the precise arrangement of these membrane deformations. Postsynaptic intramembranous specializations have been observed infrequently. This may be related to the lack of a well-developed postsynaptic density at Aplysia synapses. In one instance the tip of an invading postsynaptic spine contained a loose aggregate of large (~ 10 nm) intramembranous particles that corresponded in extent with the clustering of presynaptic vesicles. This arrangement appears to be very similar to that described at vertebrate excitatory synapses.

The findings from both cytochemical and freeze-fracture studies suggest that the organization of Aplysia and vertebrate active zones can be quite similar and argue against fundamental mechanistic differences in synaptic transmission between molluscan and vetebrate neurons. (Supported by an Irma T. Hirschl Career Scientist Award.)

37.1 LOCALIZATION OF VAGAL CARDIOMOTOR NEURONS IN NEONATAL PIG. D.A. Hopkins, P.M. Gootman, N. Gootman*, S.M. DiRusso* and M.E. Zeballos*. Dept. of Anatomy, Dalhousie University, Halifax, N.S. B3H 4H7 and Dept. of Physiology, Downstate Medical Center, Brooklyn, NY 11203.

The neonatal pig has been used extensively as a model for the postnatal maturation of the neural control of circulation (Gootman et al., Reviews in Perinatal Medicine, 1979). To date there have been no anatomical studies of the locations of cardiac preganglionic neurons in the pig. In the present study the distribution of the medullary cells of origin of the cervical vagus (N=3) and of physiologically identified cardiac nerves (N=6) has been studied in neonatal pigs ranging in age from one day to two months. Following anesthetization with sodium pentobarbital or halothane, the cervical vagus was exposed or a thoracotomy was performed and cardiac nerves were electrically stimulated to determine their function. The cervical vagus and cardioinhibitory nerves were injected with 5-20 µl of a 30% horseradish peroxidase (HRP) solution. After 1-3 days survival, transverse sections were cut through the medulla oblongata and processed for HRP histochemistry (Mesulam, 1978). In addition, the brain of a 7-day old piglet was embedded in paraffin and serial sections were cut and stained with cresyl violet or cresyl violet and luxol-fast blue.

After injections in the cervical vagus, retrogradely labelled neurons were observed in the ipsilateral dorsal motor nucleus (DMV), in and ventrolateral to the nucleus ambiguus (NA) as well as occasionally in intermediate regions between the two major nuclei. There were also small numbers of labelled neurons in the nucleus of the solitary tract. After injections in cardiac nerves the majority of labelled neurons were found in very distinct and tightly packed groups of neurons ventrolateral to the NA. Fewer were seen in the NA and scattered ventrolateral to the NA. Only small numbers of labelled neurons were present in the DMV. The distribution of labelled neurons after cardiac nerve injections appeared to be similar at each age studied. Comparisons of serial paraffin sections and labelled sections suggest that a morphologically distinct cardiac nucleus is present in the pig. The characteristic appearance and location of the nucleus make it ideal for further anatomical and physiological studies of cardiac preganglionic neurons. Supported by MRC of Canada, NLBI Grant HL 28064 and the American Heart Association, Nassau Chapter.

37.2 DISTRIBUTION WITHIN THE BRAINSTEM OF VAGAL AFFERENTS IN THE DOG. C.L. Chernicky, K.L. Barnes, J.P. Conomy and C.M. Ferrario. Division of Research and Department of Neurology, Cleveland Clinic Foundation, Cleveland, Ohio 44106.

The distribution of vagal afferents was examined in the brainstem of 6 mongrel dogs (7-12 kg) with horseradish peroxidase (HRP) histochemistry. HRP was applied to the right or left nodose ganglion or the vagosympathetic trunk within 2 cm distal to the ganglion by either implantation of 30% HRP-acrylamide gel pellets or multiple microinjections of 30-50% HRP in TRIS buffer (pH 8.6). After 6-8 days the animals were anesthetized again (pentobarbital, 30 mg/kg, i.v.) and perfused transcardially with 1.0 L of normal saline (37°C) followed by 4.0 L of 5% glutaraldehyde in phosphate buffer (37°C) and a final wash of 2.5 L of cold sucrose buffer. The brainstem and ganglia were removed immediately and stored overnight in sucrose buffer at 4°C. Both the nodose ganglion and the brainstem (from C_2 to the caudal pons) were serially sectioned at 40-60 µm. Sections were processed for HRP histochemistry using tetramethyl benzidine (TMB) as the chromagen and examined for HRP reaction product with both bright and darkfield illumination.

The afferent fibers of the vagus nerve enter the dorsolateral medulla as a single bundle about 6 mm anterior to the obex. As the fiber bundle travels dorsomedially, afferents separate into a number of fascicles which reconverge and travel caudally in the solitary tract (TS). Labeled fibers exit the solitary tract throughout its rostral-caudal extent and are distributed within the following nuclei: the ipsilateral nucleus tractus solitarius (NTS), dorsal motor nucleus of the vagus (DMV), nucleus intercalatus (NI) and area postrema (AP). Fibers are also seen crossing in the nucleus commissuralis of Cajal and the commissure of the area postrema at the obex to enter the contralateral NTS and AP. The ipsilateral medial NTS and AP receive the heaviest projections of vagal afferents, while only moderate labeling is seen in the contralateral NTS and AP. No HRP reaction product was found in the contralateral DMV, NI or TS.

Although the distribution of the canine vagal afferents is similar to that in the cat, the heavy labeling in the area postrema more closely resembles that shown in the monkey.

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37.3 CYTOARCHITECTURE OF THE RAT NUCLEUS TRACTUS SOLITARIUS: CATECHOLAMINES, BLOOD VESSELS AND PEPTIDES. L.Y. Koda, J.F. McGinty, E.L.F. Battenberg, and F.E. Bloom. The Arthur V. Davis Center, The Salk Institute, La Jolla, CA 92037.

Clinical and experimental evidence has implicated central monoamine and peptide systems in blood pressure regulation. Discrete monoamine and peptide terminal plexuses are located in pressor-related areas of the medulla, particularly in the nucleus tractus solitarius (NTS) and central grey. The NTS complex contains the A2 noradrenergic and more rostrally the C2 adrenergic cell body groups. In addition, various peptides implicated in autonomic function are found in the region of the NTS. The present study is aimed at mapping the distribution of blood vessels and of catecholamine- and peptide-containing cell bodies and terminals within the NTS. Sprague-Dawley rats (200g) were prepared for cryostat glyoxylic acid induced monoamine fluorescence (GIF) or immunohistochemistry. Where blood vessel distribution was examined rats were additionally perfused with 3-5 ml of ice-cold 0.2% pontamine sky blue in Ringer's solution. Coronal serial frozen sections were prepared through the NTS. The GIF cell bodies within the NTS complex were found caudal to the obex and are therefore considered to be the A2 cell body group as defined by Dahlstrom and Fuxe. The A2 cell body group is composed of 900 (890 + 43 n = 3) small to medium sized (20-30 µm diameter) neurons. In general, they are located on the dorsal and lateral edges of the dorsal motor nucleus of the vagus. They extend bilaterally from 200 µm to approximately 1600 µm caudal to the obex where they meet dorsal to the central canal and continue down the midline approximately 200 µm caudal to obex. The cell population is densest 600-1000 µm caudal to the obex where 15-20 cell bodies are found bilaterally in a 20 µm thick section. The NTS also contains a dense GIF terminal plexus. Serial sections of the GIF terminal plexus have been analyzed, and a detailed mini-atlas of the region developed. Blood vessel density has also been quantified over the NTS complex. Qualitative examination reveals that the NTS is less vascular than the surrounding central grey. Gamma-MSH immunoreactive (ir) fibers moderately innervate or pass through the NTS. Rostral to the obex the gamma-MSH ir are seen intermittently, but are mainly dorso-medial within the nucleus. Caudal to the obex, the fibers move ventrally and laterally to form a moderately dense plexus just lateral to the dorsal motor nucleus of the vagus (possibly in the nucleus intercalatus of Staterini). Gamma-MSH ir fibers continue just dorsal to the central canal into the spinal cord. The immunohistochemical distribution of other peptides e.g., enkephalin and VIP is now being added to the detailed NTS maps of catecholamines and peptides (USPHS grants HL25457 and MH 29466).

37.4 REGIONAL CHANGES IN GLUCOSE METABOLIC RATE IN CNS ELICITED BY ELECTRICAL STIMULATION OF THE NUCLEUS TRACTUS SOLITARIUS IN RAT. D.J. Reis, C. Iadecola*, S. Mraovitch*, L. Tucker* and D.A. Ruggiero. Lab. of Neurobiology. Cornell Univ. Med. Coll. New York, NY 10021.

The intermediate one third of the nucleus tractus solitarius (NTS) is a major site of cardiovascular integration. While major ascending projections of NTS have been mapped by traditional anatomical techniques (e.g. Ricardo and Koh, Brain Res. 153:1, 1978), polysynaptic pathways from the "cardiovascular NTS" have not. We have used the [^{14}C]-2-deoxyglucose method (2-DG) to map changes in metabolic rate (GMR) elicited by electrically stimulating vasodepressor sites in NTS. Rats were anesthetized with chloralose (40 mg/kg), paralyzed, and artificially ventilated with blood gases maintained. The intermediate third of lat. n. of NTS was stimulated at most sensitive sites with pulse trains (2 sec on/ 1 sec off, 20uA, 10Hz, 55 min). At onset of stimulation, AP fell to 61 $\pm$ 8 from 121 $\pm$ 5 mmHg (n=5; $p<.005$) stabilizing after 10 min at 93 $\pm$ 5 mmHg. 2-DG was administered 10 min after the start of stimulation, timed arterial samples withdrawn and animals killed 45 min later. Operated non-stimulated rats were controls. Densitometry was performed by a TV-based image analysis system. After NTS stim. (n=5), GMR significantly ($p<.05$) increased in 25 areas. In Group I (increase $\geq$ 40%), most pronounced increases (percent of control) were ipsilateral in: lat. n.of NTS (53%), lat. commissural n. of X (60%), lat. parabrachial n. (46%) paraventricular n. (37%), lat. hypothal. area (40%), n. stria terminalis (60%), and lat. preoptic area (40%). Contralaterally, GMR increased in all of the above areas except for portions of lat. NTS other than the mirror focus. In Group II (increase 20-40%) GMR was increased in n. ambiguus (20%), area postrema (37%) ventromedial (22%) and parafascicular thalamic n. (31%), zona incerta (33%) and central n. of amygdala (35%). Group III (GMR was unchanged) included parvocellular, gigantocellular, and vestibular nn., all adjacent to electrode sites, cerebellar nuclei, and medial parabrachial n. We conclude: (a) Stimulation of the cardiovascular NTS increases GMR throughout brain in regions primarily corresponding to anatomically defined monosynaptic projections. (b) The exceptions, e.g. thalamus and preoptic region, may represent polysynaptic spread. (c) The absence of changes in GMR in medial parabrachial n. suggests that 2-DG and anatomical data may differ. (d) The absence of stimulus spread indicates that the 2-DG method can be used in conjunction with electrical stimulation to map anatomical projections. (e) Information from cardiovascular afferents remains largely confined to interconnected systems mediating cardiovascular and visceral control.

37.5 DEOXYGLUCOSE MAPPING OF CENTRAL NERVOUS SYSTEM STRUCTURES DURING CENTRAL CHEMORECEPTOR MEDIATED HYPERCAPNIC RESPONSES IN THE RAT. C.V. Rohlicek*, J. Ciriello and C. Polosa*. Department of Physiology, McGill University, Montreal. Canada H3G 1Y6.

Physiological experiments suggest the presence of CO_2 receptors in the region of the ventral surface of the medulla. However, the actual anatomical structures associated with these receptors and the neural pathways involved in the respiratory and cardiovascular responses to activation of these receptors remain uncertain. In the present study, the deoxyglucose method was used to functionally map central structures activated by hypercapnia. Peripherally chemodenervated male Wistar rats anaesthetized with urethane were instrumented with femoral venous and arterial catheters and tracheal cannulae and allowed either to spontaneously breath room air or paralyzed and made hypercapnic by artifically ventilating with a gas mixture of 10% CO_2 and 90% O_2. In the latter group, the increased arterial pressure and electrical activity in the recurrent laryngeal nerve were used as indication of the activation of central chemoreceptors. [^{3}H]deoxyglucose (300-500 µCi/100g) was injected intravenously as a single bolus and after 45 min the animals were decapitated and the brains removed and quickly frozen on dry-ice. Serial transverse sections at 8µm were cut with a cryostat (-26°C) and one in every 10 was dried on cover glass and placed in contact with [^{3}H] sensitive film for 3-9 weeks. Autoradiographs were developed and analyzed for areas having the greatest metabolic activity as indicated by the density of photographic emulsion. Sections of the brainstem from hypercapnic rats, in comparison to those of the control rats, revealed marked increases in density bilaterally in the ventrolateral region of the solitary n. rostral to the obex, around the region of the n. retroambiguus, and in a region of the ventrolateral medulla extending rostrocaudally from the obex to the level of the intramedullary rootlets of the facial nerve. The cervical and thoracic cord of both the hypercapnic and control groups was more dense than the lumbar cord. In both groups the dorsal aspect of the dorsal horn and the region of the lateral horn, as well as lamina X in the thoracolumbar cord of the hypercapnic rats, were more dense than the surrounding grey matter. These data provide a functional map of central structures activated by hypercapnia suggesting that these structures are involved in the CO_2 responses of respiratory and sympathetic neurones.

Supported by the Canadian MRC and Heart Foundation.

37.6 IDENTIFICATION OF SPECIFIC CELLS IN THE CNS THAT ARE FUNCTIONALLY ACTIVE DURING CARDIOVASCULAR REFLEXES USING THE [^{14}C] DEOXYGLUCOSE TECHNIQUE.

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Recent CNS mapping studies of cardiovascular reflexes in our laboratory using the [^{14}C] deoxyglucose technique of Sokoloff, et al. (J. Neurochemistry 28:897-916, 1977) have demonstrated that functional metabolic activity increases substantially in specific nuclei within the CNS; i.e. nucleus tractus solitarius, nucleus ambiguus, external cuneate nuclei, inferior olivary nuclei, paramedian reticular nucleus, parabrachial nucleus, interpeduncular nucleus, hippocampus, and insular cortex, in response to stimulation of carotid sinus and vagal afferents. Although metabolic activity appears to be increased throughout an entire nucleus or region of the CNS, the localization of specific cells or groups of cells within a nucleus that are part of a specific reflex pathway cannot be obtained from gross observations of the autoradiographic data. Therefore, a technique has been devised to locate specific cells or groups of cells that are functionally and metabolically activated in specific reflexes. The frozen 20 um brain slices that are used to produce the autoradiographs are stained using a modified cresyl violet staining procedure and photomicrographs of these stained sections magnified 40X, 80X, or 160X are then montaged. The corresponding autoradiographs of the same sections are densitometerized using an aperature size of 100 um^2. An overlay of the numerical printout of the densitometer scan can then be photographically scaled on clear acetate to the same magnification as the photomicrograph of the stained section. The numbers with the highest values represent the areas of greatest metabolic activity. Therefore, since each number corresponds to an area 100 um^2, specific cells or groups of cells can be identified and functionally related to the reflex that was activated. Data obtained from serial brain sections using this type of analysis can be used to construct 3-dimensional plexiglass models of the cellular structure of various brain segments and specific nuclei. More importantly, specific cells or groups of cells within those nuclei that are involved in a specific reflex pathway can be identified. This method of analysis of [^{14}C] deoxyglucose data allows one to determine the physiological function of specific cells or groups of cells within the CNS. (Supported by NIH Young Cardiovascular Investigatator Grant HL 21042 and the VA Medical Research Service).

37.7 SUBNUCLEAR ORGANIZATION AND TOPOGRAPHY OF FOREBRAIN INPUTS TO THE NUCLEUS TRACTUS SOLITARIUS OF THE RABBIT. Gerald A. Higgins* and James S. Schwaber. Dept. of Anatomy and Neurobiology, University of Vermont School of Medicine, Burlington, Vermont 05405

Recently we have described in the rabbit direct projections from the central nucleus of the amygdala (CE) and bed nucleus of the stria terminalis (BST) that selectively innervate certain regions of the nucleus tractus solitarius (NTS) and dorsal vagal nucleus (DVN). Stimulation of the CE of the rabbit produces a profound, short latency decrease in heart rate that is mediated by the vagus nerve (Kapp et al, Soc.Neurosci.Abstr.,Vol.6,p.817, 1980). This effect may be mediated directly by forebrain inputs to vagal motoneurons, or indirectly by affecting baroreceptor afferents within the NTS. The aim of this report is to examine the morphological substrate for forebrain-visceral afferent interactions within the NTS.

Cytoarchitectonic studies show that the NTS of the rabbit is composed of eight distinct subnuclei: dorsomedial, commissural, intermediate, interstitial, lateral, medial, parvocellular and ventrolateral.

In initial experiments using standard neuroanatomical techniques, afferents were traced to the NTS from the CE, BST, aortic nerve (AN), carotid sinus nerve (CSN) and cervical vagus nerve (VN). The dorsomedial subnucleus contains medium-sized, fusiform cells, abundant fibers, and receives heavy projections from the CE, BST and AN, as well as inputs from the CSN and VN. The intermediate subnucleus is composed of a dorsal zone receiving VN input and a ventral zone receiving CE and BST afferents. The ventrolateral subnucleus contains medium and large-sized, darkly staining neurons and receives CE, BST, CSN and VN inputs. The medial subnucleus receives AN, CSN and VN afferents but is spared by CE and BST inputs. The interstitial subnucleus receives CSN and VN inputs. The lateral subnucleus and regions bordering the DVN and area postrema/IV ventricle receive CE and BST afferents. The commissural subnucleus receives minor projections from all these afferent sources. The parvocellular subnucleus receives VN input.

Additionally, regions of the NTS receiving forebrain inputs project to other cardioregulatory nuclei. For example, the dorsomedial, ventrolateral and ventral intermediate subnuclei project to the region of the intermediolateral cell column of the thoracic spinal cord. The ventral intermediate subnucleus, parts of the medial subnucleus and the NTS-DVN border region project to the BST. The NTS also projects to the vagal motor nuclei.

Forebrain inputs to regions of the NTS involved in cardiovascular regulation provide a substrate for early modulation of the baroreceptor reflex.

(Supported by USPHS grant NS16107 and AHA grant 79-1017)

37.8 ULTRASTRUCTURAL LOCALIZATION OF SEROTONIN IMMUNOREACTIVITY WITHIN THE FELINE NUCLEUS TRACTUS SOLITARIUS. B. Maley and R. Elde. University of Minnesota Medical School, Minneapolis, MN 55455

In a previous account we have shown a differential distribution of serotonin (5-HT)-like immunoreactivity at the light microscopic level within various subdivisions of the cat nucleus tractus solitarius (NTS). The immunoreactivity in NTS appears as varicose fibers or punctate structures. Using a modification of the Sternberger peroxidase, anti-peroxidase (PAP) method we have identified 5-HT-like immunoreactivity at the ultrastructural level in the NTS.

Subsequent to perfusion of 4% paraformaldehyde-0.1% glutaraldehyde in 0.12M phosphate buffer, 100 µm coronal sections of the NTS were stained using rabbit anti-5-HT followed by the PAP procedure, reacted with 3-3'diaminobenzidine and hydrogen peroxide, osmicated, dehydrated and embedded in Spurr's resin. Absorption controls revealed the antiserum to be specific for 5-HT-like immunoreactivity. At the electron microscopic level the morphology of 5-HT-like immunoreactive varicosities was similar throughout all subdivisions of the NTS. Structures typically labeled within NTS measured less than 1 µm and contained a population of dense core vesicles and a variety of clear vesicles. The label was found to be distributed along the membranes of vesicles, mitochondria and the plasmalemma of the 5-HT varicosities. Although the labeled 5-HT varicosities were found to be distributed throughout the neuropil of the NTS, they were infrequently observed to be in synaptic contact with neuronal structures. When found in contact, 5-HT terminals were presynaptic to spines and dendrites of NTS neurons.

Results from this study indicate a distinctive morphology for 5-HT profiles in NTS. This sets them apart from substance P or methionine-enkephalin identified terminals which we have previously described for NTS. Furthermore the paucity of synaptic contacts involving 5-HT terminals suggests a modulator role for serotonin in NTS.

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37.9 ULTRASTRUCTURAL LOCALIZATION OF MET-ENKEPHALIN, SEROTONIN AND SUBSTANCE P IN THE CAT INTERMEDIOLATERAL CELL COLUMN. V. Holets and R. Elde. Department of Anatomy, University of Minnesota Medical School, Minneapolis, MN. 55455

Met-enkephalin (ME), serotonin (5-HT) and substance P (SP) immunoreactive fibers are prominent in the intermediolateral cell column (IML) in the thoracic spinal cord of the cat, and are thought to play a role in the integration and control of the preganglionic sympathetic neurons. Using an immunohistochemical method to identify the ME, 5-HT and SP immunoreactive terminals in the IML of the cat at the electron microscopic level, the synaptic relationships of the three putative neurotransmitters with the IML neurons were investigated.

Pre-embedding staining for the localization of ME, 5-HT and SP immunoreactivity was accomplished using a modification of the Sternberger peroxidase, anti-peroxidase (PAP) technique. Adult cats were perfused with buffered 4% paraformaldehyde-0.1% glutaraldehyde, 100 µm transverse Vibratome sections of the thoracic spinal cord were cut, stained using rabbit anti-ME, -5-HT or -SP antisera, processed for the PAP procedure, osmicated, dehydrated and embedded in Spurr's resin. Specificity of staining was determined using antisera pretreated with the homologous antigens. The absorption controls indicated that the immunoreactivity observed was specific for each antisera.

ME and SP immunoreactive terminals have similar characteristics in IML. They measured 0.5-2 µm, and contained round clear vesicles (approximately 50 nm in diameter) and a few dense core vesicles. The ME and SP terminals were observed to make synaptic contacts with dendrites, dendritic spines and perikarya in IML. Reaction product was seen over the vesicles, as well as along the membranes of mitochondria and the plasmalemma of ME and SP positive boutons. The 5-HT immunoreactive terminals measured 0.2-1.5 µm, and contained large, dense core vesicles. They were found in apposition to IML neuronal elements, including cell bodies, dendrites and dendritic spines, but rarely formed synaptic contacts with these structures. The reaction product was observed over the vesicles and other cytoplasmic structures in the 5-HT positive terminals similar to that which was observed in the ME and SP terminals.

The presence of ME, 5-HT and SP immunoreactive terminals and synaptic contacts with IML neuronal elements suggests that these putative neurotransmitters may have a direct influence on the IML neurons in the cat thoracic spinal cord.

Supported in part by DA01248, a grant from the 3M Foundation, and a Scholars in Neuroscience Award from the McKnight Foundation.

37.10

WITHDRAWN

37.11 ORGANIZATION OF CELLS GIVING RISE TO FIBRES TO THE STELLATE GANGLION IN THE CAT. S.B. Backman, J. Ciriello and J.L. Henry. Dept. of Physiology, McGill Univ., Montréal, Canada, H3G 1Y6.

While early anatomical and electrophysiological studies indicated that the cell bodies of sympathetic preganglionic neurones are located in the spinal intermediate grey region, recent HRP studies have demonstrated their organization to be more complex than previously thought. However, it remains controversial how far rostral these cell bodies extend and also what intraspinal path their axons follow before they exit from the cord. These controversies were approached in this study by using the retrograde transport of HRP combined with both a more sensitive method for the visualization of reaction product and longer survival times than previously used. Thirty µl of a 20-50 % solution of HRP in either distilled water or in 2% dimethylsulfoxide were injected into the right stellate ganglion. After survival periods of 45-120 hrs, 40 µm transverse, saggital or horizontal frozen sections were cut and processed according to the tetramethyl benzidine method of Mesulam (J. Histochem. Cytochem., 26:106-117, 1978). Labelled neurones were seen in segments C_7-T_8 predominantly ipsilateral to the site of injection in the main body of the lateral horn, the finger-like projections extending into the lateral funiculus, a thin band extending medially from the lateral horn and the region of the central canal. Additionally, a few labelled neurones were also observed ipsilaterally in the ventral horn of segments C_6-T_3 and others contralaterally near the central canal. Labelled neurones in the main body of the lateral horn of thoracic segments were in a single group whereas those in the cervical segments which appeared to belong to the main body were in 3 groups. In thoracic segments cells of the main body were spindle shaped and had their dendrites aligned primarily rostrocaudally whereas in cervical segments they were triangular and their dendrites were aligned mediolaterally. Cells medial to the main body were morphologically similar to those in the cervical segments. Dendrites of these medial cells extended laterally into the main body while others extended to either side of the central canal. The efferent path of most axons was along the lateral border of the ventral horn, some axons travelling first laterally through the dorsal part of the ventral horn to join the main bundle. With regard to the rostrocaudal paths of these axons, in experiments where the sympathetic chain was cut just caudal to the T_1 ramus, labelled neurones were observed to end abruptly at the caudal end of T_1 demonstrating that axons do not travel rostrally within the cord before their exit.

(Supported by the Canadian Medical Research Council and Canadian Heart Foundation).

37.12 ASSOCIATION OF VASOACTIVE INTESTINAL POLYPEPTIDE-IMMUNOREACTIVE NERVE FIBERS WITH THE CARDIOVASCULAR SYSTEM OF GUINEA-PIGS. R.E. Papka*, N.G. Della*, J.B. Furness*, and M. Costa*. (SPON: J. Willoughby). Dept. Anat., Univ. KY, Lexington, KY 40536, Ctr. Neuroscience and Depts. Morph. and Physiol., Flinders Univ. of South Aust., Bedford Park, South Aust. 5042

Recent immunohistochemical studies have shown certain neuropeptides are widely distributed in the body. One of these, vasoactive intestinal polypeptide (VIP), can cause dilation of certain blood vessels. In spite of this fact there has not been a systematic study of the association of VIP-immunoreactive fibers with cardiovascular tissue. In the present work, the distribution of VIP-immunoreactive nerves was studied in the heart and blood vessels of guinea-pigs in whole mounts.

A double antibody labelling technique was used. Preparations were first exposed to antiserum raised in rabbit against authentic VIP, diluted 1:200. An FITC-conjugated sheep anti-rabbit antiserum at a 1:40 dilution was used as the second antiserum. The following tissues were examined: pericardium, atria, bicuspid valves, major arteries and veins and individual vascular beds, e.g. cerebral, uterine, mesenteric, renal and skeletal.

In the pericardium VIP-immunoreactive nerve fibers were quite sparse; individual, delicate varicose fibers were not necessarily associated with blood vessels. Immunoreactive fibers in the bicuspid valves formed a very fine plexus. In the atria such fibers were readily evident but did not form a definite plexus; the fibers were single or two fibers travelled together. Major elastic arteries had a sparse plexus of VIP-immunoreactive fibers which extended to the adventitia-media junction. The density of immunoreactive nerves varied in different regional vascular beds. Uterine arteries had a dense perivascular plexus of fibers (however, these fibers terminated 2.0-2.5mm proximal to the uterine wall). A substantial number of fibers were associated with cerebral vessels although they were fewer than those with the uterine arteries. A moderate perivascular plexus of fibers was present on the larger mesenteric vessels supplying the gut; however regional variation was noted in that the VIP-immunoreactive nerve fibers of arteries to the large bowel remained dense up to the gut wall whereas those around arteries supplying the small bowel progressively decreased in number until only a rare fiber was evident at the small bowel wall. Renal vessels and those in skeletal muscle (e.g. femoral artery) had only an occasional fiber associated with them. The present study was established that VIP-immunoreactive nerve fibers are widespread in the cardiovascular system and that there are marked differences in the density of innervation of different beds.

37.13 THE RETE MIRABILE: AN SEM/TEM STUDY. E. M. Burns, RSM, Ph. D., B. Braverman*†, M. D., T. W. Kruckeberg*, M. S., P. K. Gaetano*, A. S., G. D. Dobben*, M. D., and M. Shulman*†, M. D. Dept. of Nursing Science Laboratory, University of Illinois Medical Center, Chicago, IL 60612, and †Illinois Masonic Medical Center, Chicago, IL 60657.

In recent years the unanesthetized goat has been used as an experimental model for the study of cerebral circulation.

The flow of arterial blood to the goat brain is as follows: external carotid → internal maxillary → rete mirabile → internal carotid → circle of Willis → cerebral arteries. Ipsilateral cerebral blood flow can be measured after blocking all extracerebral branches of the internal maxillary artery, by an electromagnetic flow probe placed around the internal maxillary artery.

The rete mirabile consists of a subdural arterial network in the cavernous sinus. It is located intra-cranially on each side of the pituitary, within the sella turcica. The rete is thought to play an important role in cerebrovascular hemodynamics. Previous physiological, pharmacological and gross anatomic studies suggest that it is innervated via the sympathetic nervous system.

The purpose of this study was to investigate the fine structure of rete mirabile innervation.

By means of scanning electron microscopy (SEM) and transmission electron microscopy (TEM) we observed nerve tracts and neuro-effector junctions.

Nerve terminals contained numerous vesicles with electron dense cores approximately 86 nm in diameter as well as numerous spherical electron lucent vesicles approximately 53 nm in diameter. This evidence, although not conclusive, indicates the possibility of adrenergic innervation of the rete mirabile.

Technical assistance of I. Kairys and L. Vedegys, Dept. of Electron Microscopy, University of Illinois Medical Center, is gratefully acknowledged.

SYMPOSIUM

38 NEUROBIOLOGY OF DENDRITES. R. Llinás (Chairman; New York University Med. Ctr.), S. J. Redman* (Australian Natl. University), D. S. Faber (State University of New York at Buffalo), J.-P. Changeux* (Institut Pasteur).

This symposium concerns broad aspects of dendritic function and plasticity. It will cover the ionic basis for their electrical excitability, their role in neuronal integration, the electrophysiological modification which occurs in dendrites following axotomy, and dendritic growth and development in the central nervous system. In particular, electrophysiological properties of mammalian central neurons will be covered by Dr. Llinás. Emphasis on dendritic excitability and the ionic mechanisms involved in neuronal oscillation will be considered. Studies presented will relate to electrical activity in cerebellar, inferior olivary, and hippocampal neurons, with special reference to voltage-dependent calcium conductances and to the calcium-dependent potassium conductance which forms the basis for the dendritic spiking in these cells. Dr. Redman's topic will be concerned with the role of synaptic axo-dendritic input in neuronal integration, taking as a model his recent work on distribution of Ia terminals on mammalian alpha motoneurons. His research has dealt quite specifically with the correlation between the morphology of Ia inputs and the electrophysiological properties of the synaptic potentials evoked by these terminals. Dr. Faber will present results that elucidate some aspects of the regulatory mechanisms which follow Mauthner cell axotomy. The electrophysiological properties of Mauthner cell soma and dendritic membranes will be described in the context of three basic issues: (1) regulation and distribution of voltage-sensitive and transmitter-sensitive membranes, (2) the topographic localization of different afferent fibers to specific regions of the target neuron, and (3) interactions of discrete postsynaptic potentials. Dr. Changeux will consider the question of synaptically mediated depolarization of the dendritic processes in mammalian neurons. The topic will relate to those aspects of embryogenesis which may be important in stabilizing certain synaptic inputs and in allowing others to be selectively removed. In particular, the question of multiple innervation of climbing fibers on Purkinje cells during ontogeny and their reduction to a single terminal in the adult animal will be considered as one of the models in which selective stabilization is discussed.

39

NOT AVAILABLE

40.1 MONOCLONAL ANTIBODIES LABEL SUBSETS OF AXONS IN THE CNS OF THE LEECH. S. Hockfield, B. Zipser and R. McKay*. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y. 11724.

Monoclonal antibodies (mABs) have been generated against the CNS of the leech, Haemopis marmorata. Several of the mABs recognize subsets of neuronal cell bodies, axonal processes and axonal endings within the leech CNS. Each ganglion in the leech nerve cord is joined to the next by a large bundle of axons (the connective). While a segmental ganglion contains about 400 neurons, the connective between each neighboring pair of ganglia contains over 5,000 axons. This study examines the organization of axons in the connective using mABs.

mABs were generated by immunizing mice with homogenized leech CNS, fusing the mouse spleen cells with mouse myeloma cells and growing the resultant cell lines as monoclonal cultures. mAB binding to leech CNS was demonstrated immunocytochemically. Connectives were embedded in gelatin, sectioned (in cross section) and processed for immunocytochemistry. Sequential serial sections were treated with different mABs and either observed at the light level or further processed for electron microscopy.

Each of the mABs used in this study recognizes subsets of neuronal cell bodies in the leech CNS. Each also recognizes subsets of axons within the connective. Comparisons between adjacent sections processed with different mABs show that each mAB labels different subsets of axons. The connective is composed of two large lateral connectives and one smaller medial connective. Lan 3-9, a mAB that labels 10 neurons in the supraesophageal ganglion, labels large diameter axons in the medial connective throughout the length of the leech nerve cord. Lan 3-2, a mAB that labels the four nociceptive neurons in each segmental ganglion, labels several bundles of very small diameter axons in the ventro-lateral part of each lateral connective. Lan 3-6, a mAB that labels the pressure neurons and some small unidentified (as yet) neurons, labels a few bundles of axons in the medio-lateral part of each lateral connective with diameters slightly larger than those labeled by Lan 3-2. These positions and axonal diameters are essentially maintained throughout the entire nerve cord and are consistent between leeches.

These results demonstrate the usefulness of monoclonal antibodies for studying the leech CNS. Axons with stereotypical positions and morphology can be identified in the connective of the leech, much like neurons can be identified in each ganglion. The leech CNS, like the vertebrate CNS, contains consistently identifiable fiber tracts that may subserve specific functions.

40.2 MONOCLONAL ANTIBODIES AGAINST THE DROSOPHILA NERVOUS SYSTEM. S.C. Fujita‡ A. Ferrus‡ S.L. Shotwell‡ and S. Benzer‡(SPON: J.M. Nerbonne). Div. of Biology, California Institute of Technology, Pasadena, CA. 91125

Monoclonal antibodies against neural tissues have revealed extraordinary specificity (e.g.Zipser and McKay, Nature, 289, 549 (1981)). To apply this powerful technique to Drosophila, where genetic manipulation is feasible, we have established hybridoma lines that produce monoclonal antibodies binding to fly neural tissues.

Mice were immunized by injecting homogenates of brains cleanly dissected from freeze-dried flies. Standard technique was used for fusion with NS-1 cells. Culture supernatants were screened by indirect immunofluorescence on unfixed, frozen sections of fly heads.

Of 177 microtiterwells tested, 81 were positive for staining in some tissue in fly head sections. 61 stained the brain. Among the latter, 20 stained only the brain, while others stained also some other tissues (retina, muscle, hypoderm, etc.). 30 independent hybridoma lines were established by limiting dilution cloning. We describe here 4 examples of lines producing antibodies that bind to the central nervous system.

Antibody B7 intensely stains nerve fibers in the brain and thoracic ganglion, and all their major nerves such as the antennal and leg nerves. Fine fibers running between muscle cells, such as indirect flight muscle and proboscis muscle, are visualized. This antibody apparently stains axons only.

Antibody D10 gives intense and rather homogeneous staining in most neuropil regions of the brain and thoracic ganglion. In the lamina only a single layer of cell bodies stain. Weak staining occurs in the walls of the ventriculus and intestine; this may be associated with nerves innervating the intestinal muscles.

Antibody 2A1C stains the brain and thoracic ganglion to varying degrees in different regions. In general, the neuropil just beneath the cortex stains most. Major nerves stain less intensely, and weakly-staining fibers can also be seen in the retina, between muscles, and in the walls of the gut.

Antibody D12A gives fairly strong staining of fibers in the entire brain and thoracic ganglion, with regional variations of intensity. It also stains the major nerves with some fibers standing out brightly. Fine fibers are also seen between muscles.

An extensive set of such antibodies can be expected to be useful in studying development of the nervous system and in defining the anatomical and molecular defects in neurological mutants. (Supported by NSF grant PCM-7911771).

40.3 CELL-SURFACE SPECIFIC MONOCLONAL ANTIBODIES TO CHICK CILIARY GANGLION NEURONS. K.F. Barald. Neuroscience Program, Anatomy Dept. University of Michigan Medical School, Ann Arbor, Michigan 48109.

The ciliary ganglion of the embryonic chick is a parasympathetic ganglion of neural crest origin that contains two populations of neuronal cells both of which are cholinergic (CG neurons).

Monoclonal antibodies to these cells were produced by injecting individual mice with 1.2×10^6 cultured ciliary ganglion neurons. After 2 subsequent boosts with the same number of neurons, isolated spleen cells were grown for 4 days in the presence of a lawn of ciliary ganglion neurons and two mitogens, dextran sulfate and lipopolysaccharide (25 and 10 µg/ml respectively).

Hybridomas isolated after fusion of the spleen cells with an NS-1 parent myeloma line and subsequent cloning, produced monoclonal antibodies directed against ciliary ganglion neuronal cell surface. Two of the clones, designated CG-1 and CG-4 produce antibodies to ciliary but not surface or internal components of seven other neuronal populations from the embryonic chick or quail. These include spinal cord neurons (SC) and other parasympathetic neurons of neural crest origin. Neither antibody reacts with cell surface or cytoplasmic components of a variety of non-neuronal cells tested including skeletal muscle myotubes and myoblasts, or cultures of heart, liver, or "fibroblasts". Neither reacts with brain.

Another hybridoma clone, CG-5, produces antibodies that react with all neuronal cells but not non-neuronal cells tested. Antibody made by a fourth clone, CG-2 can be used to identify a small population of spinal cord neurons in addition to CG neurons.

The antibody made by clone CG-1 detects an antigen expressed in vitro by a small fraction ($\leq$5%) of cultured cranial neural crest cells from the embryonic chick isolated 31 hrs. after fertilization. The CG-1 antibody is cytotoxic for the crest cells it labels, as well as for the majority (>95%) of cultured ciliary ganglion neurons from embryos 8 days in ovo in the presence of complement and small amounts of antibody CG-4. In contrast, none of the cells from the trunk neural crest tested in vitro bound detectable amounts of either antibody. Neither antibody alone or in combination was cytotoxic for these cells in the presence or absence of complement.

Antibody CG-1 binds to chick ciliary ganglion neuronal cell bodies and neurites at all times tested in cell cultures isolated from embryos 7-18 days in ovo. None of the other neuronal or non-neuronal cells tested, with the exception of the ciliary ganglion neurons from embryonic quail, expressed the antigens at any time. This suggests that the neural crest contains a population of cells that can be distinguished early in development by its cell surface components.

40.4 MONOCLONAL ANTIBODIES AGAINST TORPEDO SYNAPTOSOMES. P.D. Kushner and L.F. Reichardt. Department of Physiology, University of California, San Francisco, San Francisco, CA 94143.

In order to gain a more thorough molecular understanding of synapses, their architecture, function and development, we have generated a library of monoclonal antibodies against the cholinergic synaptosome of the Torpedo ray electric organ. This organ, massively innervated by cholinergic terminals, is the purest source of synaptosomes of one transmitter type. Synaptosomes were prepared using standard isolation techniques modified to insure as pure a presynaptic fraction as possible (Hooper, Deutsch and Kelly, in prep.). Balb/C mice were immunized on days 1 and 22 IP with 50 ug protein and on day 59 IV with 20 ug protein. Fusion with NS1 cells and hybridoma selection with HAT medium were standard.

From one fusion 192 cell lines tested positive for binding to the highly purified synaptosomes, using the standard plate assay and an iodinated anti-kappa probe. Of these lines, 130 were cloned. Specificity tests, again using the plate assay, were performed in collaboration with R. Kelly. Homogenates of elasmobranch whole brain, electromotor nucleus, purified vesicles, and liver, revealed that most of the antibodies were nervous system specific: fewer than 10% bound liver and of these less than 5% cross reacted strongly with liver. Results indicated several different classes of antibodies, those binding only synaptosome and those binding other tissues in addition, for instance, vesicles, electromotor nucleus, etc.

Immunocytochemistry confirmed that most of the antibodies bound the innervated face of the electric organ. Likewise different binding patterns along this inenrvated face indicated different classes of antibodies.

With immunocytochemistry we have looked at the binding patterns of members of the library on a variety of frog and rat tissues, both central and peripheral to identify possible localization to synapses or cholinergic neurons. At the neuromuscular junction of the frog we see a variety of staining patterns. Some antibodies appear to be directed against a determinant within the terminal, others to a determinant in the muscle, others to both. The frog sympathetic ganglion with its well-defined regions of axosomatic synaptic contacts has been particularly useful in revealing interesting antibodies. Many of the antibodies cross react with the rat CNS and reveal distinctive patterns. For instance, there is a class of antibodies which stain the perimeters of the neurons of the dentate nucleus quite strongly.

We are currently identifying the antigens and specificities defined by the more interesting clones.

40.5 IMMUNOCYTOCHEMICAL LOCALIZATION OF CYTOPLASMIC ACTIN TO POST-SYNAPTIC STRUCTURES AT THE NEUROMUSCULAR JUNCTION (NMJ). Beverly W. Lubit, James H. Schwartz, and Zach W. Hall, Center for Neurobiol. and Behav., Columbia University, New York, NY 10032 and Div. of Neurosciences, Dept. of Physiol., Univ. of California, San Francisco, CA 94143.

Actins are ubiquitous, highly conserved proteins that fall into two classes: muscle actins and nonmuscle or cytoplasmic actins. Because actin is highly conserved, most antibodies to it cross-react extensively with all actins. However, an antibody to actin has been raised that has an unusual specificity: this antiactin recognizes cytoplasmic vertebrate actin but not skeletal muscle actin (1). It is therefore an ideal probe for studying the distribution of cytoplasmic actin in skeletal muscle. Previous immunocytochemical studies of human skeletal muscle showed that cytoplasmic actin is present and associated with membranes. Now we have investigated whether cytoplasmic actin is concentrated at or near the postsynaptic surface of the NMJ. Unfixed cryostat sections of rat diaphragm muscle were stained with rhodamine-labeled α-bungarotoxin to identify the endplate region and with purified antiactin which was subsequently detected with fluorescein-labeled second antibody. We have found that cytoplasmic actin or an actin-like molecule is concentrated at NMJs of normal and denervated adult rat muscle fibers. Its distribution corresponds exactly to the distribution of the acetylcholine receptors (AChRs). The high concentration of cytoplasmic actin in postsynaptic structures at the neuromuscular junction could be related to the clustering or anchoring of receptors. It could also serve to maintain the elaborately folded structure of the postsynaptic membrane. A thin layer of electron dense material has been described attached to the cytoplasmic face of the membrane and the underlying cytoplasm contains filaments which are thus far unidentified. To explore these possibilities, we studied diaphragm muscle at embryonic day 18, after receptors have clustered but before extensive folding of the postsynaptic membrane occurs, and at postnatal days 6 and 12, during the time that folds develop. We found cytoplasmic actin at all three developmental stages; its distribution in each case coincided with the distribution of the AChRs although the staining pattern at endplates became more complex with development. Thus the association of receptors with cytoplasmic actin exists prior to the development of the junctional folds. These results suggest that cytoplasmic actin may somehow stabilize AChRs in the postsynaptic membrane of the NMJ. Supported by grants from NIH, NSF and MDA.

1 Lubit, B.W. and J.H. Schwartz. An antiactin antibody that distinguishes cytoplasmic actin from skeletal muscle actin. J. Cell Biol. 86: 891-897, 1980.

40.6 MONOCLONAL ANTIBODIES WHICH RECOGNISE MEMBRANE GLYCOPROTEINS OF RAT NEURONAL CELL TYPES. C. J. Barnstable. Dept. of Neurobiology, Harvard Medical School, Boston MA 02115.

Monoclonal antibodies have been prepared against a slycoprotein fraction from rat cerebral cortex. A crude membrane fraction was dissolved in 2% sodium deoxycholate. Glycoproteins were isolated from the 6,000,000 g-min supernatant by affinity chromatography on a column of Lens culinaris (lentil) lectin-Sepharose 4B. BALB/c mice were immunised with this material and monoclonal antibodies produced by fusion of spleen cells with P3-NSI/1-Ag4-1 plasmacytoma cells.

The cell types recognised by the various antibodies have been investigated by immunocytochemical localisation at the light microscopic level. The biochemical identification of the antigens has been carried out by immunohistochemical labelling of glycoproteins separated by polyacrylamide gel electrophoresis and transferred onto nitrocellulose filters. The reactivity of the antibodies with cells in monolayer cultures set up from embryonic rat cortices will be presented and compared with the developmental expression of the antigens in vivo.

One antibody has been found that, in retina, specifically reacts with most of the cells in the ganglion cell layer. The identity of the retina cells has been studied by double-labelling experiments. Ganglion cells that project to the superior colliculus were labelled by retrograde transport of either granular blue or lucifer yellow-labelled wheat germ agglutinin. The fluorescing cells were compared with those labelled by antibody and were shown to form almost completely overlapping populations. Other experiments are in progress to determine whether the unlabelled cells represent displaced amacrine cells or ganglion cells that project exclusively to other brain regions.

Antibody 2G12 also reacts with living ganglion cells that have been dissociated from young retinas by digestion with proteolytic enzymes. Analysis of these dissociated cell preparations using a fluorescence activated cell sorter has been carried out and the use of this and other separation techniques compared for their ability to provide pure bulk preparations of ganglion cells. The results clearly show that antibody 2G12 represents a class of antibodies that can be used to isolate specific neuronal cell populations for a wide variety of chemical, pharmacological and physiological studies.

(Supported by NIH grants EY 03735 and NS 17309 and NSF grant BNS 8100340)

40.7 MONOCLONAL ANTIBODIES AGAINST CHOLINE ACETYLTRANSFERASE (ChAT): INTRASPECIES AND INTERSPECIES REACTIVITIES. Allan I. Levey* and Bruce H. Wainer. University of Chicago, Pritzker School of Medicine, Chicago, IL 60637.

Monospecific antibodies to choline acetyltransferase (ChAT), an enzyme specific to cholinergic neurons, should be useful for immunohistochemistry, purification of ChAT, and as molecular probes for studying the enzyme. We recently reported (Levey, A.I., Aoki, M., Fitch, F.W., and Wainer, B.H., Fed. Proc. 40: 270, 1981; Brain Res., in press.) use of the hybridoma technique to develop monoclonal antibodies to bovine ChAT. Immune Lewis rat spleen cells fused with the mouse myeloma cell line SP2/0 resulted in the production of two stable hybrid cell lines secreting monoclonal IgG2a antibodies reactive with bovine ChAT. We now report intraspecies and interspecies anti-ChAT reactivities of rat monoclonal antibodies secreted by the stable hybridoma cell line AB1. Antibody reactivity was measured by immunoprecipitation of enzyme in a double antibody assay. The amount of enzyme bound to antibody was calculated from measurements of enzyme activity remaining in the supernatants. Monoclonal antibody AB1 reacted equally well with crude soluble ChAT from bovine caudate nuclei and spinal cord, and with the two types of bovine caudate ChAT activity (Bov I and Bov II) resolved on CM-Sephadex ion exchange chromatography. Using bovine ChAT as a reference (100%), the following percent cross-reactivities were observed with ChAT obtained from each of the following species: Human, 69%; Ovine, 38%; Cat, 74%; Guinea pig, 84%; Mouse, 30%; and Rat, 0%. The effect of binding of monoclonal antibody AB1 in solution on enzyme activity was also measured. Aliquots of ChAT were incubated overnight in the presence of either AB1 or normal rat serum. The percent inhibitions observed were: Bovine, 0%; Human, 64%; Ovine, 0%; Cat, 22%; Guinea pig, 47%; Mouse, 38%; and Rat, 0%. The rank ordering of binding of AB1 to enzyme molecules from different species did not correlate directly with the rank ordering of the capacity to inactivate enzyme activity. These results can be used to operationally define two markers (binding vs. inactivation) which reflect interspecies differences in ChAT. The applicability of monoclonal antibody AB1 for immunohistochemical identification of cholinergic neurons in the appropriate species is presently being evaluated. (Supported in part by USPHS HD-04583; The Whitehall Foundation; The Children's Research Fund and The Nancy Pritzker Fund, Univ. of Chicago; and a Lederer Foundation Predoctoral Fellowship, A.I.L. recipient.)

40.8 IMMUNOCYTOCHEMICAL LOCALIZATION OF RAT CHOLINERGIC NEURONS USING A RADIOLABELED MONOCLONAL ANTIBODY TO CHOLINE ACETYLTRANSFERASE. M. E. Ross, D.H. Park, G.N. Teitelman, D.J. Reis and T.H. Joh. Lab of Neurobiology, Cornell Univ. Med. Coll. New York, NY 10021.

We sought to develop a specific and sensitive method for immunocytochemical localization of choline acetyltransferase (CAT). We have produced radiolabelled monoclonal antibodies (Abs) to CAT in order to take advantage of their purity and specificity.

CAT was highly purified from rat striata (Park et al. Neurosci. Abs. 1981) and monoclonal Abs to CAT were produced by the similar manner as described elsewhere (Ross et al. Brain Res. 208: 431, 1981). Mouse lymphocytes, sensitized to CAT, were fused with murine plasmacytoma (NS1) cells. Four percent of hybridomas secreted CAT antibody, detected by an enzyme linked immunosorbant assay (ELISA). Hybridomas (CMCAT) were cloned by limiting dilution. The specificity of the Abs were established by the following criteria: (1) the Abs reacted on an ELISA with pure CAT as the antigen, but not with other proteins separated out during purification of the enzyme; (2) on immunoelectrophoresis, they produced a single precipitin arc against crude striatal homogenate; (3) using rabbit antimouse IgG, Abs precipitated CAT activity in vitro.

All monoclonal Abs, however, failed to localize CAT staining cells by the PAP technique. Therefore, a ^{3}H-labeled Ab was used for immohistochemical localization. A clonal line (CMCAT-11) Ab was radiolabeled in situ by culturing the hybridoma cells for 24 hours in media containing a mixture of ^{3}H labeled amino acids. Using the Ab, moderate to large size perikarya, dendrites and terminals were specifically labeled in characteristic cholinergic structures in both the fetal and adult rats. These included diaphragmatic neuromuscular junctions, anterior horn cells of spinal cord, and neurons of hypoglossal nucleus, pontine and medullary reticular formation and cerebral cortex. Thus, radiolabelled monoclonal autibodies to CAT were useful for the highly specific localization of cholinergic neurons in both PNS and CNS of rat.

Thus, cholinergic neurons in rat can be immunocytochemically localized with a high degree of specificity by use of a raido-labeled monoclonal antibody. Since this antibody can be used along with an indirect (e.g. PAP) method, it should provide a useful method for identification of two antigens in the same section.

(Supported by grants HL 18974, HL 07378, and MH 24285)

40.9 DISTRIBUTION OF GLUTAMATE DECARBOXYLASE POSITIVE NEURONS IN THE RAT CEREBELLAR NUCLEI. E. Mugnaini and W. Oertel, Dept. of Biobehavioral Sciences, U.Conn., Storrs, CT 06268 and Lab. Clinical Sciences, NIMH, Bethesda, MD 20205

In addition to excitatory projection neurons, the cerebellar nuclei of the rat are thought to contain few short-axon neurons. Small neurons that take up exogenous ^{3}H-GABA (Chan-Palay, Cerebellar Dentate Nucleus, Springer, Berlin, 1977) may correspond to the local circuit neurons, which are seen after Golgi impregnation. In a recent immunocytochemical study using an antiserum to rat brain glutamic acid decarboxylase (GAD) and the unlabeled antibody enzyme method of Sternberger, we observed weakly GAD-immunoreactive small cells in the rat cerebellar nuclei. These data suggest the presence of a small population of GABAergic neurons in the rat cerebellar nuclei. In the present study on the rat, we analyzed the distribution of GAD-immunoreactive neurons in the four cerebellar nuclei using the same antiserum after colchicine treatment. Colchicine blocks axoplasmic transport of GAD and enhances somatal immunoreactivity (Ribak et al., Brain Res. 140:315-332, 1978). The animals were perfused, under sodium pentobarbital anesthesia, with a buffered aldehyde solution; Vibratome sections (25 μm) of the cerebellum were processed for GAD-immunohistochemistry as described elsewhere (Oertel et al., Neuroscience, in press). After topical application of colchicine (10-30 μg) stronger specific immunoreactivity was observed than after intraventricular injection (150-250 μg colchicine). With either procedure few GAD-immunoreactive neurons were found in the nucleus medialis, but an unexpectedly dense population of GAD-immunoreactive neurons appeared in the nucleus interpositus anterior and posterior and in the nucleus lateralis. In the latter, GAD-immunoreactive neurons predominated at the hilus. The dorsal part of the lateral vestibular nucleus contained scattered, small GAD-positive cells - a pattern, resembling that of the cerebellar medial nucleus. Preliminary studies indicate that a similar distribution of GAD-positive neurons is present in the cerebellar nuclei of the cat.

Thus, small neurons which are able to synthesize GABA and are presumably inhibitory, are unequally distributed in the four cerebellar nuclei and abound in the nuclei interpositus and lateralis. The possibility that some of the GAD-immunoreactive neurons are projection neurons will be discussed.

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40.10 PENTAPEPTIDE PROCTOLIN (H-ARG-TYR-LEU-PRO-THR-OH): IMMUNOLOGICAL DETECTION AND NEURONAL LOCALIZATION. C.A. Bishop and M. O'Shea. Dept. Pharmacol. Physiol. Sci., The University of Chicago, Chicago, IL 60637.

Proctolin (H-Arg-Tyr-Leu-Pro-Thr-OH) is a highly bioactive peptide and may be a neurotransmitter or neuromodulator. We have produced several proctolin antisera and applied them to detecting and localizing proctolin-like immunoreactivity (PLI) in the CNS of the cockroach, locust and other animals. Radioimmunoassay (RIA), capable of detecting 50 fmoles proctolin, was used to quantify PLI distribution. Immunocytochemistry (peroxidase-antiperoxidase or PAP technique) was used to determine the cellular localization of PLI in sectioned (6μm) and whole ganglia.

Results show that PLI is exclusively localized to neurons and moreover that the distributions of PLI as determined by RIA and immunocytochemistry are positively correlated. For example, in the cockroach, concentrations of PLI are highest in the genital ganglia (~0.8 pmoles/mg) and lowest in the cerebral ganglia (<0.05 pmoles/mg). The genital ganglia contains approximately 80 immunoreactive cell bodies and a widespread and dense distribution of arborizations and varicosities, but the cerebral ganglion contains less than ten immunoreactive cells and few varicosities.

We will describe the anatomy of the major components of the putative proctolin system in the cockroach and locust. Immunoreactive cell bodies, for example, are found in symmetrical pairs on the lateral margins in the cockroach abdominal ganglia. The lateral cell bodies appear either singly or in small clusters of 3-5 cells. An anterior lateral cluster consistently stains with the PAP technique and has contralaterally projecting immunoreactive axons. Immunoreactive cell bodies are also found close to the midline on the dorsal and ventral surfaces. Immunoreactive varicosities are distributed primarily in the dorsal neuropile and many of the neurons appear to have axons projecting to peripheral nerve roots and to other ganglia. A significant finding is that individual cell bodies occur in constant position from animal to animal. They are, therefore, potential subjects for an "identified neuron" approach to the function of a neuropeptide.

Results of a current developmental analysis of selected immunoreactive neurons and of a survey of invertebrate and vertebrate phyla for the presence of PLI will be reported.

(Supported by National Institutes of Health grants NS-06684 and NS-16298.)

41.1 EFFECTS OF LITHIUM ON MOTONEURONS, SYNAPTIC POTENTIALS, AND EXTRACELLULAR POTASSIUM IN THE ISOLATED FROG SPINAL CORD.
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The mechanism of the therapeutic action of Lithium (Li) is still unclear. In order to obtain more information on the actions of Li upon neuronal excitability and ion concentrations, we carried out experiments in the isolated circumfused frog spinal cord using conventional intracellular recordings from motoneurons (MN) and both intra- and extracellular recordings with ion selective microelectrodes (ISMEs). The preparation is useful as it allows controlled variations of ion concentrations through the bath.

Application of LiCl in the bathing solution for 30-60 min (up to 15 mmol/l, 22°C) reduced the amplitude of polysynaptic postsynaptic potentials (poly- PSPs) evoked by dorsal root (DR) single shock stimulation (up to 30%). The effect was accompanied by an increase in spontaneous synaptic activity and an elevation of the extracellular potassium (K_e) with a maximum in deeper dorsal horn layers (upto 0.5 mmol/l). The elevation of K_e also occured in the presence of TTX (3 µmol/l), indicating that it was not the result of the enhanced spontaneous activity. The membrane potential of MN was only slightly depolarized by LiCl (upto 3 mV). Experiments with Li-ISMEs demonstrated that at a depth of 400 µm (= MN pool) steady state concentrations of Li_e were reached at about 60 min, and of Li_i between 60 and 120 min. The intracellular/extracellular concentration ratio in MN was 0.5-0.8.

In a further series of experiments, we tested the action of Li upon an acute load of the Na^+-K^+-pump evoked by repetitive activation of synaptic input. During repetitive stimulation of DR (10/s, 10s) MN depolarized and K_e rose. After the stimulation, a membrane hyperpolarization appeared while K_e was still elevated. This hyperpolarization seems to reflect the action of an electrogenic Na^+-K^+-pump. Single shocks to DR during this period induced poly-PSPs with a clearly increased amplitude. LiCl (15 mmol/l) as well as ouabain (10 µmol/l) reduced or abolished both the posttetanic membrane hyperpolarization and the posttetanic facilitation of poly-PSPs.

The results agree with an interaction of Li with the neuronal Na^+-K^+-pump in frog spinal MN, and additionally demonstrate consequences of this effect upon synaptic potentials. Although the blockade of posttetanic facilitation of poly-PSPs may be relevant to the clinical action of Li, it has still to be shown that corresponding effects occur at therapeutic concentrations in man.

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41.2 NA^+/K^+ TRANSPORT IN RAT CEREBRAL CORTICAL SLICES: EFFECT OF ADRENERGIC AGENTS. P.H. Wu* and J.W. Phillis. Department of Physiology, College of Medicine, University of Saskatchewan, Saskatoon, Saskatchewan Canada S7N OWO

Norepinephrine hyperpolarizes central neurons. This hyperpolarization can be blocked by various adrenergic receptor antagonists and inhibitors of (Na^+,K^+)ATPase. A receptor-mediated activation of a membrane sodium pump has therefore been proposed as the basic mechanism by which norepinephrine exerts its central action. To gain further support for this hypothesis, the effect of norepinephrine on ion fluxes in brain slices was investigated.

Brain slices (0.35 mm thick) prepared by a McIlwain tissue chopper were incubated with ^{22}Na (2µCi/ml) for 30 min at 37°C in 5 ml Krebs improved Ringer 1 solution. After loading, the slices were rapidly rinsed in non-radioactive incubation solution. Subsequent efflux of ^{22}Na was monitored by transferring the slices at 5 minute intervals through a series of tubes containing non-radioactive incubation solution in the presence or absence of test drugs. Potassium uptake into brain cortical slices was carried out by incubating tissue with ^{42}K (0.2-2µCi/ml) and KCl to a final concentration of 5 mM for a period of 10 min at 37°C. After incubation and rinsing, the slices were dissolved and radioactivities were measured.

Norepinephrine (10^{-7}-10^{-5}M) increases the efflux of ^{22}Na from brain slices in a dose dependent fashion ($0.01>p$). ^{22}Na efflux and norepinephrine evoked ^{22}Na efflux were inhibited by ouabain (10^{-3}M). Phentolamine or propranolol partially but significantly *blocked norepinephrine evoked ^{22}Na efflux ($0.01>p>0.001$).* Norepinephrine enhancement of ^{42}K influx into rat brain slices was also a dose dependent response ($0.05>p>0.01$). Ouabain inhibited the rate of ^{42}K entry both in the presence and absence of norepinephrine. Propranolol and phentolamine effectively antagonized norepinephrine stimulated ^{42}K influx into brain slices. Norepinephrine did not alter the rates of influx and efflux of ^{22}Na and ^{42}K respectively. Various adrenergic agonists, including oxymetazoline, naphazoline, clonidine, tramazoline, methoxamine, phenylephrine, L-isoproterenol and methoxyphenamine were potent stimulants of ^{22}Na efflux from rat brain cortical slices. This facilitation of ^{22}Na efflux from brain slices by norepinephrine is stereospecific as demonstrated by the very weak effect of D-norepinephrine on ion fluxes. Our results are consistent with the hypothesis that norepinephrine hyperpolarizes central neurons by activating an ouabain sensitive, receptor-mediated, sodium pump. Supported by the Canadian Medical Research Council.

41.3 EFFECTS OF CATECHOLAMINES ON PRINCIPAL GANGLIONIC CELLS IN THE GUINEA-PIG INFERIOR MESENTERIC GANGLION. Stuart E. Dryer* and David L. Kreulen, Department of Pharmacology, University of Arizona, Tucson, AZ 85724.

Catecholamines have been shown to alter synaptic transmission in sympathetic and parasympathetic ganglia. The following experiments were performed to elucidate effects of catecholamines on the inferior mesenteric ganglion of the guinea-pig. Intracellular recordings were made from guinea-pig inferior mesenteric ganglion (IMG) in vitro. Synaptic potentials were evoked by stimulation of lumbar colonic, lumbar splanchnic, hypogastric and intermesenteric nerves with bipolar platinum electrodes. In 63% of cells tested superfusion with 10^{-6} M norepinephrine (NE) produced a 5-10 mV hyperpolarization that was slow in onset, reaching a maximum in about two minutes. This effect was not associated with a change in input resistance. In 12% of the cells tested there was a 5-8% mV depolarization with no change in input resistance. In 25% of cells tested 10^{-6} M NE resulted in no change in membrane potential or input resistance. In all cells tested dopamine (DA) 10^{-6} M or apomorphine 10^{-5} M produced no change in membrane potential or input resistance. These same cells did not respond to 10^{-6} M NE. These results suggest that NE modulates synaptic transmission in the guinea pig IMG.
Supported by USPHS grants HL27781 and BRSG 2 S07 RR05676-13.

41.4 DIAZEPAM AND (-)PENTOBARBITAL POTENTIATE INHIBITORY RESPONSES TO GABA IN CULTURED MOUSE SPINAL NEURONS BY CHANGING CHANNEL KINETICS. R.E. Study* and J.L. Barker, (Spon: J.D. Newman). Laboratory of Neurophysiology, NINCDS, NIH, Bethesda, Md. 20205

Voltage clamp recordings from mouse spinal neurons grown in culture were used to study the mechanism by which diazepam (DZ) and (-)pentobarbital (PB) potentiate inhibitory responses to the amino acid neurotransmitter γ-aminobutyric acid (GABA). Fluctuation analysis and micropatch recording techniques have shown that the membrane mechanism underlying the inhibitory response to GABA in these cells involves the activation of two-state Cl^- ion channels (Nature 274 (1978) 596; Biophys.J. 33 (1981) 14a). Clinically relevant concentrations of both drugs (1-15µM DZ and 50-200 µM PB) were applied by passive diffusion or low levels of pressure (< 1 lb/in.^{2}). GABA was applied by iontophoresis. Standard fluctuation analysis methods were applied to potentiated GABA responses to estimate the electrical properties of the elementary ion channels underlying the responses. Current responses (ΔI) were associated with an increase in current variance (σ^2). σ^2 was directly proportional to ΔI with GABA alone and during potentiation by the drugs. The ratio $\sigma^2/\Delta I$, which allows estimation of the amplitude of an elementary current event, i, did not change during potentiation. Since neither drug changed the reversal potential of the inhibitory response to GABA, the results suggest that the conductance of the Cl^- ion channels is not altered by the drugs. Spectral analysis of current variance revealed that most power spectral density plots were well fit by a single Lorentzian equation, consistent with a model in which the variance reflects the activity of a population of ion channels whose durations are exponentially distributed with a mean duration, τ. τ was calculated from $\tau = 1/2\pi f_c$, where f_c is the half-power frequency of the spectrum. τ remained constant for responses to GABA alone. τ was either unchanged or increased slightly in responses potentiated by DZ, while it was consistently and markedly increased in a dose-dependent manner during potentiation by PB. However, the drug-induced changes in τ could not alone account for the augmentation. By taking into account the frequency of channel openings ν, which can be calculated from $\nu = \sigma^2/[i^2\tau]$, we have been able to account quantitatively for the potentiation. DZ increased ν while PB almost always decreased ν. Our results indicate, therefore, that DZ and PB potentiate GABA responses by changing channel kinetics rather than conductance, and they do so in different ways.

41.5 FLUCTUATION ANALYSIS CF DIRECT INHIBITORY EFFECTS OF DIAZEPAM AND (-)PENTOBARBITAL CN THE EXCITABILITY OF CULTURED MOUSE SPINAL NEURONS. J.L. Barker and R.E. Study* (Spon: J.H. Neale). Lab. Neurophysiology, NINCDS, NIH, Bethesda, Md. 20205

Intracellular recording methods were used to study the direct, inhibitory effects of the benzodiazepine diazepam (DZ) and the barbiturate (-)pentobarbital (PB) on the excitability of mouse spinal neurons growing in tissue culture. The drugs were applied by passive diffusion or by low levels of pressure (<1 lb./in^2) from blunt micropipettes containing known drug concentrations dissolved in the bathing medium. At clinically relevant concentrations (1-15μM DZ and 50-200μM PB) both drugs increased Cl^- ion conductance in some cultured neurons (8 of 65 cells studied with DZ and 10 of 33 cells studied with PB). In contrast, 10-50μM γ-aminobutyric acid (GABA) increased Cl^- ion conductance in every cell studied. The two-electrode voltage clamp technique using microelectrodes filled with 3M KCl was employed to study the membrane current responses evoked by the drugs. With the cell clamped to holding potentials over the range -50 to-90mV both drugs and GABA evoked inward current responses in a dose-dependent manner. The inward current responses were each associated with an increase in current variance. The drug responses and their associated variance were analyzed mathematically using a model which assumes that the macroscopic responses reflect the random variation in the number of open two-state Cl^- ion channels, as has been demonstrated for GABA responses in these cells (Nature 274 (1978) 596; Biophys. J. 33 (1981) 14a). Power spectral density plots of current variance were often well-fit by a single Lorentzian term of the form $S(f)/S(0) = 1/[1 + (f/fc)^2]$ where f is the frequency, S(f) is the spectral density at frequency f, S(0) is the zero frequency asymptote of the spectrum, and f_c is the corner, or half-power frequency. Such spectral behavior would be expected if the durations of the putative ion chanrel events activated by the drugs were exponentially distributed about a mean, τ and τ was inversely related to fc ($\tau = 1/2\pi fc$). τ s for DZ events were similar or slightly longer than τs estimated for GABA-activated channels on the same cells while τ s for PB were significantly longer than those estimated for GABA. Estimates of the average conductance of ion channels activated by the drugs did not differ significantly from those estimated for GABA-activated channels. The results suggest that these clinically important drugs can directly inhibit neuronal excitability by activating Cl^- ion channels much in the manner of a natural ligand like GABA. Although "GABA-mimetic", it is not clear whether the drugs utilize GABA receptor-coupled Cl^- ion channels.

41.6 d-TUBOCURARINE COMPETITIVELY ANTAGONIZES GABA IN THE HIPPOCAMPUS Frank J. Lebeda, John J. Hablitz and Daniel Johnston, Dept. of Neurology, Baylor College of Medicine, Houston, TX 77030

d-tubocurarine (dTC), when topically applied to the mammalian central nervous system, causes seizures. Since other convulsant agents are believed to antagonize γ-aminobutyric acid (GABA) mediated inhibition in the hippocampus, we examined whether dTC exerted its effects through a similar mechanism. We have demonstrated that dTC is a potent convulsant in hippocampal slices, suppresses spontaneous inhibitory activity and antagonizes GABA-mediated events.

Intracellular recordings were made from CA3 neurons in the guinea pig hippocampal slice preparation maintained in vitro. Either a 3 kHz single-electrode clamp system with 2 M Cs_2SO_4 microelectrodes or a standard preamplifier with 4 M KAc microelectrodes was used. GABA was iontophoresed onto locations proximal to the cell body layer using conventional methods. Tetrodotoxin (2 μM) was added to the perfusion medium during the GABA iontophoresis experiments.

When 50 μM dTC was added to the bathing medium, characteristic repetitive discharges were observed. These events occurred spontaneously and in response to orthodromic stimulation. Furthermore, these paroxysmal events fit all of the criteria for giant EPSPs (Johnston and Brown, Science 211: 294, 1981), including reversal in polarity at about 0 mV. Spontaneous inhibitory potentials were found to be blocked by dTC. In additional experiments, the effect of dTC on responses to iontophoretically-applied GABA were examined. Dose-response curves were plotted as the log of the charge delivered to the GABA pipette versus the % maximum decrease in input resistance. Bath-applied dTC (500 μM) produced an apparent parallel shift to the right in the GABA dose-response relation in a manner similar to, but less potent than, bicuculline methiodide (50 μM). In contrast, picrotoxin (500 μM) decreased the maximum response and increased the ED50 (dose producing a half-maximal response); this mixed form of inhibition (competitive and noncompetitive) has also been seen in lobster muscle (Constanti, Neuropharmacol. 17: 159, 1978). Finally, bath application of several cholinergic antagonists (atropine, scopolamine, gallamine, α-bungarotoxin or decamethonium) produced none of the field potential changes characteristic of dTC.

The present results suggest that dTC produces its excitatory effects by antagonizing a GABAergic rather than a cholinergic system, and that the three convulsants investigated interact with at least two different components associated with the GABA receptor/ionic channel complex in the hippocampus. (Supported by The Epilepsy Foundation of America and NS15772 and NS11535.)

42.1 DOPAMINE RECEPTORS AND ANTIDEPRESSANT DRUG ACTION. Tyrone LEE and Siu W. TANG. Department of Psychopharmacology, Clarke Institute of Psychiatry and Department of Pharmacology, University of Toronto, Toronto, Canada.

Antidepressants are effective blockers of transmitter uptake at synapses, e.g. noradrenaline and serotonin, and have been shown to induce beta-adrenergic receptor subsensitivity. However, Serra et al. (Life Sci. 25:415, 1980) and Chiodo and Antelman (Nature 287:451, 1980) have demonstrated that repeated antidepressant treatment in rats also induced subsensitivity of dopamine autoreceptors. In order to investigate the possible involvement of dopamine in antidepressant drug action, the effect of chronic treatment in rats of desipramine (DMI) and amitriptyline (AMT) on presynaptic dopamine receptors was studied.

Adult male Wistar rats (150-175 gm) were treated with either DMI (20 mg/kg i.p.) or AMT (10 mg/kg i.p.) once daily for 7 days. Control animals received equivalent volume of saline. All animals were sacrificed by decapitation 24 hours after the last dose. The brains were immediately removed, dissected and crude homogenates of the striatum were prepared. Presynaptic dopamine receptors were measured by using ^{3}H-dopamine (0.25-2.5 nM) and specific binding was defined by 1 μM apomorphine. The data were analyzed by Scatchard plots and the results were expressed as follows in density (B_{max}) in femtomoles/mg protein and affinity constant (K_D) in nanomoles/liter.

^{3}H-DOPAMINE	SALINE	DESIPRAMINE	AMITRIPTYLINE
B_{max}	68.2±4.8* (6)	72.8±3.1 (7)	74.4±2.9 (7)
K_D	1.74±.15 (6)	1.55±.09 (7)	1.75±.14 (7)

*Mean ± S.E.M.
Number of replicate assays are indicated in parentheses.

Under the conditions used in this study no significant difference in density or affinity of the presynaptic dopamine receptors was observed between the control animals and animals treated with antidepressants. However, dopamine may modify antidepressant action via different population of dopamine receptors with longer onset of action at a different locale of the rat brain. Such a possibility is being investigated.
(Supported by the Clarke Institute of Psychiatry.)

42.2 **EFFECTS OF N-ETHYLMALEIMIDE ON ^{3}H-SPIROPERIDOL BINDING TO RAT STRIATAL MEMBRANES.** Rita M. Huff and Perry B. Molinoff. Dept. of Pharmacology, Univ. Co. Health Sci. Ctr., Denver, CO 80262.

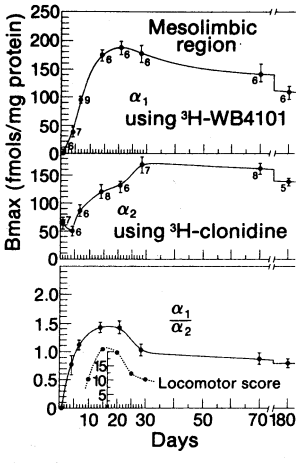
^{3}H-Spiroperidol (SPD) appears to bind to at least two types of striatal dopamine (DA) receptors, an adenylate cyclase linked subtype and a noncyclase linked subtype. Guanine nucleotides cause a 3- to 5-fold decrease in the affinity of DA receptors for agonists (Nature 275: 453, 1978). This decrease in affinity may reflect an interaction of one of the DA receptor subtypes with adenylate cyclase. A role for thiol groups in the binding of ^{3}H-DA to its receptors has been described (BBRC 96: 953, 1980). When incubations were carried out in the presence of N-ethylmaleimide (NEM), a sulfhydryl alkylating agent, a decrease in the specific binding of ^{3}H-DA has been observed. Pretreatment of striatal membranes with NEM also causes a dose dependent inhibition of DA stimulated adenylate cyclase activity (Fed. Proc. 40: 653, 1981). In the current study the possibility that NEM was selectively alkylating thiol groups associated with just one of the subtypes of DA receptors was examined. Membranes were prepared from rat striatum, incubated with NEM (10 μM - 1 mM) for 30 minutes at 37°, and then washed prior to being used in binding assays. The inhibition constant (K_i) for DA displacement of ^{3}H-SPD binding was increased up to 70-fold following pretreatment with NEM. The Hill coefficients for DA displacement of ^{3}H-SPD binding increased from 0.48 in controls to 0.94 with 1 mM NEM. The K_i values of other agonists, including N-propylnorapomorphine and apomorphine, were also increased in a dose dependent manner following treatment with NEM. Following incubation with NEM, however, GTP did not affect the K_i value for DA. Pretreatment with NEM also resulted in a dose dependent decrease in the density of ^{3}H-SPD binding sites (B_{max}); 10 μM NEM decreased the B_{max} by 15% and 1 mM NEM decreased the B_{max} by 42%. No change in the affinity (K_d) of the receptor for ^{3}H-SPD was observed after incubation with 10 μM NEM, but pretreatment with 1 mM NEM increased the K_d value by 2.5-fold. GTP did not alter the density or affinity of ^{3}H-SPD binding sites. The increases in the K_i values of agonists for ^{3}H-SPD binding sites following NEM pretreatment are greater than the increases in the K_i values observed in the presence of GTP. Thus, the effects of NEM on ^{3}H-SPD binding are not completely analogous to those of GTP. The K_i value for α-flupenthixol, an antagonist reported to be selective for cyclase-linked DA receptors, was increased 2-fold following incubation with 1 mM NEM. Similar results, however, were also obtained in studies using domperidone and bromocriptine, antagonists of noncyclase linked DA receptors. The findings do not support the hypothesis that pretreatment with NEM selectively alters DA receptors linked to adenylate cyclase.
This work was supported by NS 15756.

42.3 ALPHA-ADRENOCEPTOR DEVELOPMENT AND HYPERLOCOMOTION IN YOUNG RATS. Elizabeth J. HARTLEY* and Philip SEEMAN. Dept. of Pharmacology, University of Toronto, Toronto, Canada M5S 1A8.

Infant rats normally become hyperactive during the third week of life and this locomotor activity declines to adult levels by 25 to 27 days of age. Since the rise in locomotion has been ascribed to the development of the catecholamine system, we examined the development of post-synaptic alpha$_1$-adrenoceptors. We also measured the development of (pre-synaptic) alpha$_2$-adrenoceptors, since their maturation might inhibit or regulate the release of noradrenaline, accounting for the decline in locomotor activity. The alpha$_1$ sites were labelled by ^{3}H-WB-4101, the alpha$_2$ sites by ^{3}H-clonidine, and we used Wistar rat pup brain tissue homogenized in 50 mM Tris-HCl.

1. On the first day of life there were no detectable alpha$_1$ sites in the mesolimbic region, hippocampus, or frontal cortex. Rapid synthesis of these sites only commenced at day 4.
2. Although alpha$_2$ sites were detectable at birth, they lagged in development 1 week after the alpha$_1$ sites.
3. Only in the mesolimbic tissue did the ratio of receptor densities (alpha$_1$/alpha$_2$) parallel the locomotor development.
4. The results support Kellogg & Lundborg (1972) who proposed that adrenoceptors develop before dopamine receptors.
5. The early presence of alpha$_1$ sites also explains clonidine activation before day 14; the later onset of alpha$_2$ sites explains clonidine depression of activity after 21 days.
6. We conclude that the rise in locomotion at day 14 is associated with development of alpha$_1$ sites; the suppression of motion at day 28 is associated with the maturation of alpha$_2$-adrenoceptors in the nucleus accumbens of the mesolimbic region.

(We thank Carla Ulpian for excellent assistance; Funded by the Hospital for Sick Children Foundation, Toronto.)



42.4 ^{3}H-LISURIDE, A POTENT DOPAMINERGIC ERGOT DERIVATIVE, LABELS D2-DOPAMINE RECEPTORS. P.F. Spano°, M. Memo°, F. Riccardi°, M. Carruba°, M. Trabucchi (Spon: I. Hanbauer). Dept. of Pharmacol., Sch. of Pharm., Univ. of Cagliari and Dept.s of Pharmacol., Sch.s of Pharm. and Med., Univ. of Milan, Italy.

Increasing evidence points to the existence of multiple receptors for dopamine (DA) in the CNS. On the basis of pharmacological and biochemical experiments we and others haved named central DA receptors D1 and D2. DA-D1 receptors are those coupled to adenylyl cyclase activity whilst DA-D2 are independent from the generation of cyclic AMP. We now report that lisuride, a potent dopaminergic ergot derivative unable to stimulate the formation of cyclic AMP, exhibits a specific binding in rat CNS. We found that ^{3}H-lisuride (26 Ci/mmol) binds to membrane preparation obtained from various dopaminergic regions. The binding appeared to be saturable, stereospecific and maximally enriched in the synaptosomal fraction. Scatchard analysis of binding data revealed receptor sites with high affinity (KDs from 1.4 to 1.9 nM) and a maximum capacity ranging from 70 to 281 pmol/mg prot. according to the various areas examined. Lisuride binding was higher in striatum and pituitary. Dopamine agonists and DA antagonists, including substituted benzamides, were potent competitors of lisuride binding in striatal and pituitary preparations. Most interesting, lisuride specific binding, contrary to ^{3}H-apomorphine binding, was not decreased in striatal membranes by guanyl nucleotides. This fact according to the present hypotheses would strongly suggest that DA receptors labelled by lisuride are not associated with adenylyl cyclase activity. Lesion studies which are now in progress in our laboratory seem to give further support to this hypothesis. In conclusion our data indicate that the characteristics of lisuride specific binding are compatible with the properties of a pharmacologically relevant DA receptor, possibly of the D2 type.

42.5 DOPAMINE RECEPTOR BINDING ON INTACT CELLS: ABSENCE OF A HIGH AFFINITY AGONIST STATE. D. R. Sibley, L. Mahan and Ian Creese, Dept. Neurosciences, Univ. Calif. San Diego, La Jolla, CA 92093.

Utilizing biochemical and pharmacological criteria, anterior pituitary dopamine (DA) receptors have been classified as belonging to the D-2 category of DA receptor subtypes. We have previously shown that the radiolabeled antagonist, ^{3}H-spiroperidol (^{3}H-SPIRO) and the agonist, ^{3}H-N-n-propylnorapomorphine (^{3}H-NPA) can label these D-2 DA receptors in bovine anterior pituitary membranes with high affinity. We now report that in intact bovine anterior pituitary cells there are no high affinity agonist binding sites as determined by ^{3}H-NPA binding or by agonist/^{3}H-SPIRO competition experiments.

^{3}H-SPIRO binding to intact cells is reversible, of high affinity, and saturable with Scatchard analysis indicating a homogeneous population of binding sites with a Kd of 0.21 ± 0.05 nM (n=3) and a Bmax of 9.0 ± 0.85 fmoles/10^6 cells (n=3). The stereospecificity and rank order of neuroleptic antagonists, catecholamines, and related drugs in competing for ^{3}H-SPIRO binding is indicative of a DA receptor. There is no high affinity, stereospecific or saturable binding of ^{3}H-NPA to these cells. In contrast, washed membranes prepared from these cells exhibit high affinity ^{3}H-NPA binding with a Kd of 0.25 ± 0.02 nM (n=3) and an appropriate pharmacological specificity.

In membranes prepared from either whole anterior pituitaries or from dispersed cells, agonist/^{3}H-SPIRO competition curves are heterogeneous with Hill coefficients <1. In the presence of guanine nucleotides, agonist/^{3}H-SPIRO curves are right-shifted and steepened with Hill coefficients =1. Computer analysis indicates that agonist/^{3}H-SPIRO curves in membranes are best explained by a two state binding model whereas in the presence of guanine nucleotides a one state model is sufficient to explain the data. Strikingly, in intact cells agonist/^{3}H-SPIRO curves have Hill coefficients =1 and exhibit affinities similar to the corresponding low affinity agonist binding state seen in membranes. Addition of guanine nucleotides does not alter the agonist/^{3}H-SPIRO curve in intact cells.

We hypothesize that high affinity DA agonist binding in bovine pituitary membranes does not represent a distinct DA receptor subtype but rather a transitional guanine nucleotide-sensitive, agonist-specific binding state of a singular D-2 receptor. In our model, endogenous or exogenous guanine nucleotides, in intact cells or membranes respectively, mediate an interconversion from the high to the low affinity agonist binding state thus effectively eliminating high affinity agonist binding as measured directly with ^{3}H-NPA or indirectly with agonist/^{3}H-SPIRO competition experiments. (Sup. by MH32990)

42.6 ASCORBATE ENABLES STEREOSPECIFIC DOPAMINERGIC RECEPTOR BINDING OF TRITIATED DOPAMINE AGONISTS. S. Leff, M. Hamblin, D.R. Sibley and Ian Creese, Dept. Neurosciences, Univ. Calif. San Diego, La Jolla, CA 92093.

Dopamine (DA) and other catecholamines oxidize readily. For this reason assay media used in early studies of DA receptor binding to membrane homogenates commonly included mM concentrations of the anti-oxidant ascorbic acid. Under these conditions extensive characterization of high affinity DA ^{3}H-agonist and ^{3}H-antagonist binding strongly suggests an association with DA receptors. Several recent reports have proposed that ascorbic acid at μM to mM concentrations may inhibit high affinity DA agonist binding to striatal membranes. Since these reports showed limited characterization of dopaminergic ^{3}H-agonist binding in the absence of ascorbic acid, we have further investigated the effects of ascorbate on DA receptor binding to membrane homogenates of bovine anterior pituitary and caudate and rat striatum.

Specific binding (determined using 10^{-6} (+)butaclamol as a blank) of the antagonist ^{3}H-spiperone was not significantly affected by ascorbate concentrations from 0.1μM to 10 mM except that concentrations of ascorbic acid >6 mM inhibit specific binding due to marked decreases in pH of the assay media. Dopaminergic ^{3}H-agonist specific binding (defined using blanks of (+)butaclamol at 10^{-6} M for ^{3}H-n-propylnorapomorphine [NPA] and 10^{-5} M for ^{3}H-apomorphine) was virtually undetectable in the absence of ascorbate. Specific binding increased with increasing ascorbate concentrations until a plateau was reached at 2-5 mM ascorbate.

While levels of total ^{3}H-agonist binding were markedly reduced in the presence of mM concentrations of ascorbate, ^{3}H-agonist binding in the absence of ascorbate was almost entirely non-stereospecific. Unlabeled DA at 10^{-5} M inhibited stereospecific and non-stereospecific ^{3}H-NPA binding to bovine striatal membranes in the presence and absence of ascorbate. These non-stereospecific binding sites were inhibited equipotently by other catecholamines such as norepinephrine, epinephrine and isoproterenol. Furthermore, DA inhibitable non-stereospecific binding failed to show a regional specificity suggestive of DA receptor binding.

Our results indicate that ascorbic acid or some other anti-oxidant must be present in in vitro assays of equilibrium DA receptor binding when using readily oxidizable agonists. We are currently investigating the effects of alkaline earth metals, anti-oxidants, and transition metal chelators on DA receptor binding. (Supported by MH32990)

42.7 STEREOSELECTIVITY IN THE BINDING OF (+), (-)2-HYDROXYAPOMORPHINE AND RELATED APORPHINES TO DOPAMINE RECEPTORS. G.W. Arana* and R.J. Baldessarini, Department of Psychiatry, Harvard Medical School and Mailman Research Center, McLean Hospital, Belmont, MA 02171; J.L. Neumeyer, V.J. Ram*, Section of Medicinal Chemistry, Northeastern University, Boston, MA 02115.

The potent DA agonists (-)2,10,11-trihydroxyaporphine [(-)2-hydroxyapomorphine (-)2-OH-APO] and (-)2,10,11-trihydroxy-N-n-propylnoraporphine (TNPA) were synthesized from the opioid thebaine. The enantiomer (+)2-OH-APO was synthesized from the aporphine alkaloid (+)bulbocapnine by a process which retained the chirality at the 6a carbon atom. Competition by these and other trihydroxyaporphines for binding of ^{3}H-labeled apomorphine (APO) (0.5 nM) was evaluated with a synaptosomal-membrane-enriched fraction (P_4) of calf caudate homogenates. Specific binding was defined by use of 6,7-ADTN (10 μM) as a blank to displace ^{3}H-APO. In competition for ^{3}H-APO binding, the more potent enantiomer of 2-OH-APO is the (-)isomer (IC_{50} = 10.0 nM) in comparison with (-)APO (1.0 nM); (-)NPA (2.5 nM); (±)NPA (5.0 nM); (-)TNPA (25 nM) and (-)2,10,11-trihydroxynoraporphine (30 nM). (+)2,-OH-APO had an IC_{50} value > 5,000 nM. These findings lend further support to previous studies in which it was shown that changes in the stereochemistry at the 6a carbon, the addition of a 2-hydroxy group and substitution of other alkyl side chains for the N-methyl group on apomorphine will have significant effects on the dopaminergic propreties of such aporphines.

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42.8 INHIBITION OF R-(-)APOMORPHINE-INDUCED STEREOTYPIC ACTIVITY AND STRIATAL (^{3}H)-SPIROPERIDOL AND (^{3}H)-APOMORPHINE RECEPTOR BINDING BY S-(+)APOMORPHINE. W.H. Riffee, R.E. Wilcox, R.A. Reynolds-Vaughn*, and R.V. Smith. Coll. Pharm., Univ. Texas, Austin, TX 78712.

Although apomorphine exists in both the R-(-)- and S-(+)-isomeric forms, only R-(-)apomorphine (R-(-)APO) has previously been shown to have agonistic pharmacological activity. Initially, the ability of S-(+)apomorphine (S-(+)APO) to antagonize R-(-)APO-induced stereotypic cage climbing behavior in mice was investigated. Cage climbing was recorded continuously for one hour on videotape and subsequently the behavior was rated and the one min scores summed over 5 min periods for the hour. Studies using a racemic mixture of the two isomers showed that the presence of S-(+)APO produced a depression of the cage climbing behavior relative to that expected if S-(+)APO were merely inactive. In subsequent experiments, S-(+)APO was administered 1 min prior to the injection of R-(-)APO and dose and time-response analyses carried out. These studies demonstrated that the S-(+)isomer possesses no agonistic activity in the cage climbing model but that it acts as an antagonist. Doses of 30 mg/kg S-(+)APO which are well below toxic range of the drug depress the cage climbing behavior induced by R-(-)APO (10 mg/kg) by 50%. The time-response analysis showed a shift to the right in time of peak effect of R-(-)APO in the presence of S-(+)APO which may reflect a higher affinity of the R-(-)isomer for the receptor involved in the cage climbing behavior. In biochemical experiments the IC50 value for S-(+)APO inhibition of striatal (^{3}H)-spiroperidol (0.2nM) receptor binding was found to be 1uM vs. an IC50 value of 400nM obtained for R-(-)APO. The IC50 value for S-(+)APO inhibition of striatal (^{3}H)-apomorphine (2.0nM) binding was found to be 600nM vs. an IC50 value of 50nM obtained for R-(-)APO. (Supported in part by grants from NIMH (NM44332) and NINCDS (NS-12259).)

42.9 BEHAVIORAL SUPERSENSITIVITY BUT DECREASED STRIATAL (^{3}H)-SPIROPERIDOL RECEPTOR BINDING AFTER CHRONIC APOMORPHINE, N-n-PROPYLNORAPOMORPHINE, AND DEXTROAMPHETAMINE ADMINISTRATION. R.E. Wilcox, W.H. Riffee, R.A. Reynolds-Vaughn*, B.J. Leamons* D.M. Vaughn, and R.V. Smith. Coll. Pharmacy, Univ. Texas, Austin, TX 78712.

Several previous reports have demonstrated that chronic administration of direct and indirect acting dopamine agonists results in behavioral facilitation to challenge doses of dopamine agonists. In the present report, we sought to evaluate a possible mechanism for this response after chronic treatment with N-n-propylnorapmorphine (NPA), apomorphine (APO), and dextroamphetamine (AMP). Mice were administered (ip) NPA (30 mg/kg), APO (30 mg/kg), AMP (4 mg/kg) or saline once daily for two weeks. One, three or five days after the last chronic injection, the animals were tested for stereotypic cage climbing to challenge doses of APO. Separate groups of animals were sacrificed also at one, three and five days after the last chronic injection for assay of striatal (^{3}H)-spiroperidol receptor binding. APO-induced cage climb dose-response curves were shifted to the left at one three, and five days after chronic APO and NPA and three and five days after chronic AMP. In contrast, (^{3}H)-spiroperidol Bmax was slightly lower after chronic administration of APO and AMP and dramatically reduced (e.g. 30% at 3 days) in those mice treated with NPA. These results suggest that the relationship between APO-induced stereotypic activity and striatal neuroleptic binding may be neither as simple nor as direct after chronic drug administration as previously thought. (Supported in part by NIMH grant MH33442 and NINCDS grant NS-12259.)

42.10 STIMULATORY EFFECTS OF VARIOUS AGENTS ON THE BINDING OF ^{3}H-DOPAMINE TO NEOSTRIATAL MEMBRANE PREPARATIONS. Richard E. Heikkila, Felicitas S. Cabbat* and Lawrence Manzino*. Department of Neurology, College of Medicine and Dentistry of New Jersey-Rutgers Medical School, Piscataway, N.J. 08854.

It is now becoming increasingly apparent that certain reducing agents such as ascorbic acid can inhibit the specific binding of dopamine agonists and dopamine antagonists to neostriatal membrane preparations (e.g. Heikkila et al, Fed. Proc., 1981). During the course of studies with ascorbic acid, we tested the capacity of several drugs, all with varying oxidation-reduction potentials, for their capacity to inhibit the binding of ^{3}H-dopamine agonists and antagonists. To our surprise we found that one of these agents, potassium nitrite, did not inhibit, but in fact greatly stimulated the specific binding of ^{3}H-dopamine to neostriatal membrane preparations. This led to a search for other compounds that might also stimulate the binding of dopamine agonists. We found that several other agents including hydrogen peroxide, sodium nitrite, the diabetogenic agent streptozotocin, methyl nitrosourea, and potassium nitroprusside, in concentrations between 0.06 mM and 6 mM greatly stimulated the specific binding of ^{3}H-dopamine to neostriatal membrane preparations. As an example, 0.06 mM, 0.6 mM and 6 mM streptozotocin stimulated the specific binding of ^{3}H-dopamine by 27%, 229% and 465% respectively. The same three concentrations of sodium nitrite stimulated specific ^{3}H-dopamine binding by 25%, 63% and by 325% respectively. Current studies include a search to find other agents that similarly stimulate the binding of ^{3}H-dopamine, ways to counteract this stimulation of binding, as well as attempts to elucidate the mechanism of the stimulatory effects. Interestingly, several of the agents that stimulate the binding of ^{3}H-dopamine are also known to stimulate the activity of guanylate cyclase.

42.11 EFFECTS OF CHRONIC AMPHETAMINE OR PHENCYCLIDINE TREATMENT ON DOPAMINE AGONIST AND ANTAGONIST RECEPTORS: AMPHETAMINE BUT NOT PCP HAS DIFFERENTIAL EFFECTS. Harold A. Robertson, Department of Pharmacology, Dalhousie University, Halifax, Nova Scotia, Canada B3H 4H7.

There is now considerable evidence that chronic amphetamine treatment of rats (5 mg/kg, twice daily) produces down-regulation in the density of neuroleptic (3H-spiroperidol or D-2 dopamine) receptor sites. On the other hand, there is also evidence the same chronic amphetamine treatment produces a behavorial super-sensitivity to dopaminergic agonists such as apomorphine (Klawans and Margolin, Arch. Gen. Psychiat. 32: 725, 1975). In this study, we examined the effects of chronic d-amphetamine (5 & 10 mg/kg, i.p., twice daily) or phencyclidine (PCP) (5 mg/kg., i.p., twice daily) on striatal dopamine agonist (^{3}H-ADTN) and antagonist (^{3}H-spiroperidol) binding.

Male Wistar rats (200 g) were treated for 21 days and killed on day 23. Striata were pooled from 6-8 rats. Dopamine agonist binding was carried out using ^{3}H-ADTN, in an incubation mixture lacking ascorbic acid (ascorbate selectively destroys agonist binding sites). Displaceable binding was defined using 10 µM dopamine. Dopamine antagonist binding was determined using ^{3}H-spiroperidol and displaceable binding was defined with domperidone.

Chronic treatment with either d-amphetamine or phencyclidine produced a significant decrease in antagonist (^{3}H-spiroperidol) binding sites (Bmax) without affecting the affinity (K_D) of the site. In the same animals, there was an increase in specific ^{3}H-ADTN binding after amphetamine but not after PCP.

These findings indicate that the effects of chronic PCP are similar to those of amphetamine with respect to the neuroleptic or dopamine antagonist binding site but that PCP has little discernible effect on the dopamine agonist binding site. These findings may also clarify the apparent discrepancy between amphetamine's effects on receptor density and the effects on the response to dopamine agonists in vivo. Several groups have now suggested that the agonist and antagonist sites are distinct and separate. Our results suggest amphetamine at least can have a differential effect on the two sites. (Supported by the Canadian MRC).

42.12 MODULATION OF DOPAMINE RELEASE THROUGH ACTIVATION OF D_2 RECEPTORS IN THE ISOLATED RABBIT RETINA. M. L. Dubocovich* and N. Weiner. Dept. of Pharmacology, University of Colorado School of Medicine, Denver, Colorado 80262.

In the central nervous system, there is evidence for two subtypes of dopamine (DA) receptors: D_1 and D_2 (Kebabian and Calne, Nature 227, 93-96, 1979). The D_1 receptor is presumably linked to an adenylate cyclase system while the D_2 receptor is not. α-Flupenthixol and fluphenazine have high affinity for the D_1 receptor while S-sulpiride and metoclopramide are potent inhibitors of the physiological effects mediated by activation of D_2 receptors. In the retina of several species only the D_1 receptor has been described. Neither domperidone nor the benzamides: S-sulpiride or metoclopramide, inhibits the DA stimulated adenylate cyclase in this tissue (Redburn et al., Life Sci. 27, 23-31, 1980, Watling and Dowling, J. Neurochem. 36, 559-568, 1981). However, we have recently shown that S-sulpiride increases the calcium-dependent release of ^{3}H-DA from the isolated rabbit retina, presumably by blocking stereoselective DA autoreceptors (Fed. Proc. 40: 32, 1981). The aim of the present study was to investigate which subtype of DA receptor is involved in the modulation of DA release from the rabbit retina. The rabbit retina was isolated and labeled with 0.1 µM ^{3}H-DA (S.A:22.8 Ci/mmol). The tissue was superfused with Krebs solution and the calcium-dependent release of tritium was elicited by electrical stimulation at 3 Hz for 1 min at 20 mA, duration 2 msec. Two periods of electrical stimulation were applied (S_1 and S_2), separated by an interval of 40 mins. The percent of total tissue radioactivity released following the first period of stimulation (S_1) was: 2.25 ± 0.22%, (n = 12) and the S_2/S_1 ratio was: 1.12 ± 0.07, (n = 12). Both apomorphine (0.01 to 10 µM), and bromocryptine (0.01 to 1 µM) significantly decreased the stimulation-evoked release of tritium when added 20 mins before S_2. In contrast the DA antagonists S-sulpiride (0.01 to 1 µM), spiroperidol (0.01 to 10 µM), metoclopramide (0.01 to 10 µM), domperidone (0.01 to 10 µM) and R-sulpiride (0.01 to 1 µM), when added before S_2, increased in a concentration-dependent manner the release of tritium elicited by electrical stimulation. The concentration of the antagonists necessary to increase the stimulation evoked release of tritium by 50% were for (µM) : S-sulpiride, 0.0084; spiroperidol, 0.018; metoclopramide, 0.024; domperidone, 0.034 and R-sulpiride, 0.089. Domperidone and spiroperidol increased the spontaneous outflow of tritium at a concentration of 1 µM or higher. Neither stereoisomers of flupenthixol (0.01 to 10 µM) nor butaclamol (0.01 to 10 µM) modified the stimulation-evoked release of tritium from the rabbit retina, while fluphenazine significantly increased tritium release at 0.1 µM. These results suggest that the DA autoreceptors involved in the regulation of DA release from the rabbit retina possess the characteristics of a D_2 receptor. Supported by USPHS Grant NS 09199, NS 07927 and AA 03527.

43.1 CHARACTERISATION OF AN ENKEPHALIN ANALOG WITH SELECTIVE POTENT EFFECTS ON ANTERIOR PITUITARY HORMONES. J. Pless*, U. Briner*, H.H. Büscher*, F. Cardinaux*, W. Doepfner*, R.C. Hill*, R. Huguenin*, P. Marbach*, M. Markó*, R. Maurer*, D. Römer and L. Tolcsvai*, Preclinical Research, SANDOZ LTD., CH-4002 Basle, Switzerland.

A comparison in the rat of the properties of a series of mono- and poly-halogenated phenylalanine enkephalin analogs with the non-halogenated derivative FK 33-824 indicates that halogenation causes a selective and marked increase in the potency of the endocrinological properties of this group of compounds. In particular, the structure MeTyr-DAla-Gly-pF-MePhe-Met(O)-ol (200-999) is a very potent stimulator of prolactin and Growth Hormone secretion and inhibitor of Luteinising Hormone secretion and ovulation (ED50 for inhibition of ovulation = 0.07 mg/kg s.c.).

Detailed investigations of other pharmacodynamic effects of 200-999 show that it interacts with opiate receptors. In vitro, 200-999 displaces ^{3}H-naloxone from its binding site in rat brain homogenates to a high extent and it inhibits electrically-induced contractions of the isolated mouse vas deferens and guinea-pig ileum preparations. In vivo, 200-999 causes naloxone-reversible analgesia and it produces morphine-like physical dependence in the rhesus monkey when given by repeated i.v. infusion. However, in these tests the increased activity of 200-999 in relation to FK 33-824 is small when compared to the differences observed in the endocrinological potencies of the two compounds. RIA studies performed in the rat indicate that 200-999 penetrates the blood-brain barrier to a greater degree than FK 33-824 thereby offering a possible explanation for the more potent CNS properties of the halogenated analog.

43.2 ELECTROPHYSIOLOGICAL ANALYSIS OF THE INTERACTION AMONG PRODUCTS OF THE B-ENDORPHIN PRECURSOR. J.M. Walker,* H. Akil, and S. Watson (SPON: G.C. Quarton). Mental Health Research Institute, Univ. of Michigan, Ann Arbor, MI 48109.

B-endorphin (B-END) arises from a 31K dalton glycoprotein termed pro-opiocortin. Other products of pro-opiocortin include one or more ACTH related peptides and a 16K dalton fragment which is under investigation. The discovery that these cells produce and package several biologically active peptides raised many new questions about the nature of synaptic transmission. In particular, the question of whether the effects of two such substances are antagonistic or co-operative is critical. We previously suggested the actions of B-END and several ACTH-like peptides appear to be similar because ACTH produces analgesia upon microinjection in the periaqueductal gray. However, in other systems ACTH antagonises the effect of morphine.

A more direct approach to this problem is to observe the effects of ACTH-like substances on single neurons receiving terminals from B-END-containing cells. The locus coeruleus is innervated by the hypothalamic arcuate neurons that contain both B-endorphin and some a-MSH-like material. Single cell recordings from these cells indicates that opiates depress their rate of firing. The questing as to the function of a-MSH in these cells remains open. We are studying the effects of centrally and peripherally administered MSH and ACTH-like substances on these neurons in order to determine how various products of pro-opiocortin interact upon release.

43.3 TOPOGRAPHY AND KINETIC ANALYSIS OF DISTINCT SUBCELLULAR CLASSES OF OPIATE RECEPTORS, B.L. Roth*, M.J. Laskowski and C.J. Coscia, Depts. Biochemistry and Physiology, St. Louis Univ. School of Medicine. St. Louis, MO 63104.

We have recently provided evidence which indicated that opiate receptors in rat forebrain may be located in two distinct subcellular loci. By several criteria, 60-70% of all opiate receptors were found to be localized in synaptic plasma membrane (SPM)-enriched fractions while 20-30% of the total opiate receptors were detected in smooth endoplasmic reticulum-golgi enriched fractions. Based on marker enzyme analyses, equilibrium density gradient ultracentrifugation, electron microscopy and sensitivity to guanine nucleotides, opiate receptor binding in smooth microsomes could not be accounted for by SPM cross-contamination. We now wish to present further information which strongly suggests that the two subcellular classes of opiate receptors are indeed distinct.

According to current concepts of membrane biogenesis, opiate receptors in smooth microsomes could be localized so that the recognition site for ligands is on the luminal surface. Assuming that the receptor is not a transmembrane protein, addition of low concentrations (20-100 µg/mg protein) of proteases should not perturb this "latent" receptor. When proteolysis was performed under iso-osmotic conditions (0.32 M sucrose, 40 mM KCl, 5 mM Hepes, pH=7.4), 60-80% of the receptors for [^{3}H]-naloxone were resistant to trypsin, α-chymotrypsin or pronase. As a positive control, SPM opiate receptors were almost completely destroyed under identical conditions. An enzyme located on the luminal surface, thiamine pyrophosphatase, was 90-100% latent to proteolysis in the absence of detergents. In the presence of 0.1% Triton X-100 and trypsin, pronase or chymotrypsin, however, this enzyme was 70% inactivated suggesting the occurrence of right-side out microsomes. The relative absence of a trans-membrane SPM marker-enzyme in this fraction indicates that these membranes are not inverted SPM's. These data support the notion that [^{3}H]-naloxone binding sites in microsomes are luminally localized and that the SPM binding sites are located on the extracellular surface of the membrane.

In kinetic studies with membranes isolated from calf caudate nucleus, we found that microsomal receptors for [^{3}H]-D-ala^{2}-D-leu^{5}-enkephalin exhibit faster rates of association and disassociation when compared with SPM receptors. In preliminary studies microsomal opiate receptors displayed relaxation times for association of 0.059 ± 0.01 min^{-1} while SPM receptors had relaxation times of 0.029 ± 0.002 min^{-1}. Taken together the kinetic and topographical data suggests that microsomal opiate binding represent a discrete subcellular population.

43.4 EFFECTS OF PHENCYCLIDINE (PCP) AND ITS DERIVATIVES ON ENTERIC NEURONS. A. R. Gintzler,* R. S. Zukin and S. R. Zukin Dept. Biochem. Downstate Med Ctr. Dept. Biochem. Albert Einstein Med Sch. Dept. Psychiatry, Mount Sinai Med Sch. N.Y.C.

The effects of PCP and related drugs on isolated intact segments of guinea pig ileum were determined. TCP, PCP and ketamine decreased the height of electrically-induced contractions (0.1 Hz) of intact segments of isolated guinea pig ilea. 30-40% of the inhibition of contraction height (0.1 Hz) was reversed by pretreatment with the pure narcotic antagonist naloxone. This naloxone reversible component showed cross tolerance with morphine. PCP pre-treatment caused a shift to the right in the dose respone curve to ACh that was not parallel with the control dose response curve. Thus PCP does not interact with the muscarinic cholinergic receptor in a strictly atropine-like competitive fashion. Binding sites for [^{3}H]PCP were detected in homogenates of the guinea pig longitudinal muscle-myenteric plexus preparation. The affinity constants and the rank order of potencies of various PCP derivatives competing with [^{3}H]PCP for binding suggests that these binding sites are very similar to those found in the central nervous system. These data suggest that the isolated guinea pig ileum may be used as an in vitro system for studying the mechanism of action of phencyclidines. Supported by Grants #DA02893 to ARG, DA01843 to RSZ, DA02587 to SRZ.

43.5 Thermodynamics of [^{3}H] Diprenorphine (DP) and [^{3}H] Etorphine (ET) Binding. R. Hitzemann, M. Goldsmith* and J. Curell*. Departments of Psychiatry and Pharmacology and Cell Biophysics, Univ. Cincinnati School of Medicine, Cincinnati, OH. 45267.

The hypothesis was tested that there are significant thermodynamic differences between [^{3}H]DP and [^{3}H]ET receptor binding. A whole rat brain membrane preparation, carefully stripped of endogenous opioids, was used to measure the equilibrium binding of [^{3}H]DP and [^{3}H]ET at 2°, 10°, 18°, 25°, 37° and 45°C. In (-) NaCl buffer, the Van't Hoff plots for [^{3}H]ET binding were linear over the range of 10° to 37°C and had a negative slope. In (+) NaCl buffer, temperature had no effect on K_A (H=0). NaCl significantly decreased the [^{3}H]ET K_D value below the (-) NaCl value only at 37° and 45°C. For [^{3}H]DP binding in both (+) NaCl buffer the Van't Hoff plots were linear over the range of 18° to 45°C and had a positive slope. NaCl significantly increased the [^{3}H]DP K_D at all temperature except 2°C; this effect was inversely proportional to increasing temperature. The equilibrium thermodynamic parameters at 37°C were as follows: (-) NaCl buffer - [^{3}H]ET-ΔG = -13.7kcal/mole, ΔH = + 2.5kcal/mole, ΔS= + 52.3 e.u.; [^{3}H]DP- ΔG = - 13.0kcal/mole, ΔH = - 8.4kcal/mole, ΔS = + 14.8e.u.; (+) NaCl buffer - [^{3}H]ET - ΔG = 13.5kcal/mole, ΔH=0, ΔS = + 43.5e.u.; [^{3}H]DP - ΔG = - 13.2kcal/mol, ΔH = - 9.8kcal/mole, ΔS = + 10.9e.u. The Bmax for both [^{3}H]ET and [^{3}H]DP significantly increased with increasing temperature in (-) NaCl buffer. In (+) NaCl buffer temperature had no effect on the [^{3}H]DP - Bmax. The [^{3}H]ET Bmax in (+) NaCl buffer progressively increased over the 2°C value at 10°, 18° and 25°C. However, at 37° and 45°C the Bmax values were not significantly different than the 2°C value but were significantly less than respective (-) NaCl values. Thus at 37°C both the affinity and Bmax for [^{3}H]ET binding were significantly decreased by NaCl. Overall, these results show that in comparison to [^{3}H]DP binding, [^{3}H]ET binding is significantly more entropy driven and that the availability of receptors for both ligands is temperature and ion dependent. Supported by NS-16061.

43.6 Phylogenetic Differences in Opiate Receptor Subtypes. M. Carroll-Buatti*and G.W. Pasternak (SPON: D. Rottenberg). Cotzias Lab. of Neuro-Oncology, Sloan-Kettering C.C. and Cornell University Medical College.

Both the subtypes and levels of opiate receptor binding differ between species. Rats have two major populations of ^{3}H-D-ala^2-D-met^5-enkephalinamide (3H-enkamide) binding sites: a high affinity site (K_D 0.2 nM) present in small numbers (Bmax 1.6 fmoles/mg tissue) and a low affinity site (K_D 3.3 nM) present at far greater numbers (Bmax 10.3 fmoles/mg tissue). Morphine binds very potently to the high affinity ^{3}H-enkamide site, displacing its binding with an IC_{50} less than 1 nM. In contrast, the low affinity site is far less sensitive to morphine and binds enkephalins much more potently. Naloxazone, an irreversible opiate antagonist, selectively inhibits the high affinity sites. Treatment of rat or turtle (Chrysemys scripta elegans) brain membranes with naloxazone in vitro lowers ^{3}H-enkamide binding by about 60%, while binding in frog membranes (Xenopus laevis) is depressed only 35% ($p<0.05$). The most striking differences were noted in goldfish membranes (Carassius auratus) where binding is virtually insensitive to naloxazone ($p<0.001$), suggesting the absence of high affinity sites. Since high affinity ^{3}H-enkamide binding is easily displaced by low morphine concentrations, displacement studies were performed in several species. Morphine significantly inhibits a portion of binding in both rat and turtle with an apparent IC_{50} of less than 1 nM in contrast to goldfish where no inhibition at these low concentrations is found. Goldfish binding is potently displaced by unlabeled D-ala^2-D-leu^5-enkephalin and by higher morphine concentrations (IC_{50} 5 nM), insuring the relevancy of binding. Finally, the saturation studies of ^{3}H-enkamide were performed in goldfish brain membranes. Unlike the curvilinear, two component Scatchard plots found in rat, goldfish have linear Scatchard plots describing a site (K_D 3.9 nM; Bmax 6.0 fmoles/mg tissue) whose affinity is very similar to the low affinity site in rat (K_D 3.3 nM). Thus, high affinity binding sites are more prevalent in higher species, correlating with their later appearance in ontogeny. The lack of high affinity sites in goldfish adds considerably to the hypothesis that high affinity sites represent a physically and pharmacologically distinct subpopulation of opiate receptors. The goldfish also offers a simple model for the examination of low affinity sites without the complicating binding to the high affinity receptors.

43.7 Mu and Delta Opiate Receptors in the CNS: A New Classification G.W. Pasternak, B.L. Wolozin*, A-Z Zhang. Cotzias Laboratory of Neuro-Oncology, Sloan-Kettering C.C. and Cornell U. Med. College

Binding studies in the CNS with ^{3}H-labeled opiates and enkephalins have suggested different types of sites for each class: mu (morphine) and delta (enkephalin). However, correlating these receptors with biochemically defined sites has remained difficult. Computer analysis of saturation studies have demonstrated high and low affinity ^{3}H-D-ala^2-D-leu^5-enkephalin (^{3}H-DADL: K_D 0.5 nM, Bmax 1.2 fmoles/mg tissue; K_D 5.2 nM, Bmax 11.0 fmoles/mg tissue) and ^{3}H-dihydromorphine(^{3}H-DHM: K_D 0.23 nM, Bmax 1.58 fmoles/mg tissue; K_D 2.9 nM, Bmax 5.84 fmoles/mg tissue) sites in rat brain. (Note that the term low affinity is a relative one. The K_D's remain under 10 nM.) Naloxazone selectively and irreversibly blocks the high affinity binding of both ligands. The mu peptide morphiceptin inhibits both ^{3}H-DHM and ^{3}H-DADL binding in a biphasic manner. The initial displacement of both ligands (IC_{50}<10nM) is abolished by naloxazone treatment of the membranes. Similar results are found displacing with morphine. Together, these experiments indicate that a mu peptide and a mu opiate bind with greatest affinity to the high affinity site of both ^{3}H-DHM and ^{3}H-DADL and strongly suggest that these ligands bind equally well and with highest affinity to the same site. However, the low affinity sites of ^{3}H-DHM and ^{3}H-DADL remaining after naloxazone treatment appear to be quite different. Morphine potently inhibits low affinity ^{3}H-DHM binding (IC_{50} 10 nM) but is quite weak against ^{3}H-DADL binding (IC_{50} 87 nM). Morphiceptin also displaces low affinity ^{3}H-DHM binding 250-fold better than low affinity ^{3}H-DADL binding. Conversely, D-ala^2-D-leu^5-enkephalin inhibits ^{3}H-DADL binding (IC_{50} 11 nM) far better than ^{3}H-DHM (IC_{50} 86 nM). Thus, the low affinity ^{3}H-DHM site selectively binds opiates and the low affinity ^{3}H-DADL site selectively binds enkephalins. In summary, these experiments define three general classes of opiate binding sites. Both ^{3}H-DHM and ^{3}H-DADL bind equally well and with highest affinity (K_D's 0.23 and 0.5 nM, respectively) to a discrete naloxazone sensitive receptor which mediates opiate, enkephalin, and beta-endorphin analgesia. Because of morphine's great potency at this site, we propose it be named mu_1. ^{3}H-DHM binds with slightly lesser affinity (K_D 2.9 nM) to a site which preferentially binds opiates, termed mu_2. ^{3}H-DADL binds with slightly lesser potency (K_D 5.2 nM) to a site which preferentially binds enkephalins. This latter site corresponds to the previously described delta receptors.

43.8 RECEPTOR BINDING STUDIES OF CNS KAPPA RECEPTORS. P.L. Wood. Douglas Hospital Research Centre, Verdun, Quebec, H4H 1R3.

Using rat brain membrane preparations, a unique kappa receptor was labelled with ^{3}H-ethylketazocine (^{3}H-EKC): KD = 3.45 nM, Bmax = 452 fmol/mg protein. Binding of ^{3}H-EKC to this site was potently displaced by kappa, partial mu and agonist/antagonist analgesics. Mu, delta, sigma and epsilon receptor agonists, however, were much less active at the kappa site. Representative K_I (nM) values for the mu, delta and kappa (^{3}H-Dihydromorphine/^{3}H-D-Ala^2-D-Leu^5-enkephalin/^{3}H-EKC) receptors were: morphine (1.3/26.1/696). D-Ala^2-D-Leu^5-enkephalin (8.3/1.6/113). β-endorphin (0.25/0.28/1835). SKF 10047 (1.4/5.0/24.1); EKC (4.1/7.6/4.7); buprenorphine (3.1/3.6/2.0); cyclazocine (0.2, 1.1, 5.0) and naloxone (1.4, 16.5, 11.1). Further studies of the inactivation of this ^{3}H-EKC binding site by N-ethylmaleimide demonstrated that kappa agonists but not mu or delta agonists could protect against receptor alkylation. Using such receptor inactivation protocols the specificity of purported kappa agonists was also examined and indicated that EKC but not MR-2034 possesses significant affinity for the delta receptor. In summary, our studies describe a kappa binding site which possesses a unique pharmacology and which can be selectively protected by purported kappa agonists from inactivation by alkylating agents. Therefore, our data support the concept of multiple opiate receptors and suggest that in addition to mu and delta sites a kappa receptor population is present in the rodent CNS.

43.9 DYNORPHIN-(1-13) CATALEPSY IS NOT MEDIATED BY A μ OPIATE RECEPTOR IN RAT BRAIN. B.H. Herman and A. Goldstein. Addiction Research Foundation, 701 Welch Road, Palo Alto, CA 94304.

In common with other opioids, lateral intraventricular (LV) administration of dynorphin-(1-13) induces catalepsy in the rat Herman et al., Life Sci. 27: 883, 1980). Naloxone was less effective in antagonizing the cataleptic effects of dynorphin-(1-13) as compared with D-Ala^2-dynorphin-(1-11) and Bc-endorphin, suggesting that catalepsy may be mediated by more than one opiate receptor in brain.

Evidence for a unique opiate receptor of dynorphin type in two peripheral tissue preparations has recently been provided (Wüster et al., Europ. J. Pharmacol. 62: 235, 1980; Chavkin & Goldstein, Nature, in press). We now report that μ opiate receptors in brain do not mediate dynorphin-(1-13) catalepsy, since no cross tolerance occurs between dynorphin-(1-13) and sufentanil (a specific μ receptor agonist).

Rats were made tolerant to sufentanil by chronic s.c. administration. Tolerance was assessed by comparing platform immobility (300-sec cut-off) and righting loss (60-sec cut-off) produced by a test dose of sufentanil (4 ug/kg) given before and after chronic sufentanil. Animals showing at least 50% reduction in both catalepsy scores were then tested with LV drugs. Controls were chronically administered saline. Scores are median times (sec). Independent groups were tested under each drug condition, and the same rats (N shown in parentheses) were tested for both catalepsy responses.

The table (platform immobility) shows that cross tolerance was obtained between systemic and central sufentanil, but not between sufentanil and dynorphin-(1-13). Indeed, dynorphin-(1-13) immobility was enhanced in sufentanil tolerant animals, and this effect is under further study. Similar results were obtained for righting.

	Test drug (LV)	
Chronic drug (s.c.)	Sufentanil (3.2 nmol)	Dynorphin-(1-13) (100 nmol)
Saline	300 (4)	119 (12)
Sufentanil	34 (7)	300 (8)
p	<.05	<.01

These data indicate that dynorphin-(1-13) catalepsy is not mediated by a μ opiate receptor.

Sufentanil was a gift from Janssen Pharmaceutica. Supported by NIDA grants DA7063 and 1199.

43.10 SPINAL CORD SEROTONINERGIC NEURONS: ACTIVATION BY MORPHINE IN THREE FUNCTIONAL REGIONS. J.W. Commissiong, Dept. of Physiology, 3655 Drummond Street, Montreal, Quebec, Canada, H3G 1Y6.

A part of the mechanism of morphine analgesia is thought to be mediated at the spinal level. The mechanism probably involves the activation of neurons in nucleus raphe magnus which project to the cord. An increased release and metabolism of serotonin (5-HT) in the cord, and consequently an increased production of 5-hydroxyindoleacetic acid (5-HIAA), the major metabolite of 5-HT in the cord, would accompany such neuronal activation. The hypothesis was tested with regard to: 1) The specificity of morphine in activating serotoninergic, noradrenergic and dopaminergic neurons in the cord, and 2) The activation of serotoninergic neurons in three major functional regions of the cord. A gas chromatographic-mass fragmentographic method was used in which 5-HIAA, methoxyhydroxyphenylglycol (MHPG) and homovanillic acid (HVA), the major metabolites of 5-HT, norepinephrine (NE) and dopamine (DA) respectively, were determined from a single injection. The results obtained after the administration of 10 mg/kg s.c. of morphine for 1 hr are summarized in Table 1.

TABLE 1

REGION	5-HIAA nmol/g ± SD (N=5)		MHPG		HVA pmol/g ± SD (N=5)	
CERV.	CONTROL	MOR.	CONTROL	MOR.	CONTROL	MOR.
D. Horn	1.7±0.2	3.4±1.2*	1.5±0.3	1.4±0.2	399±82	329±59
V. Horn	1.2±0.1	2.5±0.8*	1.3±0.2	1.2±0.1	280±35	299±70
THOR.						
Z. Int.	1.6±0.3	3.9±0.9*	-	-	379±57	389±74

* $P < 0.01$ when compared with control.

The effect of morphine was dose-dependent and was reversible by naloxone (4 mg/kg i.p.). There were no consistent changes in the levels of 5-HT, NE or DA. Two conclusions follow from these results. 1) Of the three spinal monoaminergic neuronal systems, only serotoninergic neurons appear to be activated after morphine. 2) The activation of serotoninergic neurons is non-specific with regard to the functional region of the cord involved, since activation occurred to the same degree in the three functional regions studied. Much caution is therefore needed in the interpretation of results in which the analgesic affect of morphine is specifically correlated with the activation of serotoninergic neurons in the dorsal horn of the cord. (Supported by the MRC of Canada).

44.1 THE ROLE OF THE FRONTAL EYE FIELDS AND THE SUPERIOR COLLICULUS IN THE COMPUTATION OF RETINAL-ERROR AND EYE-POSITION INFORMATION. P.H. Schiller,* J.H. Sandell, and S.D. True*. (SPON:B. Dawson). Dept. of Psychology, M.I.T., Cambridge, MA 02139.

Recent studies have shown that in man and monkey the acquisition of visual targets by saccadic eye-movements is accomplished not only by relying on a retinal-error signal but also by utilizing eye-position information (1,2). Where these two signals are combined in the chain of events leading to a saccade is not yet clear, although it has been shown that both retinal-error and eye-position information are available at the level of the superior colliculus (SC) (3).

The aim of our experiment was to determine whether or not the frontal eye fields (FEF) and the SC are essential for computations based on both retinal-error and eye-position signals. To do this we used the Mays-Sparks paradigm (2). Monkeys were trained to acquire briefly flashed visual targets. Prior to the initiation of the saccade either the FEF or the SC was electrically stimulated to move the eyes, in total darkness, to a new location. Under such conditions animals corrected for eye displacement and reached the actual position of the already extinguished target, suggesting that both retinal-error and eye-position signals were utilized. We then removed the SC in some animals and the FEF in others. We found that following these ablations animals were still able to make proper corrections when the remaining structure was electrically stimulated. This, in combination with earlier work (4) suggests that the retinal-error signals supplied by either the SC or the FEF is combined with the eye-position signal at a station subsequent to the SC, probably somewhere in the brainstem.

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44.2 PROJECTIONS FROM THE GRANULAR FRONTAL CORTEX TO BRAINSTEM OCULOMOTOR NUCLEI IN THE MONKEY. G.R. Leichnetz and R.F. Spencer. Department of Anatomy, Medical College of Virginia, Virginia Commonwealth University, Richmond, Virginia 23298.

We have previously described the origin, course and termination of projections from the granular frontal cortex to the superior colliculus (SC). However, HRP gel implants into subregions of the prefrontal cortex (PFC) in both old and new world monkeys also demonstrated corticofugal trajectories that were followed to the rostral interstitial nucleus of the medial longitudinal fasciculus (riMLF), interstitial nucleus of Cajal, oculomotor nucleus, nucleus of Darkschewitsch, and rostral paramedian pontine reticular formation. But it was difficult to determine the specific subregions of PFC from which these anterogradely-labelled projections to specific nuclei originated.

Therefore, stereotaxic microinjections of brainstem preoculomotor nuclei were made to answer this question and to determine whether PFC subregions project to more than one, perhaps several, of these targets. Unilateral HRP injections into the oculomotor nucleus resulted in large numbers of retrogradely-labelled cells (60-80 cells in a single section) within the rostral bank of the arcuate sulcus and few, if any, on the crown of the prearcuate cortex. There was also a lightly-labelled and almost continuous stratum of layer V pyramidal cells on the dorsal convexity and medial prefrontal cortex from the lip of the principal sulcus to the cingulate sulcus. There were no labelled cells in the banks of the principal sulcus, inferior portion of the prearcuate cortex between the caudal principal sulcus and inferior ramus of the arcuate sulcus, or in the orbitofrontal cortex. Injection of the riMLF, on the other hand, resulted in only occasional retrogradely-labelled cells in the rostral dorsal convexity and medial prefrontal surface. Concentrations of cells were observed in the dorsal bank of the superior ramus of the arcuate sulcus which continued to its caudal terminus, and at these more caudal levels the densely-labelled cells were continuous with an almost uninterrupted stratum of layer V pyramidal cells which extended into the dorsal convexity of Area 6 and onto the medial surface to the cingulate sulcus, the abundance of which we attributed to probable spread of the injection into the subjacent dorsomedial red nucleus. Only an occasional cell was observed in the rostral bank of the arcuate sulcus, and again there were none in the banks of the principal sulcus or in the orbitofrontal cortex.

These preliminary results seem to suggest that prefrontal projections to the superior colliculus and oculomotor nucleus may arise from the same cortical area, but the projection to riMLF appears to come from a different region.

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44.3 FRONTAL EYE FIELDS IN MONKEY: CLASSIFICATION OF NEURONS DISCHARGING BEFORE SACCADES. C. J. Bruce and M. E. Goldberg. National Eye Institute, NIH, Bethesda, MD 20205.

The frontal eye fields of the Rhesus monkey have been shown to participate in saccadic eye movements. Microstimulation evokes saccades and lesions impair the ability to make saccades. Neurons have an enhanced response when the monkey makes a saccade to a stimulus in the neuron's receptive field, but not when the monkey attends to the stimulus without making an eye movement. In order better to understand the role of the frontal eye fields in visually guided saccades, we studied the temporal discharge patterns of frontal eye field neurons in Rhesus monkeys performing tasks requiring visual fixation and saccades.

51% of neurons discharged before visually guided saccades to limited areas of the contralateral visual field. Of these presaccadic neurons, 23% were purely visual: they discharged in response to the stimulus and their discharge was unaffected by the presence or absence of eye movements to it. In contrast, 7% were purely motor. They did not respond to visual stimuli and when the monkey was induced to perform the necessary saccade in the dark (by omitting the target and rewarding the eye movement) these cells responded as briskly as when the animal made the same saccade to a visual target.

The largest number (70%) of the presaccadic neurons were visuomotor in that their discharge was affected by both visual stimululation and eye movements. Most visuomotor neurons gave vigorous responses when the monkey made a saccade to a visual target, but discharged much less to either the eye movement or the visual stimulus alone. The response began 100 msec or less after the visual target onset and continued through the saccade whether or not the stimulus was still present. For some neurons the response terminated sharply at or before the end of the saccade. For others it continued up to 200 msec longer.

Some visuomotor neurons began to respond before the saccade target onset if the task repeatedly required a certain saccade. This anticipatory discharge ceased when several trials not requiring the saccade were given.

We analyzed the effect of orbital position by requiring the monkey to make similar saccades from different orbital positions or to reach the same orbital position using different saccades. Each neuron studied discharged in association with a particular range of directions and amplitudes of eye movements, not to movements which achieved particular orbital positions.

These results are consistent with the hypothesis that the frontal eye fields provide subcortical structures with trigger and targeting signals for saccadic eye movements.

44.4 FRONTAL EYE FIELDS IN THE MONKEY: EYE MOVEMENTS REMAP THE EFFECTIVE COORDINATES OF VISUAL STIMULI. M. E. Goldberg and C. J. Bruce. National Eye Institute, NIH, Bethesda, MD 20205.

Man and monkey can make accurate saccades to briefly flashed targets present only before an intervening saccade. Such performance implies a dissociation of the direction of the evoked saccade from the retinal location of the target, and logically entails a vector subtraction of the intervening saccade from the retinal target location. We have previously described visuomotor neurons in the monkey frontal eye fields which discharge optimally before saccades to visual targets, We studied the response of these neurons while monkeys executed such double saccades in order to see how a dissonance between retinal stimulus location and required eye movement direction effects their discharge.

Monkeys previously trained on a simple saccade task were trained to make two successive eye movements to serially flashed spots each of which appeared and vanished before the monkey made the first eye movement. The second eye movement had coordinates which could only be calculated if the retinal location of the target were adjusted by the dimensions of the first eye movement. For each neuron studied we first determined its visual receptive field and the direction and magnitude of saccade associated with its optimal response. We then studied the same neuron in double jump experiments in which the stimuli were arranged either (1) that neither stimulus was in the receptive field of the neuron, but the second eye movement was the correct one, or (2) the second stimulus was in the receptive field of the neuron, but neither movement was correct.

Most visuomotor neurons gave a strong response during the right movement-wrong receptive field case. No neurons were more responsive in the right receptive field-wrong movement case than in a control task using the same stimuli without any eye movements. Since these neurons require visual input for their optimal responses, in the right movement-wrong receptive field case the visual input comes from an area of the retina not usually capable of driving the cell. These data suggest the critical signal from the frontal eye fields is not the retinal location of a target, but the movement that the target will evoke.

The frontal eye field also contains a class of neurons discharging after certain saccades. These postsaccadic neurons continue to discharge for up to several seconds after the saccade. If the animal makes a second eye movement, their discharge is immediately truncated so that they signal only the most recent saccade. We suggest that these neurons carry the information necessary for the remapping of the responses of the visuomotor neurons in the double saccade paradigm.

44.5 UTILIZATION OF AN EYE POSITION SIGNAL BY SACCADE-RELATED BURST NEURONS IN THE MONKEY SUPERIOR COLLICULUS. David L. Sparks and John D. Porter*, Dept. of Physiology and Neurosciences Program, University of Alabama in Birmingham, Birmingham, Al. 35294.

Recent studies (Mays and Sparks, Science, 208, 1980) provide convincing evidence that visual saccade targets are localized in a spatial, as opposed to a retinocentric, frame of reference. Computation of the vector of a saccade is based upon both retinal and eye position information. The present experiment is concerned with the question of where in the CNS retinal and eye position signals are combined.

Two rhesus monkeys with implanted eye coils were trained to look to brief (125 ms or less) visual targets for water reinforcement. Microelectrodes were introduced into the superior colliculus bilaterally and unit activity was monitored until a cell with saccade-related activity was isolated. On random trials, in the interval between target offset and saccade onset, electrical stimulation of the opposite colliculus drove the eyes to another position in the orbit. The monkey then made a short-latency compensatory movement of saccadic velocity to the location of the (now absent) target. On stimulation trials, the target coordinates were arranged so that the vector of the compensatory saccade was in the movement field of the unit being studied. Most units with saccade-related bursts also exhibited a burst prior to compensatory movements in their movement field. The discharge prior to compensatory saccades was generally reduced when compared to that observed for control movements. Burst neurons that did not fire before compensatory saccades were shown to require prior activation by a visual stimulus within their movement field (visually-triggered burst cells, Mohler and Wurtz, J. Neurophysiol., 39: 1976).

Saccades made to compensate for the stimulation-induced perturbation must be based upon a combination of retinal and eye position information. Accordingly, neurons which discharge prior to these compensatory saccades must be part of the neuronal circuitry after eye position and retinal signals have been combined. While we do not dismiss evidence that local feedback of an eye position signal is used to modify the output of pontine burst neurons (Van Gisbergen et al., J. Neurophysiol., 45: 1981), our data indicate that an eye position signal is also combined with retinal information at or prior to the level of the superior colliculus.

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44.6 RESPONSE OF SUBSTANTIA NIGRA CELLS RELATED TO SACCADES TO REMEMBERED TARGETS. O. Hikosaka* and R. H. Wurtz (SPON: D. L. Robinson). Laboratory of Senosorimotor Research, National Eye Institute, NIH, Bethesda, MD 20205.

Cells in the pars reticulata of the substantia nigra of rhesus monkeys show high frequency, tonic discharges. Some of these cells show a response (decrease in discharge rate) during a visually guided saccade task in which the monkey makes a saccade to a currently present spot of light, and the decrease is coupled either with the stimulus onset or with the saccade onset (Hikosaka and Wurtz, Neurosci., 6:15, 1980). We found other types of cells which were characterized by: 1) weak or no response during a visually guided saccade task; 2) stronger response during a task which requires saccades to a previously present spot of light (delayed saccade task). In this delayed saccade task a spot of light (cue stimulus) is flashed for 50 msec while the monkey is fixating; this spot indicates the target for a subsequent saccade. When the fixation point goes off about 1 sec later, the monkey has to make a saccade (delayed saccade) to the no longer present spot of light; reward is given if the correct saccade is made.

The first type of cell decreased its discharge rate in response to the cue stimulus. This response was distinguished from a simple visual response because the response became much weaker or faded away when the task was changed so that the same spot of light was no longer the cue for the delayed saccade.

The second type of cell decreased its discharge rate in relation to the delayed saccade. The decrease in discharge rate started 50-200 msec before the saccade onset. Some of these cells showed no response during the visually guided saccade task although the resulting saccades were physically the same while others showed weaker response before visually guided saccades. None of these cells showed significant change in discharge rate in relation to spontaneous saccades made in the dark.

The third type of cell was characterized by the maintained decrease in discharge rate which started after the cue stimulus and ended with the delayed saccade.

All of these three types of cells showed the strongest decrease in discharge rate when the delayed saccade was directed to a part of the visual field contralateral to the substantia nigra studied.

These experiments suggest that the substantia nigra pars reticulata participates not only in the initiation of visually guided saccades but also in the initiation of saccades guided by short-term memory of target location.

44.7 AXONAL PROJECTION OF VISUAL-OCULOMOTOR CELLS IN THE SUBSTANTIA NIGRA TO THE SUPERIOR COLLICULUS. R. H. Wurtz and O. Hikosaka*. Laboratory of Sensorimotor Research, National Eye Institute, NIH, Bethesda, MD 20205.

We have shown that cells in the lateral part of the pars reticulata in the monkey substantia nigra decrease their discharge rate in relation to saccades to real or remembered visual targets. This area roughly corresponds to the anatomically determined area which has a clear projection to the superior colliculus. The present study attempts to determine: 1) what type of pars reticulata cells project to the superior colliculus; 2) to which part or layer of the superior colliculus single pars reticulata cells project. We used microstimulation techniques to antidromically activate pars reticulata cells from a movable stimulating electrode in the superior colliculus of awake rhesus monkeys. We first inserted a fine tungsten microelectrode through a guide tube into the superior colliculus and determined the visual and oculomotor properties of cells recorded along the electrode track. This allowed us to determine the location of the electrode tip: from purely visual responses in the superficial layers and to purely motor responses in the deeper layers. Then we recorded single cells in the pars reticulata with another microelectrode and determined the relation of these cells to visual stimuli and to saccades to those stimuli. During recording of the single pars reticulata cells, we moved the electrode in the superior colliculus while applying single pulse stimuli. Many pars reticulata cells which decreased their discharge rate in relation to visual stimuli or saccades were activated antidromically from the ipsilateral superior colliculus. Antidromic responses were identified by: 1) fixed latencies to single and double pulse stimuli with a short interval; 2) collision with spontaneous spikes. We measured the threshold and latency for antidromic activation at 100 micron steps through the superior colliculus which yielded depth-threshold and depth-latency plots. Latency of antidromic response usually ranged between 0.7 and 1.3 msec. The depth-threshold plot typically showed multiple low threshold peaks of less than 20 uA. These peaks usually corresponded to the depths where visual-motor or motor cells were recorded. These multiple peaks indicate that the axons of pars reticulata cells arborize to several branches in the intermediate and deep layers and possibly terminate on some cells in these layers.

We suggest that pars reticulata cells which decrease discharge rate in relation to saccades to real or remembered visual targets have monosynaptic connection with superior colliculus cells which increase discharge rate in relation to visually or non-visually guided saccades.

44.8 BURST NEURONS IN THE MESENCEPHALIC RETICULAR FORMATION (MRF) ASSOCIATED WITH VISUALLY-TARGETED AND SPONTANEOUS SACCADES. David Waitzman and Bernard Cohen, Dept. of Neurology, Mount Sinai School of Medicine, New York, N.Y. 10029.

Single unit activity was recorded in the MRF of alert rhesus monkeys trained in a visual fixation/saccade task (Waitzman & Cohen, Neurosci., 5: 389, 1979). Sites of recording were identified both histologically and via microstimulation which produced characteristic fixed and variable amplitude saccades (Matsuo et al., Neurosci., 6: 15, 1980). Most MRF burst neurons began firing before contralateral spontaneous and task related saccades which brought the eyes to positions of fixation within ±30 degrees of the horizontal meridian. The number of spikes in the 32 msec period before the onset of movement was related to saccadic direction, not to the size of the horizontal component of movement. However, histograms ordered by size of horizontal component of movement, revealed that the highest peak firing rates were associated with saccades of specific sizes. For example, one group of cells had peak frequencies of approximately 600 Hz which occurred 15 msec before the onset of 2°-4° on-target saccades. These neurons also fired in association with larger on-target saccades but the peaks of firing were after the onset of movement. This group of neurons was active for 2°-4° spontaneous saccades but peak frequencies were about 300 Hz and occurred 1-2 msec before the onset of movement. Similar groups of neurons were identified which were most active prior to saccades in other ranges: 6° to 8°, and 10° to 12°. Another prominent feature of these neurons was the inhibition of firing that preceded off-target saccades to the ipsilateral side. During ipsilateral on-target saccades the neuron was not inhibited and spontaneous firing levels were maintained. In neurons associated with small (2°-4°) and medium (6°-8°) sized saccades the spontaneous level of firing increased during fixation of the target. These results demonstrate that the MRF has burst activity which leads spontaneous and visually-targeted eye movements. This lead activity contains information about the direction of the upcoming movement. MRF neurons that are excited to their peak firing rate before the onset of movement may help determine the size of the upcoming saccade. If the MRF affects the level of excitability of the saccade generating mechanism in the pons, then the reciprocal excitation and inhibition of MRF units during on- and off-target saccades could account for the production of contralateral saccades. During fixation the enhanced spontaneous firing rates in the MRF on both sides could provide damping activity that suppressed saccade generation in any direction.

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44.9 NEURONAL CORRELATES OF VERGENCE EYE MOVEMENTS. Lawrence E. Mays. Department of Physiological Optics and Neurosciences Program, University of Alabama in Birmingham, Birmingham, AL 35294.

The oculomotor system which controls vergence eye movements is functionally and neurologically distinct from the conjugate control systems. Signals which control the angle between the eyes appear to be derived independently of conjugate signals and then combined with them at the medial and lateral rectus motoneurons.

The purpose of the present study is the identification of neuronal activity which may control disjunctive eye movements. Monkeys were trained to look at a small target light for a water reward. The horizontal and vertical positions of both eyes were measured using the search coil technique. By placing target arrays at near (19 cm) and far (83 cm) distances from the monkey it was possible to elicit convergence and divergence eye movements on command. Extracellular microelectrode recordings were made in pontine and midbrain areas during disjunctive and conjugate eye movements and fixation. Units were encountered near the oculomotor nucleus which had a firing rate closely related to the angle of convergence. The activity of these cells was unrelated to the direction of conjugate gaze. These responses are consistent with the expected characteristics of a vergence signal. However, the possible role of these units in other components of the near responses (e.g. accommodation) has not yet been determined.

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44.10 SPATIAL COORDINATES OF VISUAL MESSAGES IN THE FLOCCULUS. J.I. Simpson, W. Graf* and C.S. Leonard* (SPON: R. Hess). Dept. Physiol. & Biophys., New York Univ. Med. Ctr., NY, NY 10016.

The visually modulated speed and rotation selective cells in the dorsal cap of the inferior olive project to the flocculus and can be divided into three classes according to eye dominance and the spatial orientation of the preferred axis of rotation. Since each Purkinje cell (P-cell) is synapsed upon by a single climbing fiber, each floccular P-cell can be assigned to one of three classes by determining the preferred axis of rotation of its complex spike (CS) response to visual stimulation. Collectively, the preferred axes of rotation, which bear a close resemblance to the principal axes of the semicircular canals, establish a natural or intrinsic reference frame potentially useful in the analysis of signal processing in the flocculus. In lightly anesthetized, immobilized rabbits we have extracellularly recorded the CS and simple spike (SS) responses of floccular P-cells to whole-world visual stimuli provided by a planetarium projector that produced a random spot pattern rotating about selectable axes. The responses of other neuronal elements were also recorded. Determination of the preferred axes of rotation for the CS responses confirmed that three basic classes of P-cells could be distinguished. In general, for a given P-cell the preferred axes of rotation for the CS and SS responses were similar. By far the most common relation between CS and SS responses was one of reciprocity, i.e. when CS activity increased SS activity decreased and vice-versa. A reciprocal relation seems not to be obligatory for the CS and SS activity of some cells increased and decreased together. While the SS responses are induced by retinal image slip, they likely represent a combination of retinal slip signals *per se* and signals related to aspects of eye movements consequent to the presence of retinal slip. In most P-cells with a binocular receptive field the spatial organization of both the CS and SS receptive fields was such as to prefer rotation rather than translation of the visual world. In a few cases, however, the sense of the direction selectivity of the SS response for each eye alone was such that translation would produce the greater modulation with binocular viewing. Responses with preferred axes similar to those of CS responses were also recorded from neuronal elements other than P-cells. In summary, the spatial organization of the visual world yielding preferred axes of rotation of floccular CS responses in vestibular coordinates is prevalent for SS responses. Supported by USPHS grant NS13742 and DFG grant Gr688/1.

44.11 VESTIBULAR CONTRIBUTIONS TO TARGET VELOCITY SIGNAL IN THE MONKEY VERMIS. David A. Suzuki* and Edward L. Keller. (SPON: E.Sutter). Smith-Kettlewell Inst. Visual Sciences, San Francisco, CA 94115.

The observation of retinal slip and smooth pursuit eye velocity-related Purkinje cell activity in lobules VI and VII, has raised the possibility of a neural target velocity correlate in the cerebellar vermis. The target velocity construct, however, requires the additional presence of a head velocity signal, the existence of which will be reported.

In order to clarify the sensory-oculomotor interactions occurring in the cerebellum, monkeys were trained to 1)suppress their vestibuloocular reflex (VOR), 2)track a small moving spot, and 3)fixate a stationary spot during movement of either a whole-field drum or a small test spot not associated with a reward. With these respective paradigms, head, smooth pursuit eye, and retinal slip velocity signals could be dissociated. Purkinje cells lacking vestibular inputs and exhibiting eye movement or eye movement plus retinal slip-related activites were observed, but this report will focus on the units exhibiting head velocity-related activity.

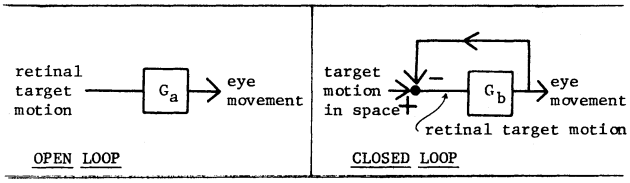
The majority of Purkinje cells receiving vestibular inputs exhibited peak firing rates during contralateral head rotations. Modulations in discharge rate were approximately in-phase with and increased in amplitude with head velocity. Some of these units also showed discharge modulations during pursuit eye movements with peak firing rates occurring with maximum contralateral eye velocity. Retinal slip velocity-related activity was observed in some units and peak activity occurred with contralateral visual stimulus movement. Thus, when head, smooth pursuit eye, and retinal slip velocity-related signals could be observed in the same Purkinje cell, peak firing rate was associated with head, eye, and image movement in the same direction (contralateral). This convergence of velocity signals implicates the presence of a neural correlate of a target velocity signal.

In a minority of the units demonstrating head velocity-related activity, peak firing rates occurred during ipsilateral head rotation. Of these units, some also exhibited pursuit eye and/or retinal slip velocity-related discharges. The eye and retinal slip-related activities usually exhibited peak firing rates during contralateral pursuit or visual stimulus movement. These units were, therefore, characterized by differing directional preferences for head and eye/retinal image movements. Such units could contribute to VOR or optokinetic functions.

The results suggest both the neccessity of broadening the label "vestibulocerebellum" to include the vermis and an involvement of vermal lobules VI and VII in the regulation of smooth pursuit eye movements.

44.12 SMOOTH PURSUIT EYE MOVEMENTS IN OPEN AND CLOSED LOOP: A LINEAR SYSTEM, ROUGHLY SPEAKING. Jordan Pola and Harry J. Wyatt. State University of New York, College of Optometry, NY, NY 10010.

Laboratory studies of the human smooth pursuit eye movement system have often been performed in the "open loop"; that is, with target motion (often sinusoidal) stabilized at the retina. In this situation, target motion at the retina, which is assumed to be the stimulus, is held constant regardless of the eye movements that result. In contrast, in the real world, a person generally makes pursuit eye movements in the "closed loop"; that is, target motion at the retina varies with the eye movement. The two conditions can be represented roughly as follows:



The open loop has significant experimental advantages; for example, gain and phase lag of the response (for constant stimulus frequency) may reflect system parameters and stimulus effectiveness. (Closed-loop gain and phase are often constrained to be near 1 and 0^{o} respectively). However, it is unclear whether the open-loop pursuit system tells us how the system performs in the real world. The relationship between open- and closed-loop performance is relatively simple for a linear system (G_a and G_b in the Figure are the same), but possible non-linear and volitional components of pursuit movements prevent us from assuming simple linearity.

We have measured open- and closed-loop pursuit movements for sinusoidal target motion, at frequencies 0.25-3.0 Hz and amplitudes $2\text{-}6^{o}$ peak-to-peak. Gain and phase lag were measured from the eye position record. (This entailed removing saccades from the records). The open-loop data were used to calculate closed-loop gain and phase lag for a linear system, and we compared calculated and experimental closed-loop results for each subject. We have observed a striking degree of correspondence between the linear prediction and the experimental closed-loop data. This suggests that (i) the steady-state pursuit system is approximately linear for small signals, and (ii) results from open-loop experiments have application in the real world.

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45.1 METOCLOPRAMIDE INCREASES CANINE PLASMA β-ENDORPHIN IMMUNOREACTIVITY AND CORTISOL: EVIDENCE FOR SECRETION FROM PITUITARY INTERMEDIATE LOBE. B. Sharp*, E. Levin*, and J. Sowers* (SPON: D. Aures-Fischer). VA Wadsworth and Sepulveda Medical Centers and UCLA Dept. of Medicine, Los Angeles, CA. 90073.

Dopamine has been shown to inhibit the secretion of β-endorphin-immunoreactivity (β-EP-I) from the intermediate lobe of the pituitary, in vitro. The in vivo physiology of this modulation including its potential influence on the secretion of intermediate lobe β-EP-I into the systemic circulation has not been evaluated.

Metoclopramide, a specific dopamine receptor antagonist, was administered iv (2.5 mg) to 5 loosely restrained male mongrel dogs. Blood was drawn via an indwelling iv cannula at -30, -15, 0, +10, +20, +45, +60, and +90 min. In a second experiment, dogs were pretreated with dexamethasone 1 mg iv 120 min prior to metoclopramide. All plasma was extracted with talc and eluted with HCl+acetone, yielding recovery efficiencies of 60% and 30% for β-endorphin and β-lipotropin (β-LPH), respectively. β-EP-I was measured by an RIA which has equimolar cross-reactivity with β-LPH.

Following metoclopramide, basal β-EP-I levels increased from 16±2 pg/ml (mean±SE) to a peak of 68±16 at +10 min ($p<.025$). Thereafter, a steady decline to 27±5 at 90 min was observed. Cortisol increased from a basal of 3.3±0.5 µg/dl to 8.7±2.2 at 90 min ($p<.05$). Pretreatment with dexamethasone did not alter β-EP-I response but completely inhibited the cortisol response.

To further characterize the β-EP-I, 2 plasma pools were collected from 4 dogs prior to and 10 min following the administration of metoclopramide. The β-EP-I extracted from each pool was chromatographed on Sephadex G-50 gel, using a 0.9x60 cm. column at 4°C. RIA of the collected fractions showed 2 immunoreactive peaks which corresponded to the elution volume of β-LPH and β-EP. The basal β-LPH/β-EP ratio was 2.5 and the peak was 0.7.

Conclusions: The marked shift in the molar ratio of β-LPH/β-EP following the administration of metoclopramide is most consistent with secretion from the intermediate lobe of the pituitary since its β-EP-I content is predominantly β-EP rather than β-LPH. Furthermore, the rapid and significant rise in plasma β-EP-I following metoclopramide suggests that, at least in species such as the dog which have a well-defined pituitary intermediate lobe, the secretion of pituitary β-EP-I is tonically inhibited by endogenous dopamine. The differential effect of dexamethasone pretreatment on cortisol versus β-EP-I secretion suggests differences in the regulation of secretion from the two cell types which constitute the dog intermediate lobe; dopamine has not been shown to alter secretion by anterior lobe corticotrophs.

45.2 ANOMALOUS β-LIPOTROPIN CONTENT IN THE ANTERIOR PITUITARY OF BRATTLEBORO RATS:ABSENCE OF β-LIPOTROPIN. R. G. Allen*, J. C. Crabbe, Jr., J. Stack*, and N. D. Gaudette*. VA Medical Center and University of Oregon Health Sciences Center, Portland, OR 97201.

The biosynthesis and processing of corticotropin (ACTH), β-lipotropin (β-LPH), and β-endorphin in the rat pituitary are well understood. In the normal rat, the major protein containing the amino acid sequence of β-endorphin is β-LPH (MW approx. 14K). Since homozygous Diabetes Insipidus (Brattleboro) rats have been shown to lack functional vasopressin, as well as having reduced content of ACTH, it was of interest to us to investigate the forms of ACTH, β-LPH, and β-endorphin contained in anterior lobe tissue obtained from vasopressin deficient rats.

Anterior lobes obtained from Brattleboro rats were extracted in 30% acetic acid in the presence of protease inhibitors, lyophilized, and then fractionated by sodium dodecyl sulfate/polyacrylamide gel electrophoresis (SDS/PAGE). The gels were sliced, eluted, and assayed for β-LPH and β-endorphin immunoactivity. We found immunoactivity migrating on the gels only at the positions of the 31-35K common precursor to ACTH and β-LPH, and a very small amount of β-endorphin migrating at 3.5K daltons. Virtually no β-LPH was seen in these extracts.

In order to further assess this striking result we grew primary monolayer cultures of anterior lobe tissue taken from Brattleboro rats in the presence of radioactive methionine for periods of 2-48 hours. At the end of the incubation periods the cells were harvested and the extracts immunoprecipitated with affinity-purified antisera to ACTH, β-LPH and β-endorphin. The immunoprecipitates were then fractionated by SDS/PAGE, and the gels sliced, eluted and counted. Again, we found no intracellular β-LPH at any time period examined. Vasopressin added to the culture medium (10 ng/ml) for 24 hours did not cause β-LPH to appear in the cells. We conclude from these results that as well as a vasopressin defect, Brattleboro rats have a major defect in the processing and/or storage of β-LPH and β-endorphin derived from the common precursor to ACTH and β-LPH.

45.3 BETA-ENDORPHIN IMMUNOREACTIVITY IN RAT PLASMA: VARIATIONS IN RESPONSE TO DIFFERENT PHYSICAL STIMULI. G.P. Mueller. Dept. of Physiology, USUHS, Bethesda, MD 20014.

Recent findings in rats indicate that pituitary beta-endorphin (B-END) mediates, in part, the behavioral analgesia caused by certain forms of stress. Since the degree of analgesia produced depends upon the type of stress employed, it is possible that the amount of pituitary B-END released may also vary according to the particular stress experienced. To investigate this possibility, comparison of the effects of several types of stressful stimuli on plasma levels of B-END-like immunoreactivity (B-END-LI) was made in male rats. Treatments were: tail-flick and hot-plate nociception testing procedures, etherization and immobilization. Plasma levels of total B-END-LI were estimated by radioimmunoassay (Mueller, P.S.E.B.M. 165 75, 1980) and characterized by gel filtration chromatography (Sephadex G-50). The antibody used (C-55) recognizes B-END and beta-lipotropin (B-LPH) standards equally but does not cross react with N-terminal fragments of B-END (i.e. met-enkephalin and α-END). Continuous immobilization maximally increased circulating B-END-LI 10 fold by 10 to 30 min of stress. As compared to control values (.18±02 ng/ml) etherization for 90 sec increased plasma levels of B-END-LI 2.8, 5.0 and 3.1 fold by 5, 15 and 30 min respectively. Tail-flick and hot plate analgesia testing procedures both resulted in a doubling in circulating B-END-LI (.16±.03 ng/ml vs. .32±.18 ng/ml and .33±.06 ng/ml respectively), however, only the rise in response to hot-plate testing was significant due to the large variation in hormone values observed in the group subjected to tail-flick testing. Chromatographic analysis of B-END-LI in plasma pools revealed that about 70% of circulating B-END-LI in control animals co-chromatographs with B-END standard; the remaining 30% resembles B-LPH in molecular size. In response to stress (immobilization), both forms of plasma immunoreactivity were increased although a relatively greater rise in the form corresponding to B-LPH was observed. In stressed animals, 60% to 75% of circulating B-END-LI co-chromatographed with B-LPH standard. Together these data suggest that both B-END and B-LPH are secreted by the pituitary under basal and stimulated conditions. The increases in total plasma B-END-LI content observed in response to different stresses varied from 2 to 10 fold and these differences may indicate that the magnitude of a plasma B-END-LI response is directly related to the degree of trauma experienced.

45.4 THE SECRETION OF PITUITARY BETA-ENDORPHIN-LIKE IMMUNOREACTIVITY IS REGULATED INDEPENDENTLY BY DOPAMINE AND GLUCOCORTICOIDS IN THE RAT. J.M. Farah, Jr.*, D.I. Sapun* and G.P. Mueller (SPON: J.M. Sarvey). Department of Physiology, Uniformed Services University of the Health Sciences, Bethesda, MD 20014.

Recent in vitro studies indicate that several mechanisms probably control the secretion of beta-endorphin-like immunoreactivity (β-END-LI) by the rat pituitary. Dopamine acts directly on the pars intermedia but not the pars distalis to inhibit the release of β-END-LI. By contrast, glucocorticoids inhibit the spontaneous release of β-END-LI from pars distalis cells but is without effect on cells of the pars intermedia (see Eipper and Mains, Endo. Rev. 1:1, 80). The purpose of the present study was to examine the possibility that dopaminergic neurons and circulating glucocorticoids may regulate independently the pars intermedia and pars distalis secretions of β-END-LI in vivo. Accordingly, dopaminergic and glucocorticoid drugs were given alone and in combination to determine their effects on plasma levels of β-END-LI in male rats. Plasma β-END-LI was measured by RIA (Mueller, P.S.E.B.M. 165:75, 1980); the antibody used (C-55) recognizes camel β-END and human beta-lipotropin (β-LPH) equally but does not detect N-terminal fragments of β-END (met-enkephalin and alpha-endorphin). Administration of the dopamine receptor blocker, haloperidol (HAL, 2.5 mg/kg, ip) maximally increased plasma β-END-LI 3.4-fold over control values by 30 and 60 min after injection (.22±.04 ng/ml to .78±.15 and .71±.11 ng/ml respectively); by 3 h after HAL, plasma levels of β-END-LI had returned towards control values. Pretreatment with the dopamine receptor agonist, bromocriptine (5 mg/kg, ip, 2 h) completely prevented the release of β-END-LI in response to HAL. However, the HAL-induced increase in circulating β-END-LI was not affected by pretreatment with a dose of dexamethasone (50 µg/ml, ip, 4 h) known to completely prevent the stress-induced release of pituitary β-END-LI. Conversely, metyrapone (100 mg/kg, ip, 30 min), a blocker of corticosterone synthesis, increased plasma levels of β-END-LI 5-fold over control values (1.31±.32 ng/ml vs. .25±.03 ng/ml); this rise was unaffected by pretreatment (2 h) with either 1 or 5 mg/kg bromocriptine. The present results demonstrate that circulating β-END-LI in rats is acutely elevated by dopamine receptor blockade in a manner which appears to be independent of glucocorticoid influence on pituitary β-END-LI secretion. Conversely, the acute effects of glucocorticoid manipulations (dexamethansone and metyrapone) on plasma β-END-LI seem to occur independent of dopaminergic regulation. These findings are consistent with the view that dopamine neurons regulate β-END-LI release by the pars intermedia in vivo whereas the glucocorticoids inhibit the secretion of β-END-LI by the pars distalis.

45.5 ADRENERGIC REGULATION OF IMMUNOREACTIVE BETA-ENDORPHIN RELEASE FROM RAT PITUITARY. D.J. Pettibone and G.P. Mueller, Dept. of Physiology, Uniformed Services University, Bethesda, MD 20014.

Stimulation of α-adrenergic receptors by clonidine in vivo evokes the release of β-endorphin-like immunoreactivity (β-END-LI) into the circulation (ENDO, in press) probably by acting directly on cells of the par distalis (ENDO SOC abs, 1981). In this report, we further characterize the adrenergic control of β-END-LI release from rat pituitary in vitro and in vivo.

Enzymatically dissociated anterior lobe (AL) or neurointermediate lobe (NIL) cells from male, Sprague-Dawley rats were cultured for 5-9 days and then incubated for 2h in presence or absence of various drugs. Release medium was assayed for β-END-LI using an RIA for camel β-END (Mueller, P.S.E.B.M. 165:75, 1980). The RIA recognizes human β-lipotropin (β-LPH) and β-END equally but does not detect N-terminal fragments of β-END (enkephalin, α-endorphin). Incubation of AL cells with any of several α-adrenergic agonists significantly enhanced release of β-END-LI; α-methylnorepinephrine (10^{-6}M) from 0.80±0.06 to 1.44±0.16, methoxamine (10^{-5}M) from 2.20±0.12 to 5.58±0.62, clonidine (10^{-6}M) from 2.20±0.12 to 5.64±0.22 ng/plate. Mixed α-, β-adrenergic agonists such as norepinephrine (10^{-6}M) or epinephrine (10^{-6}M) also significantly enhanced release of β-END-LI from AL cells. The epinephrine-induced release (1.74±0.11 to 9.75±0.72 ng/plate) appears to be mediated by α-adrenergic receptors because phenoxybenzamine (10^{-5}M), but not propranolol (10^{-5}M), blocked the response.

Release of β-END-LI from NIL cells was not affected by clonidine (10^{-6} to 10^{-10}M) (ENDO SOC Abs, 1981), however β-adrenergic activation by isoproterenol (10^{-7}M) increased β-END-LI release from 20.18±2.32 to 39.02±1.69 ng/plate, an effect blocked by propranolol (10^{-5}M). Isoproterenol also released β-END-LI from AL cells but the concentration required was 100-fold greater than that which activated NIL release.

Intraperitoneal administration of isoproterenol (1 mg/kg) to rats increased plasma β-END-LI (0.38±0.03 to 1.83±0.19 ng/ml) 30 min post-injection. Treatment with propranolol (2 mg/kg, ip) 45 min before isoproterenol (200 µg/kg, ip) blocked the 3-fold rise in plasma β-END-LI, suggesting a β-adrenergic involvement.

These results demonstrate that α- and β-adrenergic activation releases β-END-LI from the pituitary in vivo and in vitro. Secretion of β-END-LI from AL or NIL cells in culture appears to be controlled predominantly by α- and β-adrenergic mechanisms, respectively. Together, these findings raise the possibility that blood-borne catecholamines may normally influence release of pituitary β-END-LI in vivo.

45.6 SEROTONERGIC NEURONS STIMULATE RENIN AND CORTICOSTERONE SECRETION VIA THE PITUITARY GLAND. L.D. Van De Kar*, C.W. Wilkinson*, M.S. Brownfield*, Y. Skrobik* and W.F. Ganong. Dept. of Physiology, University of California, San Francisco, CA 94143.

Brain serotonin has been shown to stimulate renin secretion in anesthetized dogs (Zimmermann and Ganong, Neuroendocrinology, 30:101, 1980). In conscious rats, the serotonin agonist quipazine and the serotonin releaser p-chloroamphetamine (PCA) caused an increase in both plasma renin activity (PRA) and corticosterone (Van De Kar et al. Neurosci. Abstr. 6:323, 1980). In order to identify the population of serotonergic neurons that stimulates renin and corticosterone secretion, the dorsal or median raphe nuclei were selectively lesioned by local injection of 5,7-dihydroxytryptamine (5,7-DHT) into desmethylimipramine pretreated rats. Two weeks later, the rats received saline or PCA (10 mg/kg i.p.) 2 hours before sacrifice. The PCA-induced increase in PRA was significantly inhibited by dorsal but not median raphe lesions. Combined dorsal-median raphe lesions also inhibited the rise in PRA caused by PCA. In contrast, the effect of PCA on plasma corticosterone was not inhibited by any of the lesions. This suggests that serotonergic neurons in the dorsal raphe mediate a stimulatory influence on renin but not corticosterone secretion.

In an effort to identify the pathways employed to exert this effect, rats were pretreated with the sympathetic blocker bretylium tosylate (10 mg/kg i.p.) 18 or 4 hours before sacrifice. This treatment did not prevent the effect of PCA on PRA and corticosterone. Adrenal enucleation performed 5 weeks before sacrifice, also failed to affect the PRA and corticosterone responses to PCA. Adrenalectomy, 70 hours before sacrifice, caused a significant increase in PRA and did not prevent the PCA-induced increase in PRA. The effect of PCA on PRA and corticosterone was completely abolished by hypophysectomy 22 days before sacrifice. These results suggest that the information from the central serotonin receptors is transmitted to the kidney via a factor that is secreted from the pituitary gland.

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45.7 EFFECT OF CHOLINOMIMETICS AND CHOLINESTERASE INHIBITORS ON PLASMA BETA-ENDORPHINS. Edward H. Mougey* and James L. Meyerhoff. Dept. of Medical Neurosciences, Div. of Neuropsychiatry, Walter Reed Army Institute of Research, Washington, D.C. 20012.

Cholinomimetic agents have been shown to release pituitary corticotrophins (1). Since corticotrophins and endorphins may share a common biochemical precursor (2), and since physostigmine is reported to have antinociceptive effects, we hypothesized that cholinomimetics or cholinesterase inhibitors might release pituitary endorphins. Accordingly, we have examined the effects of oxotremorine, nicotine, neostigmine, and physostigmine on plasma beta-endorphin immunoreactivity. Male albino Sprague-Dawley rats weighing 300 ± 10 grams were individually housed in a room with an ambient temperature of 23 ± 2°C and a 12/12 light/dark cycle (0700-1900 light). Food and water were available ad libitum. The rats were habituated to intraperitoneal injections of saline for 4 days and on the 5th day subjected to intraperitoneal injection of oxotremorine (1.2 mg/kg), nicotine (1.2 mg/kg), physostigmine (0.375 mg/kg), neostigmine (0.375 mg/kg) or saline. Ten minutes following injection the rats were decapitated, the blood collected in cold heparinized tubes, and centrifuged for 10 min at 4°C. The plasma was transferred to tubes containing traysylol and stored at -35°C. The samples were extracted to concentrate the immunoreactive fraction and then assayed for beta-endorphin immunoreactivity using antibody produced in rabbits in our laboratory. Significant increases in beta-endorphin immunoreactivity were produced by all four drugs. The finding of a response to neostigmine, which does not cross the blood brain barrier, suggests that direct effects on pituitary receptors must be considered along with several other possible mechanisms, including stress which has been demonstrated to release adrenocorticotropin and beta-endorphin concomitantly (3).

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45.8 DESENSITIZATION OF THE ACTH RESPONSE TO EPINEPHRINE IN RAT ANTERIOR PITUITARY CELLS IN CULTURE. V. Giguère* and F. Labrie* (SPON: S. Radouco-Thomas), MRC Group in Molecular Endocrinology, CHUL, Québec, Canada G1V 4G2.

Recent data obtained in our laboratory show that ACTH secretion in rat anterior pituitary cells in primary culture is stimulated by highly specific α_1-adrenergic mechanisms. The ACTH response to epinephrine (EPI) is inhibited by the specific α_1-adrenergic antagonist prazosin at a K_d value of 0.06 nM while the α_2 antagonist yohimbine is 1,200 times less active. Many hormones which elicit a response in their target cells by initially binding to cell surface receptors have been found to decrease the magnitude of their responses during prolonged incubation. In order to examine the phenomenon of desensitization of the response to EPI in culture rat anterior pituitary cells, we have studied the ACTH response to EPI during prolonged incubation with the catecholamine. During a 3-h incubation period, rat anterior pituitary cells respond to increasing concentrations of EPI with a 10- to 12-fold stimulation of ACTH release at an ED_{50} value of 15 nM. First exposure of the cells to 1 µM EPI during 3 h leads to a decrease in the ACTH response to a second challenge with 30 nM EPI. Desensitization to EPI reaches a plateau at about 30% of the control stimulation after 2 h of preincubation. The desensitization by EPI is obtained at an ED_{50} value of 10 nM. The reduction of the ACTH response to EPI is not due to a decrease in pituitary cell ACTH content since EPI pretreatment reduces ACTH content by only 10%. We next studied the dose-response curves to EPI in control and 1 µM EPI-treated cells. The ED_{50} value for EPI stimulation of ACTH secretion is 3-fold higher in cells pretreated with the catecholamine while the maximal response to EPI is inhibited by 60%. When 1 µM phentolamine, an α-adrenergic antagonist, is present during preincubation with 1 µM EPI, no decrease in ACTH response to EPI is observed during the second incubation with EPI alone. Preincubations with phentolamine alone has no effect on a later stimulation by EPI, thus indicating that the occupancy of the α-adrenergic receptor is not sufficient to cause desensitization. When cells are preincubated for 3 h with 1 mM IBMX, a phosphodiesterase inhibitor, the ACTH response to 30 nM EPI is inhibited by 40%. The present data show that stimulation of the α_1-adrenergic receptor controlling ACTH secretion causes rapid desensitization to further stimulation by EPI. While α-adrenergic stimulation induces an almost complete inhibition of the response to a second stimulus, increased cyclic AMP levels induced by IBMX can also cause some desensitization (40%), thus suggesting that stimulation of cellular activity independant of receptor binding is also responsible for desensitization to specific stimuli.

45.9 ENKEPHALIN SYSTEMS IN THE MEDIAN EMINENCE OF THE MOUSE: EFFECTS OF NEONATAL MSG TREATMENTS. M.A. Romagnano, Theresa L. Chafel*, W.H. Pilcher* and S.A. Joseph. The Neuroendocrine Unit, Univ. of Rochester Sch. of Med., Rochester, NY 14642.

The immunocytochemical distribution of enkephalin (Enk) was examined in the mediobasal hypothalamus of control mice and monosodium glutamate (MSG) treated mice using the unlabeled antibody enzyme method. Mice were neonatally treated with MSG according to the protocol of Pilcher (previously described). As adults, both control and experimental animals were perfused with Bouin's fixative, and the brains were serially sectioned at 50 μ on an Oxford Vibratome. Sections were immunocytochemically stained in Enk antiserum used at a dilution of 1:2000 (antiserum from Drs. Sundberg and Morris). Alternate sections were stained with ACTH antiserum to determine the extent of the lesion. We have previously shown that in the rat and mouse, the opiocortin bed nucleus is glutamate-sensitive and neonatal treatment with MSG destroys the cell bodies and fiber projections which normally stain with ACTH antiserum. In this study, only animals which demonstrated a complete lesion were used.

In addition to other areas which have been previously described (Wamsley et al., Brain Res., 190:153-173, 1980), Enk immunoreactive fibers in the mediobasal hypothalamus of control mice were found in the median eminence (ME). Both the internal and external zones of the ME contained numerous Enk fibers. In contrast, MSG-lesioned mice displayed a complete lack of immunoreactivity throughout the ME.

The presence of enkephalinergic fibers in the external zone of the ME in close proximity to portal vessels and loss of these fibers after MSG treatment suggests a possible role for enkephalin neuropeptidergic interaction with other neuroendocrine systems.

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45.10 TIME COURSE FOR DEVELOPMENT OF INCREASED PLASMA CONCENTRATIONS OF CORTICOSTERONE FOLLOWING HIPPOCAMPECTOMY IN ADULT FEMALE RATS. M. M. Wilson, S. E. Greer*, and D. L. Keith*. Univ. of Portland and Univ. of Oregon Health Sci. Ctr., Portland, OR 97203

In a previous study, we observed increased basal PM plasma corticosterone (B) and ACTH concentrations in female rats whose hippocampus had been removed by suction (Wilson, M. M., et al., Brain Res., 197:433, 1980). There were no differences between hippocampectomized and cortex-lesioned controls in the AM and both groups showed AM-PM fluctuations indicative of an intact circadian rhythm. The increased PM basal samples were surprising because they have not been reported in numerous experiments on hippocampectomized rats. We tested the hypothesis that B concentrations did not increase until at least one month after surgery when neural reorganization has occurred. PM blood samples were obtained from adult female rats 8 days after they were housed in our controlled environment animal quarters (lights on 6 AM-6 PM). Rats were then assigned to intact (I), cortex-removed (CORTX) and hippocampectomized (HIPPX) groups by a randomized block design. Both dorsal and ventral hippocampus were removed by suction. In the CORTX control group, that portion of parietal cortex removed in the HIPPX groups was suctioned off to expose the dorsal hippocampus. Rats were housed 2/cage in a random order. No one entered the animal room for 24 hrs prior to the experiment. Experiments were done from 5:00-6:20 PM. Rats housed together were removed from the cage simultaneously and taken to an adjoining room where they were placed in jars saturated with ether until unconscious. Blood samples (1 ml) were drawn from the exposed external jugular vein within 3 min from the time the rats were first disturbed. Samples were collected weekly from 1-6 wks after surgery. All samples were run in the same radioimmunoassay for B to eliminate any effect of interassay variation. In hippocampectomized rats, plasma B concentrations were higher 1 wk after surgery than before surgery. Pre- and post-lesion values did not differ in the other groups. At 1 wk after surgery, there were no differences between groups in the PM plasma B concentrations. Corticosterone concentrations in the HIPPX group increased over the 6 wk post-surgery period with plasma concentrations at 5 and 6 wks higher than those at 1, 2, and 3 wks. Control groups did not vary with time. At 5 and 6 wks after surgery, PM plasma B concentrations were higher in HIPPX than in control groups. The results indicate that there is an immediate effect (within 1 wk) of removing the hippocampus on PM plasma B concentrations, but the full effects of hippocampectomy are not apparent until 5 wks after surgery.

45.11 SOMATOSTATIN RELEASE IN THE MEDIAN EMINENCE AS DETERMINED BY PUSH-PULL PERFUSION IN THE UNANESTHETIZED RAT. N.W. Kasting, J.B. Martin and M.A. Arnold*. Neurology Res. Lab., Mass. Gen. Hosp. and Harvard Med. Sch., Boston, MA 02114

Somatostatin is a tetradecapeptide known to inhibit the secretion of growth hormone (GH) from the anterior pituitary. Somatostatin is released from hypothalamic neuronal terminals in the ME where it reaches the anterior pituitary via the portal circulation. Research utilizing deafferentation techniques, lesions, or passive immunization with anti-somatostatin serum has indicated that somatostatin may have an important role in the rat in stress-induced GH suppression but its role in non-stress pulsatile GH release in plasma is not clear. It may function to decrease trough levels of GH. The present research was designed to investigate dynamic in vivo release of somatostatin from neuronal terminals in the median eminence (ME) in the unanesthetized, freely-behaving rat and to clarify the relationship of somatostatin secretion to plasma GH levels. To accomplish this goal, 300 g SD male rats were surgically implanted with chronic venous catheters and chronic intracranial guide cannulae directed towards the ME. After rats had recovered from surgery, they were placed in a sampling cage in which a spring-ball bearing assembly allowed the rats free movement and enabled tubing to pass out of the cage for sampling. Push-pull perfusion was used to evaluate immunoreactive somatostatin (IRS) release in the ME. Perfusion samples were collected at 15 min intervals and assayed for IRS by a specific and sensitive RIA. At the end of each 15 minute perfusion period a venous plasma sample was collected to be assayed for GH and prolactin (PRL). The experimental perfusion lasted for 4 h from 1000 h to 1400 h and the rats had free access to food and water. Results indicate that IRS is released in the ME in a pulsatile and episodic fashion with an inter-peak interval of about 1 h. Four hour determinations of mean plasma GH levels and mean IRS release showed a significant negative correlation. The results tended to cluster into two groups, one with low IRS release, normal pulsatile GH release and low PRL, and the other with high IRS release, low plasma GH levels and high PRL, indicative of a stressed animal. Peaks of IRS release often were concurrent with peaks of plasma GH. Gel chromatography indicated that the IRS was composed of several molecular weight species. These results suggested that somatostatin has an important role in stress-induced GH suppression and that there is also a short loop feedback of GH on somatostatin neurons. Furthermore, somatostatin appears to be released in several molecular weight forms, probably representing prohormone or precursor forms of the molecule.

45.12 MODULATION AND FUNCTIONAL ROLE OF GABA BINDING SITES AND UPTAKE SYSTEM IN RAT ANTERIOR PITUITARY. G. Racagni J.A. Apud, C. Civati, D. Cocchi+, V. Locatelli+ and E.E. Müller+. Inst. of Pharmacology and Pharmacognosy and Inst. of Pharmacology+, Univ. of Milan, Italy.

Evidence has been accumulated that γ-aminobutyric acid (GABA) plays a role in the regulation of anterior pituitary (AP) function and, particularly, of prolactin (PRL) release. In this context, we have presented data supporting the existence of a dual GABAergic control of PRL secretion in the rat; one stimulatory exerted on a central nervous system (CNS) site, and the other inhibitory, occurring at the level of the AP. Further, we have shown that GABA is present in the AP and derives from medio-basal hypothalamic (MBH) structures, which release the aminoacid into the hypophyseal portal vesvels. In this presentation, we report evidence on the measurement of GABA in the pituitary portal circulation and the existence at AP level of a GABAergic uptake system and different populations of GABA binding sites. GABA concentrations in the stalk blood were determined by a mass fragmentographic technique and the values are 677 ± 68 pmol/ml. GABA in the AP is taken up by an effective uptake system, temperature dependent. When AP homogenates were incubated at 15°C in a medium containing ^{3}H-GABA ($5 \cdot 10^{-8}$M) there was a rapid accumulation of radioactivity in the tissue, after 60 min. Previous studies by our group have indicated that AP does possess the enzymatic machinery to break down the aminoacid, i.e. GABA-T. The presence of this enzyme may account for the high doses of GABA required for significant inhibiting PRL secretion, we now report experiments performed on in vitro pituitaries in the presence of a specific GABA-T blocker, ethanolamine-O-sulphate, showing that GABA is able to decrease PRL release at lower concentrations (10^{-6}M). To characterize the pituitary GABA receptors, the binding of ^{3}H-muscimol (^{3}H-M) to crude mitochondrial AP membrane fractions, extensively washed with triton X 100, was examined. ^{3}H-M binding was saturable and occurred with a high (K_D= 2.8 nM and B_{max}= 45 fmol/mg prot) and low (K_D= 29 nM and B_{max}= 155 fmol/mg prot) affinity. Stereotaxic lesions at the level of MBH elicited a significant increase of the number of ^{3}H-M binding sites, suggesting that these receptors have become supersensitive. These results indicate that the inhibitory action of GABA at the pituitary on PRL secretion may represent a receptor mediated and a physiological action.

46.1 SYNAPTIC CONNECTIONS BETWEEN LEECH SWIM-INITIATING NEURONS AND THE SWIM CENTRAL PATTERN GENERATING CIRCUIT. Janis C. Weeks
Dept. of Biology, UCSD, La Jolla, CA 92093 (present address: Dept. of Zoology, Univ. of WA, Seattle, WA 98195).

Previously, swim motor neurons, pattern-generating "oscillator" interneurons and a single swim-initiating interneuron (cell 204) were identified in the leech (Ort et al. 1974; Friesen et al. 1978; Weeks & Kristan, 1978). Friesen and collaborators proposed a model for the swim central pattern generator (CPG) circuit involving the four oscillator cells, but more recent results (Weeks, in press) indicate that this description is incomplete and that other CPG cells must exist. In the present experiments, synaptic connections were sought between cell 204 and the oscillator cells, and between cell 204 and two newly identified CPG cells. These results provide the first indications of how the activity of swim-initiating cells causes activation of the rhythmic CPG circuit.

Pairwise intracellular recordings from cell 204 and oscillator cells revealed no synaptic connections in either direction. Cell 204 affects oscillator cells only insofar as its activity serves to evoke rhythmic swim motor output. Hence, cell 204 does not initiate swimming by way of effects on oscillator cells but rather must act via other CPG cells. Accordingly, new CPG cells were sought and two were identified and characterized.

Both new cells (cells 205 & 208) are unpaired intersegmental interneurons anatomically similar to cell 204. Cell 205 not only participates in pattern generation, but also has swim-initiating capabilities similar to those of cell 204 and is the first bifunctional swim cell of this type to be discovered. Cells 204 & 205 are not synaptically linked. The second new CPG neuron, cell 208, provides a major monosynaptic (as tested by TEA injection) source of rhythmic excitation to dorsal excitor motor neurons during swimming. Cell 208 is most interesting in that it is directly excited interganglionically by cells 204 & 205 and accordingly provides the first known link between swim-initiating cells and the CPG. Because of these inputs cell 208 is activated early on during swim initiation, unlike the oscillator cells which come on only at the onset of rhythmic motor bursts.

In other experiments both cell 205 and 208 were found to receive mechanosensory input. Cell 205 is polysynaptically excited by T (touch), P (pressure) and N (nociceptive) sensory neurons, and has a rectifying electrical connection with the multimodal giant fiber (S cell) system. Cell 208 is inhibited by P and N cells.

Friesen et al. (1978) J.exp.Biol. 75:25-43; Ort et al. (1974) J.comp.Physiol. 94:121-156; Weeks (in press) J. Neurophysiol.; Weeks & Kristan (1978) J.exp.Biol. 77:71-88.

46.2 MOTOR CONTROL IN THE LEECH: A NEWLY IDENTIFIED INTERNEURON SUBSERVING VENTRAL FLEXION. M.P. Nusbaum and W.B. Kristan, Jr., Dept. of Biology, UCSD, La Jolla, CA. 92093.

A new bilaterally paired intersegmental interneuron has been identified in the segmental ganglia of the gnathobdellid leeches Hirudo and Macrobdella. This cell, the Ventral Flexor interneuron (VF cell), when stimulated in brainless, semi-intact preparations causes the posterior end of the leech to flex ventrally and to one side. Stimulation of it's bilateral homologue causes the back end to flex to the other side. The anterior end of the leech shows no behavioral response to VF cell stimulation. These behavioral responses result from synaptic connections from the VF cells to the ensemble of identified motor neurons that innervate the dorsal and ventral longitudinal muscles. These are the muscles used by the leech for swimming and shortening, as well as for a ventral flexion. Each VF cell excites all of the ipsilateral ventral exciter motor neurons, and bilaterally inhibits the dorsal exciter motor neurons, in all ganglia posterior to it's own.

Serial VF homologues are weakly coupled such that an anterior VF cell excites a posterior VF cell, but there is no connection in the reverse direction. There is only weak electrical coupling between the bilateral VF homologues so that common presynaptic input is necessary to activate these cells coordinately. This suggests that the VF cells may be part of neuronal circuits for different behaviors, both local and whole-body.

Both VF cells receive bilateral mechanosensory and photosensory excitation, intra-and intersegmentally. Much of both of these sensory inputs are mediated by the multi-modal interneuron, the S cell. This cell receives strong mechanosensory and photosensory excitation and has a monosynaptic excitatory connection onto the VF cells.

There are also reciprocal inhibitory interactions between the VF cells and cells in the swim circuit. Depolarizing the swim-initiating interneuron, cell 204, results in a barrage of ipsp's in the VF cells. During swimming, the VF cells receive strong phasic inhibition. In turn, depolarizing a VF cell occasionally stops a swim.

Experiments are now under way to further elucidate this reciprocal inhibition, as well as to determine the contribution of the VF cells to natural behaviors.

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46.3 Rate Modification in the Leech Heartbeat Central Pattern Generator. Edmund A. Arbas Biological Laboratories, Harvard Univ. Cambridge MA. 02138

The central pattern generator (CPG) which drives contractions of heart tubes in the leech, Hirudo medicinalis, is well characterized in terms of its neuronal elements, and the synaptic interactions which produce its basic output rhythm. Heart tube contractions are elicited by rhythmic bursts of action potentials from Heart Excitor (HE) motorneurons. The HE cell activity rhythm is controlled by cyclical barrages of inhibitory synaptic potentials from an ensemble of Heart Interneurons or HN cells. To understand the dynamics of the CPG further, I have examined several pathways through which the period of the rhythm is modified, using intracellular recording and electrical stimulation of neurons in isolated nerve cords as well as in semi-intact preparations in which only a few ganglia were dissected for recording.

I find that, in general, stimuli which increase motor activity also lead to decreases in the cycle period of the heartbeat CPG. Profound decreases in the burst period of HE cells (of up to 50%) are observed in specific association with swimming behavior. Such decreases are observed whether swimming is manifested "spontaneously" in semi-intact preparations, or elicited by mechanical stimuli applied to the body wall, electrical stimulation of nerve roots or swim-initiating interneurons (Weeks & Kristan 1978 J. Exp. Biol. 77;71-88).

The interaction between the swim system and the heartbeat CPG is, in great part, centrally mediated. Reductions in HE cell burst period are observed in the absence of peripheral feedback, on activation of the swimming motor program in isolated nerve cords. The reduction of HE cell burst period in every case reflects accellerated cycling of the HN cell ensemble.

46.4 LEECH HEARTBEAT: TIMING AND COORDINATION BY INTERSEGMENTAL INTERNEURONS. E.L. Peterson. Biol. Labs., Harvard Univ., 16 Divinity Ave., Cambridge MA 02138.

In each of the first 7 segmental ganglia of the leech Hirudo medicinalis there is a bilateral pair of heart interneurons (HN cells). These 7 pairs control the firing of the motor neurons which drive the rhythmic constrictions of the two lateral hearttubes. The 4 most rostral pairs constitute the heartbeat 'timing oscillator' by virtue of their capacity to reset and entrain the activity of all cells in the heartbeat system. The timing oscillation is generated in the following way. The interneurons originating in ganglion 3 (cells HN(L,3) and HN(R,3)) fire impulse bursts in antiphase, a relationship sustained by a balance between strong monosynaptic reciprocal inhibition and the endogenous properties of these two cells. Similarly cells HN(L,4) and HN(R,4) inhibit one another strongly and burst in antiphase. Finally a cell HN(1) or HN(2) both inhibits and is inhibited by the ipsilateral HN(3) and HN(4) cells. The apparent function of the connections between cells HN(1)/HN(2) and HN(3)/HN(4) is to phase-lock the oscillation of the HN(4) pair to that of the HN(3) pair thereby ensuring a coordinated output to the rest of the heartbeat system. Specifically, with each cycle the cessation of the HN(3) burst releases the ipsilateral cells HN(1) and HN(2) from inhibition. The resulting activity of the latter cells delays the ipsilateral HN(4) cell's burst sufficiently to hold cell HN(4) in its characteristic phase relationship to cell HN(3). Thus the timing oscillator can be viewed as a pair of oscillators--one the HN(3) cell pair, the other the HN(4) cell pair--coupled by the interganglionic coordinating interneurons HN(1) and HN(2). (Supported by NIH 1 RO1NS 15101-01 to R.L. Calabrese and a NATO postdoctoral fellowship to E.L.P)

46.5 SYNAPTIC RELATIONS UNDERLYING SWALLOWING AND REGURGITATION IN NAVANAX, AN OPISTHOBRANCH MOLLUSC. M.B.Zimering,* D.C.Spray and M.V.L. Bennett, Dept. Neurosci., Einstein Col. Med., Bx., NY.

Navanax inermis ingests prey by a rapid pharyngeal expansion mediated by radial muscles of the pharynx. Ingestion is followed by swallowing or regurgitation. Swallowing results from a wave of contraction that spreads anteroposteriorly along succesive bands of circumferential muscles of the pharynx (CMs). Regurgitation may result from a similar wave of contraction in the reverse direction, but more often it results from a simple, synchronous contraction of more caudal CMs.

CMs are controlled by neurons in the buccal ganglia,CMNs, which innervate distinct CM bands in specific regions of the pharynx. There are 7-8 homologous CMNs in each buccal ganglion; 4 controlling CMs in anterior, middle, midposterior, and posterior regions of the pharynx unilaterally (LUA & RUA, LUM & RUM, LUMP &RUMP, LUP &RUP), and 3-4 controlling CMs in similar regions bilaterally (LBA & RBA, LBM &RBM and/or LBMP & RBMP, LBP & RBP). The midposterior regions overlap with both the middle and the posterior regions. Electrotonic coupling occurs between at least 30 classes of CMN pairs; coupling coefficients were determined for each of these pairs ($n \geq 3$) and were as large as 0.25. Patterns of connectivity are as follows: within one ganglion CMNs innervating muscles unilaterally and bilaterally at the same level are coupled, except for UP and BP CMNs. CMNs innervating adjacent muscle groups are coupled except for BP CMNs. CMNs innervating nonadjacent muscles are at most weakly coupled. Between opposite ganglia corresponding CMNs generally are coupled except for UP CMNs (BP CMNs not tested), and noncorresponding CMNs are at most weakly coupled.

Electrotonically coupled CMNs can exhibit "effective sign reversal of coupling" when there is much inhibitory activity in them (Science 194: 1065, '76). CMNs which are not electrotonically coupled also can exhibit reversed coupling. Systematic investigation of reversed coupling ($n \geq 3$) showed bilateral symmetry between ganglia of interactions within each ganglion, but both classes of reversed coupling between corresponding CMNs on opposite sides (LUM & RUM, LUMP & RUMP) were stronger from left to right. U and B CMNs at the same level did not exhibit reversed coupling except for RUM and RBM where the relation was reciprocal. Other intraganglionic relations were strongly directional anteroposteriorly, with CMNs exhibiting reversed coupling with each CMN innervating muscles at more posterior levels, i.e. A to M, MP and P; M to MP and P; MP to P. These relations imply that under conditions of a high rate of background inhibitory activity depolarization and hyperpolarization of more anterior cells inhibits and excites respectively more posterior cells; actions presumably important in swallowing. In the absence of inhibitory activity, electrotonic coupling may facilitate regurgitation or pharyngeal emptying prior to the rapid ingestion phase. Supported (in part) by NIH grant 5T 32GM7288.

46.6 CONSEQUENCES OF ELECTRICAL COUPLING AND NON-SPIKING RELEASE OF TRANSMITTER FOR CIRCUIT ANALYSIS AND TRANSMITTER IDENTIFICATION. J.S. Eisen and E. Marder. Biology Dept., Brandeis University, Waltham, MA 02254.

Both electrical coupling and non-spiking transmitter release have been described in invertebrate and vertebrate nervous systems. However, the implications of these two phenomena for circuit analysis and transmitter identification have not been yet fully explored. In the stomatogastric ganglion of the spiny lobster, the 2 PD motor neurons are electrically coupled to the AB interneuron. Depolarization (with or without action potential activation) evokes a complex IPSP in another motor neuron, the LP. Action potentials in the LP cell evoke discrete IPSPs which can be recorded in both the PD and AB neurons. Based on these data alone, there are 9 possible alternative representations of the PD-AB-LP circuit. Therefore, we have used the Lucifer Yellow cell fill/kill technique (Miller & Selverston, Sci 206: 702-704, 1979) to selectively remove either the AB or the PDs from the circuit, and we have been able to eliminate 8 of the 9 alternative representations for the PD-AB-LP circuit.

When the AB neuron is killed, the PDs still evoke an IPSP in the LP. Likewise when both PDs are killed the AB alone evokes an IPSP in the LP. Therefore, the compound IPSP recorded in the LP under physiological conditions is due to transmitter released from both the PD and AB neurons. When the AB neuron is killed, LP action potentials still evoke discrete IPSPs in the PDs. However, when the PDs are killed, LP action potentials no longer evoke IPSPs in the AB, suggesting that the IPSP normally recorded in the AB cell is actually an electrotonic coupling potential from the PD. The PD-AB group also evoke IPSPs in another follower cell, the VD motor neuron. In this case, Lucifer fill/kill experiments indicate that the IPSP recorded in the VD cell is evoked predominately, if not completely, by AB-released transmitter.

Current evidence suggests that the PD and AB cells release different transmitters. Our data show that transmitter identification in any vertebrate or invertebrate local circuit containing electrically coupled neurons capable of non-spiking transmitter release is possible only if one knows, with certainty, which of a group of electrically coupled cells is actually releasing transmitter. Supported by NSF BNS-78-15399 and the McKnight Foundation.

46.7 MODULATION OF ELECTRICAL COUPLING AMONG CELLS WITH DIFFERENT POSTSYNAPTIC ACTIONS ON THE SAME FOLLOWER CELLS: A MODEL FOR "SYNAPTIC SWITCHING". E. Marder and J.S. Eisen. Biology Dept. Brandeis University, Waltham, MA 02254.

The stomatogastric ganglion of the lobster contains 2 PD motor neurons which are electrically coupled to the AB, an interneuron. In the previous paper (Eisen & Marder, above) we showed that the compound IPSP recorded in the LP motor neuron resulting from depolarization of the PD-AB cell group is due to transmitter released from both the PD and AB cells.

The 2 PDs and the AB together evoke a complex IPSP in the LP cell. This IPSP is multiphasic, containing an early picrotoxin-sensitive component which reverses at E_K, and a later, picrotoxin-resistant component which fails to reverse, even at membrane potentials 20 to 40 mV more hyperpolarized than E_K. We used the Lucifer Yellow cell fill/kill technique (Miller & Selverston, Sci 206: 702-704, 1979) to kill either the single AB cell or 2 PD cells. We found that after killing the 2 PD cells, the AB-evoked IPSP in the LP cell was picrotoxin-sensitive and reversed at E_K. After killing the AB cell, we found that the PD-evoked IPSP in the LP cell showed no apparent reversal potential, at cell membrane potentials tested (-40mV to -140mV) and was picrotoxin-resistant. Thus the PD and electrically coupled AB cells have remarkably different postsynaptic actions on the same follower cell.

The strength of the electrical coupling between the PDs and the AB appears to be influenced by inputs from the central nervous system (Ayers, personal communication). We predict that changes in this coupling provide a mechanism by which under some conditions the IPSPs in the LP cell will be 1) primarily evoked by the PDs 2) primarily evoked by the AB or 3) evoked by a combination of the two, with different consequences for the postsynaptic cell.

The PD neurons make cholinergic neuromuscular connections, while all present evidence suggests that the AB-transmitter is glutamate. In the special case, when the electrically coupled cells have different postsynaptic actions because they release different transmitters, our model predicts that a postsynaptic effect might switch from one mediated primarily by one transmitter to one mediated primarily by the other transmitter.

We suggest that neuronal circuits containing electrically coupled neurons capable of non-spiking transmitter release provide hitherto unexplored mechanisms for synaptic modulation and plasticity.

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46.8 AN IDENTIFIED LOCAL INTERNEURON IN THE NEURAL OSCILLATOR OF CRAYFISH SWIMMERETS. D. H. Paul. Department of Zoology, University of California, Davis, CA 95616.

The swimmerets are paired appendages on the underside of the abdominal segments of crayfish. Each swimmeret is under the control of a neural oscillator in its own hemiganglion. The two oscillators in a ganglion can be coupled across the midline and to those of adjacent ganglia, so that when the appendages beat vigorously their movements are tightly coordinated. Using Lucifer dye filled microelectrodes to penetrate processes in the swimmeret neuropil, I have discovered six structurally and physiologically different local, nonspiking interneurons. There appears to be one of each type per hemiganglion. One of these, the MAIN cell - Medial (in the swimmeret neuropil), Axonless, InterNuncial cell - is an integral part of the swimmeret oscillator. It has a characteristic spontaneous behavior: abrupt hyperpolarizations caused by trains of IPSPs anticipate the onset of power-stroke bursts; the cell depolarizes as the IPSP frequency declines and it is relatively depolarized during return-stroke (RS) bursts as well as during tonic RS activity in non-bursting nerve cords. In silent preparations the MAIN cell's resting potential is -65 to -70 mV. During bursting it oscillates up to 10 mV around a -50 to -55 mV membrane potential. Injection of small depolarizing currents (≤1nA) stops bursting of all ganglia and drives tonically RS motoneurons. The reinstated bursting that begins following the depolarization is reset relative to the prestimulus pattern. The MAIN cell is dye- and electrically coupled to at least one, decussating RS motoneuron. When the nerve cord is not bursting, small hyperpolarizing currents (1nA) initiate and sustain the normal swimmeret bursting pattern in all ganglia; these hyperpolarizations also induce the usual patterning of IPSPs observed in this cell during spontaneous bursting. The MAIN cell is morphologically distinct from the other local interneurons by the shape, large size, and orientation of its integrating segment from which a characteristic array of primary branches extend dorsally (Fig. 1).

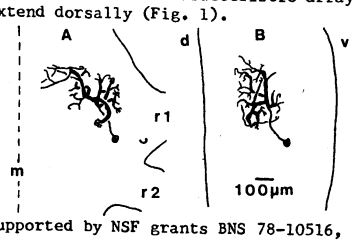

Fig.1. MAIN cell in right hemiganglion. A, dorsal aspect, B, sagittal view from right side. d, dorsal; m, midline; r1, root 1 (innervates swimmerets); r2, root 2; v, ventral.

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46.9 CENTRAL CONTROL OF A RHYTHMIC INSECT BEHAVIOR WHOSE NEURAL ACTIVITY PERSISTS IN THE ISOLATED GANGLION. Karen J. Thompson, Department of Biology, University of Oregon, Eugene, OR 97403.

Movements corresponding to oviposition digging behavior are elicited upon transection of the ventral nerve cord in female grasshoppers. The terminal abdominal ganglion drives these movements and is capable of expressing oviposition motor output in the absence of sensory information.

All nerves of the terminal abdominal ganglion can be cut and the ganglion removed from the animal for extracellular ovipositor motor nerve recording. The pattern of activity in the isolated ganglion is found to closely resemble the naturally occuring one. This is true for burst period, spiking discharge frequencies, and for phase relationships among the four muscles of the ventral ovipositor valves.

The oviposition pattern generator appears to be under the control of more rostral ganglia. This control is thought to be inhibitory because cutting the ventral nerve cord anterior to the terminal abdominal ganglion is followed by continuous digging behavior. An attempt was made to mimic presumed higher center inhibitory control by electrically stimulating the cut connectives. Oviposition ceased during stimulation and for one to many cycles afterwards, depending upon the strength of the stimulation.

Intracellular recordings from motoneurons of a major ovipositor muscle, the depressor, show electrical activity consisting of membrane potential oscillations of approximately 6 s. periodicity. The motoneurons discharge with spiking activity at the peak of the depolarizing wave. Membrane potential changes were imposed on motoneurons to determine if they are involved in pattern generation. These motoneurons were hyperpolarized to suppress their discharge. This did not affect the timing of subsequent bursts. Thus, the depressor motoneurons do not appear to be an integral part of the pattern generator. Instead, pattern generator activity must depend upon interneurons.

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46.10 FICTIVE SCRATCH AND FICTIVE FLEXION REFLEXES DISPLAY DIFFERENT MOTOR NEURON ACTIVITY PATTERNS IN THE TURTLE. Paul S.G. Stein, Gail A. Robertson* and Joyce Keifer*. Department of Biology, Washington University, St. Louis, MO 63130.

Gentle rubbing of specific regions of the shell in the low-spinal turtle will elicit a scratch reflex in which the foot reaches up and rubs against the stimulated site (Valk-Fai and Crowe, J. Comp. Physiol. 125:351,1978). The same stimulus will elicit a rhythmic motor program, termed the fictive scratch reflex, in an immobilized turtle (Stein and Grossman, J. Comp. Physiol. 140:287,1980). Gentle pressure applied to the dorsum of the foot will elicit a flexion reflex in which the foot withdraws from the location of the stimulus probe. The same stimulus will elicit a fictive flexion reflex motor program in an immobilized turtle. Electroneurographic recordings from muscle nerves and intracellular recordings from spinal motor neurons (MNs) reveal that these reflexes differ in their patterns of MN co-activation. This result indicates that the motor program responsible for the generation of the scratch reflex is different from that utilized for the flexion reflex in the turtle, Pseudemys scripta elegans.

The A1 phase of the fictive scratch is characterized by co-activation of MNs to iliotibialis (IT-KE), ventral puboischiofemoralis internus (VP-HP), and anterior iliofemoralis (AI-HP). These MNs maintain their activity until the middle or late portions of the A2 phase. During the A2 phase there is activation of MNs to femorotibialis (FT-KE) and ambiens (AM-KE). These MNs are active until the end of the A2 phase. Thus early in the A2 phase MNs to all five of these muscles are co-active. These MNs are inactive during the B phase of the fictive scratch.

In contrast, during the fictive flexion reflex MNs to AI-HP and VP-HP are depolarized and produce action potentials. At the same time, MNs to IT-KE, AM-KE and FT-KE are hyperpolarized. This demonstrates that the activation pattern produced during fictive flexion is different from that during fictive scratch. Therefore, in turtle, the synergies observed in the flexion reflex can not be utilized to construct a model of the scratch reflex program generator. Deliagina et al (J. Neurophysiol. 45:595,1981) have recently presented data which supports a similar conclusion in the cat. Sherrington (Quart. J. Exp. Physiol. 3:213,1910) claimed that a bipartite division of hindlimb musculature into flexors and extensors according to flexion reflex activation patterns can be utilized to describe the scratch reflex synergies in cat. This claim is not supported by the recent cat data and does not provide an adequate description of our turtle data. Supported by NIH Grant NS-15049 to PSGS and NIH Training Grant in Neurobiology NS-07071 to GAR.

46.11 SENSORY PATHWAYS ACTIVATING THE SCRATCH REFLEX IN THE TURTLE. Lawrence I. Mortin*, Joyce Keifer* and Paul S.G. Stein. (SPON: Viktor Hamburger). Department of Biology, Washington University, St. Louis, MO 63130.

Gentle mechanical stimulation of specific shell bridge regions (SP1, SP2 and SP3) of the low-spinal turtle, Pseudemys scripta elegans, elicits a rhythmic scratch reflex (Valk-Fai and Crowe, J. Comp. Physiol. 125:351,1978). This behavior can be monitored by electromyographic recordings (EMGs). Immobilized turtles produce a fictive scratch reflex which can be monitored by electroneurographic recordings (ENGs; Stein and Grossman, J. Comp. Physiol. 140:287,1980). Electrical stimulation of the carapace of a tortoise, Testudo graeca, excites cutaneous neurons in the shell (Rosenberg, Comp. Biochem. Physiol. 66A:227,1980).

We have implanted pairs of silver wires (4 mil) into shallow holes drilled into the shell bridge of low-spinal P. s. elegans at SP1, SP2 and SP3. Trains of electrical pulses (5-15 V, 1 msec pulses, 10-50 Hz for 50-500 msec) can elicit the goal directed scratch reflex as monitored by EMGs. Similar results have been obtained independently in Crowe's laboratory (personal communication). We have also found that this electrical stimulation will elicit a fictive scratch reflex. ENGs from segmental cutaneous nerves in the midbody region (D3-D6) reveal a temporally and spatially dispersed volley in response to each electrical pulse (4-22 msec latency range). This pattern of sensory activation remains the same when the nerve is cut central to the recording electrode. ENGs from cutaneous nerves D3 to D6 were used to map the dermatomes of the shell bridge. Each dermatome is directly lateral to the spinal root entry zone of its nerve. In the center of each dermatome mechanical stimulation excites only one cutaneous nerve; along the edges of each dermatome stimulation activates two adjacent segmental nerves ("overlap zone"). In general, the D3/D4 overlap zone contains SP3; the D4/D5 overlap zone contains SP2; and the D5/D6 overlap zone contains SP1.

The pathway of the central descending signal which initiates the scratch reflex was investigated with spinal cord hemisections at specific levels between D3 and D7. These resulted in the loss of scratch sensitivity in all ipsilateral shell bridge regions innervated exclusively by cutaneous nerves anterior to the cut. No contralateral loss of scratch sensitivity was found. The descending signal for the scratch reflex must run mainly ipsilaterally in the spinal cord. A similar result is seen in the cat, where the central pathway descends ipsilaterally in the caudal portions of the thoracic spinal cord (Deliagina, Neurophysiology 9:470,1977). Supported by NIH Grant NS-15049 to PSGS and NIH Training Grant in Neurobiology NS-07071 to LIM.

46.12 THE MARKOV ORDER OF INTERSPIKE INTERVALS RECORDED FROM RAT CEREBELLAR NEURONS: CORRELATIONS WITH MEASURES OF CENTRAL TENDENCY AND VARIABILITY. C. J. Sherry (Biology), W. R. Klemm (Veterinary Anatomy), and D. L. Barrow* (Mathematics), Texas A & M University, College Station, Texas 77843.

Interspike intervals (ISIs) were collected into a conventional non-sequential interval histogram. The histogram was then divided into thirds such that each third contained an equal number of ISIs. The first step was to have the computer determine if an interval belonged to the shortest, middle, or longest group and to code this relationship as a 1, 2, or 3, respectively. These numbers were then tallied into a series of conditional probability matrices, where the sequential relationships were specified as probabilities in a 'digram' matrix for 2 adjacent numbers through a 'pentagram' matrix for 5 numbers. The Markov order of each neuron was calculated using the Chi square statistic (Theoret. Biol. 29:427, 1970). The Markov order of each neuron was then tested for correlation with measures of central tendency (mean interval, median interval, modal interval, spikes per second) and variability (10-90 percentile range, 25-75 percentile range). The correlation coefficients were -0.56, -0.43, -0.26, 0.71*, -0.89*, and -0.62*, respectively. Multiple correlation methods were also used to determine the relationship between Markov order and mean-median (0.87*), 10-90 range-spikes per sec. (0.89*), 10-90 range-median (0.89*). (*indicates $p < 0.05$)

The neurons that were best described by an order 3 or 4 Markov process had very similar non-sequential distributions. For example, the average 10th percentile was 9.2 msec. (SD 1.6), the 25th percentile (18.2±1.8), the median (17.6±2.9), the mean (23.6±3.9), the 75th percentile (26.4±4.2), the 90th percentile (41.4±6.5), and the 75-90 percentile range (15.0±2.5). The neurons that were best described by an order 1 or 2 Markov process had a higher average median (56.7±53.8) and particularly a larger 75-90 percentile range (45.0±20.4).

This seems to imply that the 'memory' of the cerebellar neurons in this sample could extend to approximately 250 msec. (i.e. 90 percent of the intervals were 50 msec. or less and therefore, the duration of any group of 5 sequential intervals, on the average, should be 250 msec. or less). When the length of the longer intervals (i.e. the 75-90 percentile range) increases, the 'memory' of the system decreases.

47.1 DEVELOPMENT OF SPATIAL RESOLUTION AND CONTRAST SENSITIVITY IN MONKEY VISUAL CORTEX. Colin Blakemore* and François Vital-Durand* (SPON: Leo M. Chalupa). Univ Lab of Physiology, Oxford OX1 3PT, England and INSERM Unité 94, 69500 Bron, France.

Visual acuity improves gradually during the first year in baby monkeys (Teller et al, Vision Res, 18: 561, 1978) and there is a similar improvement in the spatial resolution of neurones in the lateral geniculate nucleus (LGN), from a maximum of 5 cycles/degree at birth to 35 c/deg in the adult. Surprisingly, LGN development is apparently entirely unhindered by visual deprivation (Blakemore & Vital-Durand, Trans Ophthal Soc UK, 99: 363, 1979).

We have now examined the maturation of spatial resolution and contrast sensitivity in the striate cortex of Old-World monkeys (mainly Erythrocebus patas). We recorded from neurones in the foveal representation from the day of birth to adulthood in normal and binocularly deprived animals, and stimulated the receptive fields with drifting gratings whose orientation and contrast could be varied, and whose spatial frequency, direction and temporal frequency of drift were under microprocessor control. In the newborn animal about half of all cells recorded showed some degree of orientation selectivity and these neurones were usually monocularly dominated. In visually-experienced animals, at all ages, the best cortical neurones had cut-off spatial frequencies similar to those for the best LGN cells; development of neural 'acuity' in the cortex normally parallels that in the LGN. As in kittens (Derrington, J Physiol, 276: 46P, 1978), contrast sensitivity was initially rather low and increased on average by a factor of 10 between birth and adulthood. Also, many cortical cells in young animals showed little low-spatial frequency attenuation and responded well to temporal modulation of the entire field. The narrowness of tuning for spatial frequency improved gradually during the first year of life.

By contrast with the LGN, deprivation seems to prevent all these maturational improvements in the cortex. With up to 130 days of binocular deprivation, cut-off spatial frequencies and contrast sensitivities of cortical cells remain at virtually the same levels as on the day of birth. These results suggest that there are two neural 'filters' for spatial information, one peripheral (perhaps in the retina) and one in the striate cortex, both maturing in parallel in the normal animal. The maintenance of maturation in the cortical 'filter' requires visual experience while that in the peripheral one does not.

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47.2 STRUCTURAL DEVELOPMENT OF FUNCTIONALLY IDENTIFIED NEURONS IN KITTEN DORSAL LATERAL GENICULATE NUCLEUS (LGN_D). Michael J. Friedlander. Dept. of Physiology and Biophysics, University of Alabama in Birmingham, Birmingham, AL 35294.

Different physiological classes (W-,X-, and Y-) of $LGN_{(D)}$ neurons in the adult cat have characteristic morphological features[1,2]. Studies of $LGN_{(D)}$ neurons in young kittens (age 1-6 weeks)[3] suggest differential developmental rates for different physiological cell classes. The present study was designed to determine how the postnatal development of physiological and morphological properties of individual $LGN_{(D)}$ neurons are related.

Experiments were done on kittens aged 3-8 weeks. Single $LGN_{(D)}$ neurons were evaluated for receptive field center size, surround inhibition, temporal sensitivity, nature of response to standing and moving targets, linearity of spatial summation, and latencies to optic chiasm stimulation. Cells were then impaled with an HRP filled micropipette and injected intracellularly with horseradish peroxidase (HRP). Vibratomed sections were reacted with DAB-H_2O_2 and injected cells were drawn at 1000X.

Preliminary results indicate that some X-like and W-like cells have adult like dendritic morphology by postnatal age 3-4 weeks. However, some X-like cells (age 3-8 weeks) have morphological features different from those seen in the adult. These features include small dendritic trees symmetrically distributed about the soma, when viewed in the coronal plane. In some cases a very tangled dendritic arborization was seen. Some of these immature X-like cells have terminal swellings on their dendrites that are otherwise devoid of appendages. Too few Y-like neurons have been recovered for analysis, to date.

1. Friedlander, Michael J., C.-S. Lin, L.R. Stanford and S. Murray Sherman 1981, J. Neurophysiol. 46: no. 1.
2. Stanford, L.R., Michael J. Friedlander and S. Murray Sherman 1981, J. Neurosci. 1: no. 6.
3. Daniels, Jerry D., John D. Pettigrew and Joyce L. Norman 1978, J. Neurophysiol. 41: no. 6.

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47.3 THE DORSAL LATERAL GENICULATE NUCLEUS OF THE NORMAL FERRET AND ITS POSTNATAL DEVELOPMENT. R.W. Guillery, D. Card Linden and Josephine Cucchiaro, Dept. Pharmacol. Physiol. Sci., The University of Chicago, Chicago, IL 60637.

The anterograde transport of ^3H proline and horseradish peroxidase has been used to study the normal retinogeniculate pathways of ferrets from birth to maturity. In adults, the lateral geniculate nucleus (dLGN) shows a "carnivore" pattern, having layers A, A1, C, C1, C2 and C3, a medial interlaminar nucleus, and a well defined perigeniculate nucleus. At birth the dLGN is unlaminated and all parts are reached by afferents from both eyes. The larger crossed component extends from the optic tract well into the perigeniculate field while the smaller uncrossed component barely reaches this field. During postnatal days 1-3, the uncrossed fibers restrict their arbors to a small, posteromedial region (the precursor of the binocular segment of the nucleus) and subsequently the crossed fibers retreat from the region occupied by the uncrossed fibers. Between postnatal days 4 and 8, the field occupied by the ipsilateral component expands to form a major focus (lamina A1) and a minor focus (C1), but arbors of the crossed component still overlap the borders of lamina A1 and occupy all parts of lamina C1. The perigeniculate nucleus and the medial interlaminar nucleus become clearly distinguishable between days 3 and 8.

Between days 8 and 15, the cytoarchitectonic borders of layers A and A1 become clearly defined, but retinogeniculate axons still extend across the A/A1 border. These axons retreat to their appropriate layer after day 15, and the nucleus reaches its essentially adult structure by about day 28. Segregation of retinofugal axons in the C layers occurs after segregation in the A layers, but cells of the C layers show signs of cytological maturity earlier than those of the A layers. As the ipsilateral and contralateral components become segregated, the nucleus changes from a comma-shaped structure on the lateral aspect of the dorsal thalamus, to an "L"-shaped structure on the posterior aspect of the dorsal thalamus.

Our results show that the crossed and uncrossed inputs initially overlap. Segregation into distinct ipsi- and contralateral zones begins well before any visible cellular lamination occurs, but the process of segregation is not completed until well after the adult pattern of cellular lamination is formed. Thus the cellular and the retinofugal lamination probably represent separate developmental processes that are to some extent independent of each other.

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47.4 INSIDE-OUT PATTERN OF NEUROGENESIS OF THE CAT'S LATERAL GENICULATE NUCLEUS. C.J. Shatz. Dept. of Neurobiology, Stanford University School of Medicine, Stanford, CA 94305.

The pattern of neurogenesis of the cat's lateral geniculate nucleus (LGN) was studied by means of ^3H-thymidine autoradiography. Neurons of the LGN are generated between embryonic day 24 (E24) and E31 (gestation is 65 days), with those destined for the A-layers generated prior to those for the C-layers (Hickey, '79). However, in the embryonic brain when the future C-layers first become identifiable on the basis of the pattern of retinogeniculate projections, they apparently lie external (lateral) to the prospective A-layers. This suggests that neurons destined for the C-layers must migrate through earlier-generated A-layer neurons en route to their appropriate position in the developing thalamus.

To verify this suggestion, fetuses were injected with ^3H-thymidine on E30 in order to label preferentially those cells destined to lie in the C-layers (Hickey,'79), and allowed to survive varying periods thereafter. That C-layer neurons were indeed selectively labeled by an E30 injection was shown in one animal which survived to 55 days postnatally. Another fetus survived 9 days, to E39. At this age, the LGN analage is an undifferentiated mass of cells and the C-layers are not evident from inspection of the histology or the retinogeniculate projection pattern (Shatz & DiBerardino,'80). However, labeled cells destined for the C-layers are found almost entirely along the medial aspect of the anlage, internal to a thick unlabeled cell margin. This margin presumably consists of earlier-generated neurons of the prospective A-layers. Two other fetuses injected at E30 survived to E54 when the C-layers, though not yet evident histologically, can be identified on the basis of the pattern of retinogeniculate input: they lie external to the A-layers along the posterolateral edge of the thalamus. As expected, the majority of thymidine-labeled cells were found within the C-layers.

This internal-to-external shift in the position of labeled cells seen between E39 and E54 suggests that a real through-migration occurs during histogenesis. Alternatively, the shift could result from a displacement of the entire LGN. To eliminate this possibility, an additional fetus was injected at E31 and killed at E47. Labeled cells in this case were seen embedded within the nucleus, confirming the suggestion that many of the neurons destined for the C-layers indeed migrate through the body of the nucleus.

The inside-out pattern of neurogenesis of the cat's LGN contrasts with the outside-in pattern found in the rhesus monkey (Rakic,'77). However, the cat's C-layers have no clear analogy within the monkey's LGN: perhaps this is a direct consequence of the difference in pattern of neurogenesis seen in the two species.

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47.5 DIFFERENTIAL REDUCTION IN THE NUMBER OF IPSILATERALLY PROJECTING GANGLION CELLS DURING THE DEVELOPMENT OF RETINOFUGAL PROJECTIONS IN ALBINO AND PIGMENTED RATS. P.W. Land, K. Hargrove*, J. Eldridge* and R.D. Lund, Department of Anatomy, Medical University of South Carolina, Charleston, South Carolina 29425.

In adult albino rats, the uncrossed retinogeniculate and retinotectal projections appear markedly reduced when compared with pigmented animals. However, at birth these pathways appear quite similar in the two strains, occupying a much larger volume of the lateral geniculate nucleus (LGN) and superior colliculus (SC) than they do in adult (Land and Lund, 1979 Science, 205:698-700; Maxwell and Land, 1981 Anat. Rec., 199, #3) and it is not until 9-10 days after birth that conspicuous differences in the uncrossed pathways become evident. These observations allow the suggestion that the albino mutation results not in an initial "misrouting" of axons at the chiasm, but rather, a disproportionate loss of ipsilaterally projecting axons during early postnatal life.

In order to test this hypothesis, we examined the number and location of the ganglion cells of origin of uncrossed retinofugal projections in both newborn and adult rats of the Sprague-Dawley albino and Long-Evans pigmented strains. Animals received 3-4 injections (total volume 1-3 µl) of HRP, propidium iodide, True Blue or Nuclear Yellow (generously donated by Dr. H. Loewe) into one optic tract (and LGN). In some cases, each tract was injected with a different compound. Eighteen to 24 hours later, animals were perfused with 4% paraformaldehyde. The retinae were removed and either flat mounted and viewed with fluorescent illumination or reacted with diaminobenzidine and H_2O_2, flat mounted and viewed with bright field illumination. The number and location of labeled ganglion cells in the retinae ipsilateral to the injection were then plotted onto drawings of the retinae. In newborn rats of both strains, an average of 5643 ± 72 cells were labeled in the lower temporal crescent of the ipsilateral retina. Moreover, 886 ± 94 labeled cells were distributed throughout the remainder of the retina. In contrast, 1937 ± 394 ganglion cells were labeled in the lower temporal retina of pigmented adults with 163 ± 61 labeled cells occurring elsewhere in the retina while in albinos, 973 ± 236 and 49 ± 4 labeled cells were found, respectively, within and outside of the lower temporal crescent.

These results show that in both albino and pigmented rats there is a substantial loss of ipsilaterally projecting ganglion cell axons, with a more severe reduction of ipsilaterally projecting cells occurring in albinos. To what extent the reduction in ipsilateral projections in either strain results from ganglion cell death or loss of an uncrossed collateral of bilaterally projecting cells is currently under investigation. (Supported by USPHS Grant R01 EY 03447 and EY 03414)

47.6 STRIPE-REARING MODIFIES DENDRITIC MORPHOLOGY OF CELLS IN VISUAL CORTEX OF THE CAT. S.B. Tieman, K. Butterfield* and H.V.B. Hirsch (SPON: H. Tedeschi). Neurobiology Research Center, SUNY Albany, Albany, N.Y. 12222.

To determine whether selective exposure to lines of one orientation modifies the shape of the dendritic fields of cells in visual cortex, we examined the dendritic morphology of neurons in Area 17 of 4 normally-reared cats and 4 cats reared viewing only vertical lines or only horizontal lines. Kittens were placed with their mothers into a totally dark room before their eyes had opened. Beginning at 4 wks of age, the kittens were brought out for daily periods of exposure wearing masks that limited the vision of each eye to a field of 3 vertical lines or 3 horizontal lines. After a minimum of 150 h of exposure, the animals were deeply anesthetized and perfused with phosphate-buffered formalin. Blocks of visual cortex were removed, impregnated by the Golgi-Kopsch procedure, embedded in celloidin, and cut at 120 µm tangential to the pial surface. The sections were stabilized by the method of Geisert and Updyke (Stain Tech., 52:137, 1977) and counterstained with gallocyanin. Complete neurons from layers III and IV were drawn with the aid of a camera lucida, and the orientations of the dendritic fields were analyzed using Sholl diagrams. In normal cats, the distributions of the orientations of dendritic fields were fairly uniform, while in stripe-reared cats, the distributions were shifted toward horizontal or vertical. The direction of this shift varied with the type of cell and the experience of the cat. Thus, for spiny stellate cells in layer IV, the orientation of the dendritic fields matched that presented during rearing. The size of the shift was somewhat larger for the pyramidal cells than for the stellate cells. We suggest that the orientation preferences of stellate cells in layer IV are determined by the spatial arrangement of excitatory inputs, as proposed by Colonnier (J. Anat., 98:527, 1964), while the orientation preferences of pyramidal cells in layer III are determined by the spatial arrangement of inhibitory inputs, as suggested by Schiller et al. (J. Neurophysiol., 39:1362, 1976). Apparently both mechanisms of orientation selectivity can be modified by early visual experience, although the mechanism based on inhibition may be more modifiable than the one based on excitation. (Supported by PHS grants EY02609 to SBT and EY01268 to HVBH).

47.7 DEVELOPMENT OF RETINOGENICULATE SYNAPSES FOLLOWING VISUAL DEPRIVATION IN THE CAT. R.E. Kalil and G. Scott*. Depts. of Opthalmology and Anatomy, Univ. of Wisconsin, Madison, Wisconsin 53706.

In cats raised with monocular eyelid suture, neurons in the dorsal lateral geniculate nucleus (LGN) connected to the deprived eye develop abnormally. For example, cell bodies in deprived layers of the LGN fail to grow to normal size, while physiologically there is a reduction in the number of Y-cells that can be recorded from these laminae. Complete deprivation by dark rearing also disrupts normal cell growth in the LGN and decreases the percentage of Y-cells that are encountered. The basis for deficits such as these is at present unknown, but one possible explanation is that deprivation affects the formation of retinogeniculate synapses which leads to subsequent changes in LGN cell growth and function.

We have studied this question with the electron microscope in a series of cats monocularly deprived (MD) by eyelid suture at 7 days for periods that ranged from 1 to 32 weeks, and in two cats that were dark reared (DR) from birth for 42 weeks. Large area photographic montages were prepared from thin sections taken from the binocular segment of lamina A near the center of the LGN. Terminals from the retina that could be identified by standard criteria (RLP, Guillery '69) were scored quantitatively according to the number and pattern of synaptic contacts which they made. In MD cats, deprived and nondeprived laminae A were compared in each animal, whereas normally reared adult controls served for comparison with DR cats.

At each of the ages studied in MD cats, the development of RLP synapses appeared to be influenced little, if at all, by deprivation. In kittens 3 weeks old and younger, deprived and nondeprived retinal terminals made like numbers of simple axodendritic contacts almost exclusively. By 5 weeks, both sets of afferents also contacted vesicle containing postsynaptic profiles, and a small percentage of each were found in encapsulated synaptic zones. At later ages, deprived retinal terminals established patterns of mature synaptic organization at about the same rate as nondeprived terminals. Similarly, a comparison of RLP synaptic organization in 42 week DR cats with normally reared adults failed to reveal any consistent differences.

Keeping in mind the limits of the present analysis, these results indicate that visual deprivation produces no major changes in the ultrastructural organization of RLP synapses, nor the rate at which they develop. These findings suggest that in the LGN the relevant changes caused by deprivation may be biochemical in nature, or may involve alterations in synaptic patterns on a scale too large to be appropriate for study with the electron microscope.

47.8 NORMAL ORIENTATION AND DIRECTION SELECTIVITY AFTER MONOCULAR DEPRIVATION IN SIAMESE CATS. N. Berman and B.R. Payne. Department of Physiology/Biochemistry and Anatomy, The Medical College of Pennsylvania. Philadelphia, PA.

In normally pigmented cats total deprivation of pattern vision during development leads to a loss of orientation and direction selectivity of cortical neurons. This alteration in cortical receptive field properties occurs during a period when there is intense competition between the two eyes for cortical space. In normally pigmented cats, each cortex receives input from both eyes and therefore competition between the inputs from the two eyes is always a possible factor in the development of cortical receptive field properties. In Siamese cats, however, an abnormal crossing of retinal ganglion cell axons at the chiasm results in a visual cortex in which virtually all of the neurons are influenced only by one eye. This situation provides the opportunity to study how monocular deprivation affects cortical neurons when there is no competition between the input from the two eyes in the cortex.

Three Siamese kittens were monocularly sutured on the fourth day of life. 3½ to 9 months later the sutured eye was opened and single neurons were recorded in area 17 contralateral to the deprived eye. Five normally-reared Siamese cats were also studied. In both groups the cortical units had receptive fields in the region from 25° into the ipsilateral half-field to 30° into the contralateral half-field. In the normally reared Siamese cats, 83 percent of the units could be driven only by the contralateral eye. In the monocularly deprived Siamese cats, 81 percent of the units were driven only by the deprived (contralateral) eye. Thus, there was no significant increase in the effectiveness of the ipsilateral (experienced) eye in driving cortical neurons. In normally-reared Siamese cats 97 percent of the units were orientation selective, while in monocularly deprived Siamese cats, 91 percent of the units were orientation selective. Finally, in normally-reared Siamese cats 74 percent of the units were selective for the direction of stimulus movement, while in monocularly deprived Siamese cats 79 percent of the units were direction selective. Our results show that monocular deprivation has no effect on the development of receptive field properties of cortical neurons in Siamese cats. Our results suggest that the susceptibility to pattern deprivation which is present in normal cats depends on competition between the two eyes for cortical space during the early postnatal period.
Supported by EY 02088.

47.9 AN ELECTROPHYSIOLOGICAL COMPARISON BETWEEN EXOTROPIC AND ESOTROPIC STRABISMUS IN CATS R. D. Freeman and T. Tsumoto, School of Optometry, University of California, Berkeley, CA 94720

Normally reared 3-week-old kittens were made exotropic (exo) or esotropic (eso) by tenotomy of medial or lateral rectus muscles, respectively. Six cats, three in each condition, were studied at ages from 4 to 7 months. The animals were anesthetized and paralyzed, and viewed a CRT screen. The deviating eye was repositioned mechanically with sutures, or with optical prisms so that the area centrales of both eyes were approximately centered on the CRT.

In the first study, visually elicited cortical evoked potentials (EP) were measured via skull electrodes during presentation of bright, high-contrast phase-reversing sinusoidal gratings. A computer averaged and performed harmonic analysis of data and controlled presentation of stimuli which were interleaved between right and left eyes. No substantial differences were found in wave forms, amplitudes, or latencies of the EP between normal and deviated eyes of exo cats. Spatial frequency-response functions were also similar but in one case, there was a slight reduction in low-frequency sensitivity. In the eso group, responses mediated by the deviating eyes were substantially reduced and spatial frequency response functions were particularly depressed at the low-frequency end. For all cats, spatial cut-off frequencies between deviating and non-deviating eyes were similar.

For the second study, bipolar stimulating electrodes were placed stereotaxically in the lateral geniculate nucleus (LGN) and optic radiation (OR), and hooked bipolar electrodes were positioned around each optic nerve (ON). A recording electrode was placed in area 17. As found previously, receptive field analysis showed that proportions of binocular cells were very low in all cases, but neither eye was significantly dominant. Of 208 cells studied in detail, approximately half responded to ON stimulation of the deviated or non-deviated eyes. Mean response latencies to right or left ON stimulation were similar in exo cats, but deviated eye latencies were significantly longer than those from the non-deviated eye in the eso animals. These differences applied to binocular as well as monocular cells. Conduction times between ON and LGN and between LGN and OR were also slower from deviated eso but not exo eyes. Interocular inhibition of the deviated by the non-deviated eye, as determined by ON stimulation with or without visual activation was generally stronger in the eso compared to exo cats.

Considered together, these results indicate that the effects of experimentally produced esotropia are physiologically pronounced compared to those from exotropia, and that peripheral as well as central pathways are affected. (EY01175)

47.10 MORPHOLOGY AND DISTRIBUTION OF RETINAL GANGLION CELLS PROJECTING TO THE LATERAL GENICULATE NUCLEUS (LGNd) IN NORMAL AND SIAMESE CATS, A.G. Leventhal, Dept. of Anat. Univ. of Utah, School of Medicine, Salt Lake City, Utah 84132.

Electrophoretic injections of horseradish peroxidase were made into physiologically characterized sites within the LGNd of normal and Siamese cats. Histochemical procedures used stained the cell bodies, dendrites and axons of retrogradely labeled ganglion cells. The regions of retina examined ranged from .5mm from the area centralis (a.c.) to the far periphery.

In normal cats, only α and β type ganglion cells are labeled by injections restricted to the A laminae. The α/β ratios (# labeled alpha cells/# labeled alpha+beta cells) resulting from injections into lam A increase from about .045 at .5mm from the a.c. to about .10 in the periphery. The α/β ratios observed outside of the a.c. following injections into different parts of lam A1 were about 15% lower at each eccentricity than those resulting from injections into corresponding parts of lam A. Also, outside of the a.c., α and β cells projecting to lam A1 (those in ipsilateral, temporal retina) are larger than those projecting to lam A (those in contralateral, nasal retina).

In Siamese cats, as in normal cats, only α and β cells are labeled by HRP injections into lam A or lam A1 and the relative numbers of α and β cells projecting to lam A are normal. However, α cells comprise an abnormally small proportion of ganglion cells projecting to the normal segment of Siamese lam A1. They range from 0% of labeled cells near the a.c. to only 4% of labeled cells in the far periphery of ipsilateral, temporal retina following injections into this region. In contrast, α cells constitute an abnormally large proportion of cells projecting to the abnormal segment of lam A1. As a result of injections into this region, they range from the normal value of about 5.0% of labeled cells .5mm from the a.c. to over 70% of labeled cells in the far periphery of contralateral, temporal retina.

Injections into the C laminae of the LGNd in normal and Siamese cats show that the morphological classes which project to these laminae in normal cats also project to these laminae in Siamese cats. Unlike in normal cats, however, examples of all of these morphological types are found far into the contralateral, temporal retinas of Siamese cats following injections into lam C1. Thus, it appears that all classes which project to the ipsilateral C lamina are effected by the Siamese abnormality.

The morphologies and sizes of cells belonging to the different classes of retinal ganglion cells in Siamese cats seem normal. There are no obvious morphological differences between the cells in temporal retina which project ipsilaterally (normally) and those which project contralaterally (abnormally).

47.11 PERIOD OF SUSCEPTIBILITY TO EFFECTS OF MONOCULAR DEPRIVATION: DIFFERENCES BETWEEN STRIATE AND EXTRASTRIATE CORTEX. Kim R. Jones*, Peter D. Spear, and Lillian Tong. Dept. of Psychology, Univ. of Wisconsin, Madison, WI 53706.

Previous studies of the critical period for effects of monocular deprivation have been confined exclusively to striate cortex. To assess the possibility that this period might be different for extrastriate regions, we monocularly lid-sutured kittens at various ages and compared the effects in striate cortex to those in the lateral suprasylvian visual area (LS) in the same animals.

Thus far, we have studied 16 animals, divided into groups of three lid-sutured at 4, 12, 18, and 26 weeks of age, and a group of four lid-sutured at 35 weeks. All kittens were monocularly deprived for a period of 4 weeks, at which time single-unit recordings in both striate and LS were made contralateral to the deprived eye. In each area, the ocular dominance distributions of from 19-62 cells (mean=35) were determined using hand-held stimuli, and compared to results obtained from three 39 week old normal kittens. Several procedures were employed to insure a representative sampling of ocular dominance. To record from as many ocular dominance columns as possible, penetrations in both areas were made tangential to the cortical surface. In addition, cells were sampled a minimum of 100 μm apart, and comparable visual field eccentricities (0°-20°) were sampled in each area.

Monocular deprivation at 4 weeks of age caused nearly all cells in both cortical areas to be driven exclusively by the nondeprived eye. In the 12 and 18 week old groups, this dominance decreased at about the same rate for both striate and LS cortex, so that at the latter age only about 40% of the cells in each area were driven strongly or exclusively by the nondeprived eye (OD classes 6 & 7). After this age, however, clear differences developed between the two areas. In both the 26 and 35 week old groups, the percentage of cells in OD classes 6 & 7 in LS cortex fell to an average of 13%, which was similar to that seen in normals. In striate cortex, results from the same animals were much more variable, with some ocular dominance distributions appearing normal and others very abnormal. In both age groups, however, the average percentage of striate cells in OD classes 6 & 7 remained above 40%, which was significantly different than the 15% seen in normal animals.

In summary, these results show that the critical period for monocular deprivation differs for striate and LS cortex, and that the effects are more prolonged in striate cortex than previously believed.

47.12 NOREPINEPHRINE IONTOPHORESIS IN CAT VISUAL CORTEX: A QUICK CHANGE IN OCULAR DOMINANCE.
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Paul Heggelund* Neurobiology Laboratory, University of Trondheim, Dragvoll-Trondheim, Norway. (Spon: J. Brockes)

We have been studying roles played by norepinephrine (NE)-containing nerve endings in regulation of neuronal plasticity in cat visual cortex. Using NE iontophoresis in place of the continuous micro-perfusion method which was intensively used in our previous studies, we wanted to know how quickly changes in ocular dominance take place.

We first studied how responsiveness of individual visual cells changes during NE iontophoresis if their receptive fields are stimulated by appropriate visual stimuli. We found 3 populations of cortical cells during NE iontophoresis: an increase, a decrease or no change in their responsiveness to proper visual stimulation. About equal numbers of cells belonged to each of these 3 groups. In the majority of such cells that changed visual responsiveness during NE iontophoresis and that had measurable amounts of spontaneous activity, the ratio of visually evoked to spontaneous activity (signal-to-noise ratio) improved by NE. This improvement was independent of an increase or a decrease in responsiveness to visual stimulation. Secondly, there was a differential effect of NE on simple and complex cells. Although most simple cells (36 of 42) changed clearly their responsiveness during NE iontophoresis (either an increase or a decrease), the effects were seen in only one-third of complex cells (6 of 18). Finally, we noted an unusually high (N=60, 68%) sampling incidence for monocular cells in our samples which were derived from either visually normal kittens which were pretreated with 6-hydroxydopamine or a normal adult cat. A control animal which repeatedly received only current injections but not NE iontophoresis, however, showed the normal ocular dominance distribution with a high proportion of binocular cells. These results suggest that breaking up the normal convergence of visual axes, which was more or less inevitable in paralyzed preparations, induced stronger effects of "squinting" than usual on cortical binocular cells which had been plasticized by concurrent NE iontophoresis. This change in ocular dominance was observed within 24 hrs after the start of NE iontophoresis.

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48.1 TESTOSTERONE REGULATES ACETYLCHOLINE RECEPTOR NUMBER IN TWO ANDROGEN SENSITIVE MUSCLES. A.L.Harrelson*,W.V.Bleisch and V.N.Luine, Rockefeller University,New York,N.Y. 10021

The "levator ani" (LA) of rodents and the syrinx of songbirds are examples of neuromuscular systems in which both the muscles and their motoneurons have high levels of androgen receptors. The muscles respond to castration with a decrease in weight, protein and acetylcholinesterase (AChE) activity (Tucek et al., 1976,J.Neurol.Sci.27:363 and Luine et al.,1980,Brain Res. 192:89). This led us to examine the response of acetylcholine receptor (AChR) number to changing hormone levels (Bleisch, this volume). In both muscles, AChR number decreases after castration of males and treatment with testosterone can increase AChR number. In the LA of 250 g male rats, 2 weeks of castration causes a 47% decrease in AChR number, from 2.79±.15pmoles/muscle to 1.49±.06 ($p<.005$). In contrast to other reports (Chin and Almon,1980, J.P.E.T. 212:553) we find no change in AChR number per protein (153±9fmoles/mg protein in intacts and 167±6 in castrates). After 1 week of castration, one week of testosterone proprionate (TP, 127ug/kg/day s.c.) increases AChR number by 29% over untreated castrates, from 1.47±.1pmoles/muscle to 1.86±.13(p .05). Again, there is no change in AChR number per protein, since these changes parallel changes in muscle protein. In contrast to AChR, total AChE activity is unaffected by these short treatments (6.58±.71 umoles substrate/hr/muscle in intacts, 6.74±.98 in castates and 7.04±.4 in TP treated castrates). Because muscle protein decreases markedly after castration, specific AChE activity is higher in treated and untreated castrates than in intacts (335±33 nmoles/hr/mg protein in intacts, 728±77 in untreated castrates and 665±42 in TP treated castrates,$p<.01$ and $p<.01$).

Unlike the rat LA, the syrinx of songbirds exists in both males and females. In zebra finches, this organ is sexually dimorphic, and there is also a dimorphism in the number of AChR in the syrinx (1069±69fmoles/syrinx in males and 280±19 in females,$p<.001$). AChR is also different on a per protein basis (771±61fmoles/mg in males and 623±29 in females, $p<.05$). After 23 days of castration, AChR number decreases in males by 42%, from 1276±72 fmoles/syrinx to 737±85 ($p<.005$). After 6 days of testosterone treatment (5mm silastic capsule), AChR number increases in intact females from 164±14 fmoles/syrinx to 470±83 ($p<.05$).

We believe that the LA and syrinx may provide novel model systems of defined synapses for the study of synaptic regulation in response to hormones.

This work was done in the laboratory of Dr. B.S. McEwen.

48.2 LAMININ AND OTHER BASEMENT MEMBRANE PROTEINS CODISTRIBUTE WITH ACETYLCHOLINE RECEPTORS ON THE SURFACE OF CULTURED MYOTUBES. M.P. Daniels, M. Vigny*, H. Bauer, P. Sonderegger*, and Z. Vogel. NHLBI; NIDR; and NICHD; N.I.H., Bethesda, MD. 20205.

The results of recent studies [Burden, S.J. et.al. J. Cell Biol. 82:412 (1979)] have suggested a role for the synaptic basal lamina in the postsynaptic organization of acetylcholine receptors (AChR). We now report a fluorescence microscopic study comparing the extracellular distribution of basement membrane proteins to the distribution of AChR in rat myotube cultures and in sections of rat diaphragm. We have used antisera against purified basement membrane proteins (kindly provided by Drs. G. Grotendorst, J. Hassel, H. Kleinman, M. Silver, and G. Martin) and fluorescein-labeled 2° antibodies. AChR distribution was visualized in the same cultures and sections with rhodamine-labeled α-bungarotoxin. In diaphragms of adult and 17 and 20 day-old fetal rats, immunoreactivities for laminin, fibronectin, heparan sulfate proteoglycan, collagen type IV and V all delineated the basement membranes of muscle, blood vessels, connective tissue, and Schwann cells. In contrast, laminin immunoreactivity on the surfaces of cultured rat myotubes was concentrated in regions of high AChR density (AChR aggregates). Most AChR aggregates were coextensive over at least part of their area with laminin aggregates. In addition, there was a much smaller number of laminin aggregates which were not coextensive with AChR aggregates. The remainder of the myotube surface was weakly stained. Concentrations of anti-heparan sulfate proteoglycan immunoreactivity were found within a majority of AChR aggregates, but the codistribution was less marked than with laminin. Staining with antisera against the other basement membrane proteins revealed much less codistribution with AChR. Detergent extraction of the myotubes in situ yielded cytoskeletons in which the laminin-AChR association was intact. When myotube cultures were treated with conditioned medium from cultures of NG108-15 neuroblastoma x glioma hybrid cells in order to induce a several-fold increase in the number of AChR aggregates [Christian, C.N. et.al. PNAS 75:4011 (1978)], the frequency with which AChR aggregates coincided with laminin aggregates was at least as high as in controls. The results of this study indicate a consistent and stable codistribution of laminin with AChR aggregates. In another study (Vogel, Z. et.al. These Abstracts) we have shown that laminin markedly enhances the AChR aggregating activity of NG108-15 cell conditioned medium. We therefore suggest that laminin plays a role in the neuronal induction of AChR aggregation.

48.3 THE EFFECT OF LAMININ, A BASEMENT MEMBRANE PROTEIN, ON THE CELL SURFACE AGGREGATION OF ACETYLCHOLINE RECEPTORS OF CULTURED MYOTUBES. Z. Vogel, M.P. Daniels, M. Vigny*, H.C. Bauer, P. Sonderegger*, and C.N. Christian*. Lab. of Biochem. Genetics, NHLBI; Lab. of Devel. Biol. and Anomalies, NIDR; and Lab. of Devel. Neurobiol. NICHD; NIH, Bethesda, MD 20205.

Recent studies have indicated that the synaptic basal lamina may contain material which induces the aggregation of acetylcholine receptors (AChR) at the former site of the neuromuscular junction during muscle regeneration, and that synaptic basement membrane preparations can induce AChR aggregation on cultured myotubes (1). The biochemical nature of the active materials in these preparations is unknown. In another study (2) we have shown that two basement membrane components, laminin and heparan sulfate proteoglycan, are largely codistributed with aggregated AChR on the surface of cultured rat myotubes.

Therefore, we have tested the effect of 3 purified basement membrane proteins, laminin, heparan sulfate proteoglycan, and collagen type IV, on the aggregation of AChR on cultured myotubes. Purified chick plasma fibronectin was similarly tested. The proteins (generously provided by Drs. J.R. Hassel, H.K. Kleinman, M. Silver and G.R. Martin) were added to primary cultures of rat myotubes or myotube cultures of the G8-1 muscle cell line, in the presence or absence of conditioned medium (CM) from NG108-15 neuroblastoma x glioma hybrid cell cultures (3). Of the proteins tested, only laminin consistently caused a significant increase in the number of AChR aggregates per myotube (A/M). A concentration-dependent increase in A/M over the range of 1-60 μg/ml of laminin was observed. Laminin markedly enhanced the AChR aggregation activity of NG108-15 cell CM. For example, 3.6 μg/ml of laminin caused a 100% increase in the A/M of G8-1 myotube cultures after 22 hrs, NG108-15 CM gave a 170% increase, and CM together with laminin gave a 600% increase. Similar results were obtained with primary rat muscle cultures. Results of immunoassays indicated that there was less than 5 ng/ml of laminin in NG108-15 cell CM. Thus, laminin itself does not appear to be the AChR aggregating factor found in CM (3). Conditioned medium, laminin, and the two combined all produced AChR aggregates of similar morphology, found on the sides and dorsal surfaces of the myotubes.

The results suggest that laminin itself can induce AChR aggregation on cultured myotubes, and further, that laminin plays a role in the induction of AChR aggregation by neuronal factors.

1. Rubin, L.L. et al. Neurosci. Abstr. 6: 330 (1980).
2. Daniels, M.P. et al. These Abstracts.
3. Christian, C.N. et al. PNAS 75: 4011 (1978).

48.4 CELL-FREE EXTRACTS OF TORPEDO INCREASE SYNTHESIS AND CLUSTERING OF ACETYLCHOLINE RECEPTORS ON CULTURED CHICK MYOTUBES. Paul A. St. John, Joe A. Connolly* and Gerald D. Fischbach. Dept. of Pharmacology, Harvard Medical School, Boston, MA 02115.

Previous results from this laboratory have shown that cell-free extracts of the chick central nervous system, but not of dorsal root ganglia, can increase the number of acetylcholine receptors (AChR) and receptor clusters on cultured chick myotubes (Jessell et al, 1979, PNAS 76: 5397). These results suggested that the active substance might be unique to cholinergic neurons. The electromotor system of the electric ray, Torpedo californica, is embryologically homologous to the neuromuscular system and, since neuro-electrocyte synapses are cholinergic, provides a very rich source of cholinergic neurons. We have examined extracts of this model cholinergic system to determine whether they too can influence receptor number.

Tissues are homogenized in saline, then centrifuged at 10,000 x g for 20 min. The supernatant is centrifuged at 200,000 x g for 120 min to produce a high-speed supernatant (HSS) and a high-speed pellet (HSP), both of which are assayed. Extracts of the electric lobe and the electromotor nerves (both HSS and HSP) and the HSS of the electric organ increase the number of AChR on un-innervated chick myotubes to 1.7-4.2 times control values, as measured by the binding of ^{125}I-α-bungarotoxin (α-BGTX). The HSP from the electric organ contains many Torpedo AChR and if these are blocked with cold α-BGTX, the extract contains no apparent activity. Extracts prepared from the rest of the Torpedo brain or spinal cord also increase the number of AChR by 2-3 fold, but extracts of skeletal muscle or liver cause no increase.

Myotubes treated with extracts from the electromotor system are more sensitive to iontophoretically applied ACh, indicating that the additional toxin binding sites are indeed functional receptors. Labeling with a fluorescent conjugate of α-BGTX reveals that treated myotubes have several fold more clusters of AChR than control cells. At least part of this increase is explained by migration of pre-existing receptors: when AChR are prelabeled with fluorescent α-BGTX before extract treatment, extracts increase the number of fluorescent receptor clusters within 8 hrs. The extracts are not trypsin sensitive and retain most or all of their activity after heating at 95°C for 5 min. Gel filtration of the electric lobe HSS on Biogel P-150 reveals one peak of activity near the exclusion limit and a second peak of lower MW. Further characterization of these extracts is in progress.

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48.5 REGULATION OF FUNCTIONAL MUSCARINIC RECEPTORS ON CHICK CARDIAC CELLS IN CULTURE. R.E. Siegel* and G.D. Fischbach. Dept. of Pharmacology, Harvard Medical School, Boston, Mass. 02115

The presence of muscarinic binding sites on isolated chick heart cells has previously been demonstrated with two muscarinic antagonists, [^{3}H]l-quinuclidinyl benzilate ([^{3}H]lQNB) and [^{3}H]N-methylscopolamine ([^{3}H]NMS). Both atrial and ventricular cultures bound similar amounts of [^{3}H]lQNB and [^{3}H]NMS. In addition, [^{3}H]lQNB autoradiography showed that the binding sites were diffusely distributed on isolated cells from both regions (Siegel and Fischbach, Soc. Neurosci. Abstr., Vol. 6, p. 358, 1980). However, little is known concerning the relationship of the binding sites to functional muscarinic receptors. We have examined the sensitivity of atrial and ventricular cells, dissociated from 8-day embryonic chick hearts and maintained in culture for 2-3 days, to the cholinergic agonist, carbachol. The sensitivity of single cells or small cell clusters was determined by intracellular recording of the hyperpolarization produced by a known concentration of drug delivered by pressure ejection. Although both cell types have a similar density of [^{3}H]lQNB binding sites, 89% of the atrial cells (R.P. = 50 ± 8mV, mean ± s.d., n=18) hyperpolarize in response to 10^{-4}M carbachol but only 26% of the ventricular cells (R.P. = 52 ± 9mV, n=19) respond to the same concentration. Thus, the muscarinic binding sites are not correlated with functional receptors.

Saline extracts prepared from embryonic chick brain cause 4- to 5-fold increases in the number of nicotinic receptors and in the sensitivity to acetylcholine of skeletal muscle fibers in culture. These extracts had little effect on the muscarinic binding sites but enhanced the sensitivity of ventricular cells to carbachol. Addition of extract for 48 hours caused small increases (15-20%) in the [^{3}H]-NMS binding sites. In contrast, the number of ventricular cells responding to 10^{-4}M carbachol increased over 100%, from 26% in the control condition to 68% (R.P. = 52 ± 11mV, n=22) in extract-treated cultures. These studies suggest that muscarinic binding sites and functional receptors are regulated in different ways.

48.6 EVIDENCE FOR A SKELETAL MUSCLE PROTEIN THAT ENHANCES NEURON SURVIVAL, NEURITE EXTENSION, AND ACETYLCHOLINE (ACh) SYNTHESIS R. Glenn Smith* and Stanley H. Appel (SPON: Henry F. Epstein). Depts. of Biochemistry, and Neurology, and Prog. in Neurosci. Baylor Coll. of Med., Houston, TX 77030

The continued development of spinal motor neurons in culture requires either the presence of skeletal muscle in co-culture, or muscle conditioned medium. In this report, we describe the effect of a protein(s) extracted from muscle tissue on the differentiation and maintenance of dissociated spinal cord neurons.

Skeletal muscle from newborn Sprague-Dawley rats was homogenized in 3 volumes of phosphate buffered saline (PBS:pH 7.4) at 4° C, and centrifuged at 32,000g for one hour. The resulting supernatant(S_1) was recentrifuged to 100,000g for 2 hours, and S_2 dialysed against PBS overnight.

Spinal cord cultures were obtained from arachnoid-free ventral cord of stage 24-27 rat embryos. After dissociation, cells were plated(1.5×10^6 cells/dish) onto polylysine coated culture dishes. Cytosine arabinoside, or fluorodeoxyuridine(1×10^{-5}M) was added to the growth medium one day after cell plating, and was maintained in that medium until the muscle extract (MX) was added.

Within 3 days of application of MX (7.5%v/v,~450µg/ml medium) to cord cultures, neuronal and glial cells begin to associate into clusters. Concomitant with this change is (1) a 1.5 fold increase in cell survival, when compared to cultures without MX, (2) a >4 fold increase in the average number of neurites/neuron, and (3) a 3.5 fold increase in the average neurite length. The crude MX acts in a concentration dependent fashion(30-500 µg/ml). MX appears to maintain neuron survival for longer than 3 months, whereas, by that time in its absence, no neurons are detectable.

MX appears to have specific effects on motor neurons. The morphologic changes induced by MX are limited to ventral cord cultures, with no apparent result on cultured dorsal spinal neurons. Further, increases in ACh synthesis (see Vaca and Appel, this volume) and choline acetyltransferase (2.4 fold after 4 days) are observed upon addition of MX to ventral cord cells.

We have begun to characterize this activity. The substance is stable to freezing(-70° C) and lyophilization, and labile to heating(60° C,1 hr:$t_{\frac{1}{2}}$=25min.) and to trypsin. It is precipitated in a 35-60% $(NH_4)_2SO_4$ fraction with retention of activity, and has an apparent molecular weight of >40,000d as determined by gel filtration chromatography. The MX does not appear to act solely by altering cell-substratum attachment, nor does it cross react with antisera directed against β-NGF.

These data demonstrate that skeletal muscle from newborn rats possesses a protein(s) that enhances neuron survival, neurite extension, and ACh synthesis, as well as supporting glial proliferation. Supported by Hartford Foundation grant 470-G09281.

48.7 INDUCED RETARDATION OF OPTIC AXON INGROWTH RESULTS IN A DELAY IN TARGET NEURON DIFFERENTIATION. E.R. Macagno. Dept. of Biological Sciences, Columbia University, New York, N.Y. 10027

Groups of embryonic photoreceptors in one side of the compound eye of Daphnia were irradiated with a UV microbeam at a stage when the cells were post-mitotic but had yet to elaborate axons. Immediately after irradiation the embryos were placed under fluorescent illumination. On the average, 16 of the irradiated photoreceptors were killed by the exposure. Previous observations suggest that an approximately equal number were rescued by the post-irradiation fluorescent illumination. The schedule of differentiation of the rescued photoreceptors was affected such that their axons arrived at the target region in the optic lamina from 2 to 10 hours after they would have normally. Serial section electron microscope analysis showed that differentiation of laminar neurons contacted by the delayed axons was also delayed by a length of time corresponding to the delay in axon arrival. These and previous observations indicate that the differentiation of laminar neurons is triggered by contact with optic axons and can be initiated over a period of several hours after these cells become post-mitotic.

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48.8 ROLE OF NERVE GROWTH FACTOR FOR THE PRE- AND POSTNATAL DEVELOPMENT OF RAT DORSAL ROOT GANGLION NEURONS.

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Sensory neurons in vitro require nerve growth factor (NGF) for survival during critical stages of their embryonic life. The investigation of the importance of NGF for the in vivo development of sensory neurons has been hampered by the lack of biochemical marker substances for these neurons. The demonstration that the undecapeptide substance P (SP) is present in sensory neurons indicates that it could be such a marker.

Administration of NGF to newborn and adult rats lead to an increase in SP content of sensory neurons including their central and peripheral endings. Moreover, retrogradely transported NGF is biologically significant, as shown by an increase in SP and general protein content of sensory ganglia from adult rats.

Administration of 50 mg/kg of purified anti NGF-antibodies to newborn animals produced a marked but reversible reduction in the SP content of sensory neurons. These changes were paralleled by similar changes in their central nerve endings in the spinal cord. Prenatal exposure of sensory ganglia to anti NGF-antibodies resulted in a marked reduction in the number of dorsal root ganglia neurons and in their SP content.

These results indicate that NGF is not only essential for normal postnatal development of at least SP-containing neurons but is also required for their survival during the prenatal period.

48.9 THE NERVE GROWTH FACTOR OF MASTOMYS NATALENSIS. T.L.J. Darling,* E.M. Shooter and D.J. Weiner*. (SPON: U.J. McMahan). Stanford Univ. Sch. of Med., Stanford, CA 94305 and Univ. of Pennsylvania, Sch. of Veterinary Med., Pennsylvania, PA 19174.

Nerve growth factor (NGF) is a peptide hormone which is necessary for the growth and maintenance of sympathetic and some sensory neurons. In the male mouse submaxillary gland, the biologically active protein, βNGF, occurs in a stable complex (7S NGF) with two other proteins and zinc ions. Little is known at the molecular level about mammalian NGF from any source other than the male mouse submaxillary gland, and the guinea pig prostate. The high levels of NGF in the glands of male mice is demonstrably a function of high levels of testosterone. The NGF of Mastomys natalensis, the multi-mammate rat of southern Africa, has been studied to determine its molecular properties. We confirm the original finding of Levi-Montalcini et al. that the submaxillary glands of both female and male Mastomys contain substantial amounts of NGF, in contrast to the situation for female mice and other female mammals. When measured by competitive radioimmunoassay (RIA) using rabbit IgG raised against mouse βNGF, the amount of NGF in the submaxillary gland of males (0.17 mg/gm wet weight) is equivalent to that in glands of females (0.13 mg/gm). When measured by the bioassay carried out with dissociated dorsal root ganglia neurons, the amount of NGF in glands of males (52 mg/gm) is again equivalent to that measured in glands of females (40 mg/gm). The disparity between the amounts of NGF detected by the two techniques prompted attempts to purify the NGF's from both glands. In each sex, 15-50% of the RIA activity is in a low molecular weight protein, and the remainder in a high molecular weight (HMW) form. Two monoclonal antibodies against mouse pNGF both cross-react with both NGF's of each sex. The low molecular weight form has an apparent molecular weight of 25,000 based on its behavior during gel filtration, and a sedimentation coefficient of 2.5S in sucrose density gradients. The HMW NGF of both males and females has an apparent molecular weight of 57,000 during gel filtration, significantly less than that of 7S NGF. Its sedimentation coefficient is 5.1S compared to a value of 6.9S for 7S NGF under the same conditions. The sedimentation properties of Mastomys HMW NGF remain constant through further purification by chromatography on DEAE cellulose. Examination of the partially purified HMW NGF by isoelectric focusing in the presence of urea on polyacrylamide gels reveals that a protein very similar in charge to βNGF has been co-purified with a second protein having an apparent pk of 4. After DEAE chromatography the specific biological activity of HMW-NGF is about one half of that of 7S NGF.

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48.10 β-NERVE GROWTH FACTOR-LIKE MOLECULE IN HUMAN PLACENTA. Mark H. Grossman, Edward Hawrot, Michael Rosenberg* and Xandra O. Breakefield. Dept. Human Genetics and Pharmacology, Yale Univ. Sch. Med., New Haven, CT 06510.

β-Nerve growth factor (β-NGF) is essential for the maintenance and differentiation of sympathetic and sensory neurons. Little is known about human β-NGF due to the extremely low and variable levels that exist in most tissues (including human fibroblasts). β-NGF-like molecules were demonstrated in human placental tissue by radioimmune and receptor binding assays. Using a standard radioimmunoassay, homogenates prepared from the trophoblast portion of a term placenta were found to have 3.3 μg immunoreactive β-NGF per gm wet weight. This assay measures the ability of proteins in the homogenates to compete with purified mouse ^{125}I-β-NGF for antiserum prepared against purified mouse β-NGF. since other proteins in the homogenates may bind ^{125}I-β-NGF, the presence and quantity of a human β-NGF-like molecule was confirmed by a two site radioimmunoassay (Suda et al., 1978). In this assay purified antibodies prepared against mouse β-NGF are bound to poly-vinyl microtiter wells, then incubated with β-NGF standards or samples for 24 hrs, and then with ^{125}I-purified anti-β-NGF antibodies overnight. β-NGF-like molecules in human placental homogenates were further tested for their ability to compete with authentic mouse ^{125}I-β-NGF for binding to β-NGF receptors on rat PC12 pheochromocytoma cells. ([^{125}I-β-NGF]= 1 ng/ml; specific binding is that which was blocked by co-incubation with 10 μg/ml mouse β-NGF.) Immunoreactive human β-NGF molecules in human placenta were about one-fifth as effective as authentic mouse β-NGF in competing for receptor sites.

These studies support the findings of Goldstein et al. (1978) and Walker et al. (1980) of a β-NGF-like molecule in human placenta. This molecule is able to cross-react with some antibodies prepared against authentic mouse β-NGF and to bind to β-NGF receptors on rat pheochromocytoma cells. Due to the relatively high levels reported here, we are now using human placenta as a source of this β-NGF-like protein for characterization of the structure and comparison with mouse β-NGF by methods of immune-precipitation, gel electrophoresis and two-dimensional peptide mapping. (This work was supported by NIH grant NS17083 and by the Dysautonomia Fdn.)

Suda, Barde and Thoenen (1978) Proc. Natl. Acad. Sci. USA 75, 4042-4046.
Goldstein, Reynolds and Perez-Polo (1978) Neurochem. Res. 3, 175-183.
Walker, Weichsel and Fisher (1980) Life Sci. 26, 195-200.

48.11 SUBUNIT COMPOSITION OF NGF FROM HUMAN PLACENTA AT TERM. M. Blum*, C. E. Beck* and J. R. Perez-Polo (SPON: J.S.Kittredge). Department of Human Biological Chemistry and Genetics, University of Texas Medical Branch, Galveston, Texas 77550.

Nerve Growth Factor (NGF) has been isolated from several species including mouse, snake and the guinea pig. In each case the biological activity was found to reside in a basic protein with a molecular weight of about 26,000. Another more acidic protein, the γ subunit, which demonstrates arginyl esteropeptidase activity can also be consistently found associated with the biologically active subunit in a complexed form in all species studied. Only in the murine 7S-NGF complex has a third more acidic subunit, α, been demonstrated thus far. No known biological activity has been associated with the α subunit. In our laboratory we have isolated NGF from human placental tissue and once again found the biological activity to reside in a basic subunit with a molecular weight of about 28,000. Only after several unsuccessful attempts to promote the dissociation of the human NGF complex using treatments known to dissociate mouse 7S-NGF (M. Blum, C. E. Beck and J. R. Perez-Polo, Society for Neuroscience Meeting, 1980), were we able to dissociate the human complex and detect arginyl esteropeptidase activity. The difficulty we have encountered in dissociating the human NGF complex probably reflects a much greater affinity of the subunits for each other as compared to the murine 7S-NGF subunits which are easily dissociated. In addition to the γ and β subunits, we have immunological evidence for the presence of an α subunit in human NGF. Thus the human NGF complex appears to be similar to the mouse NGF in that it contains an α, β and γ although the subunits have a much greater affinity for each other.

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48.12 FURTHER STUDIES OF THE EFFECTS OF MATERNAL ANTI-NGF ON THE DEVELOPING GUINEA PIG. E. M. Johnson, Jr. P. A. Osborne*, R. E. Rydel*, R. E. Schmidt and J. Pearson. Depts. of Pharmacology and Pathology, Wash. U. Med. School, St. Louis, MO and Dept. of Pathology, NYU Med. Center, New York, NY.

We have previously reported (Science 210, 916-918, 1980) that female guinea pigs (g.p.) immunized with mouse nerve growth factor (NGF) make antibodies against mouse NGF which cross-react with g.p. NGF. The antibody is transferred to the offspring in utero. Animals born to anti-NGF producing mothers showed a marked decrease in neuronal numbers in sympathetic, dorsal root, and trigemminal ganglia, but no decrease in nodose, ciliary or sphenopalatine ganglia. The offspring had gross sensory deficits. In the most severely affected litters the newborns invariably died within a few days of birth for reason(s) not yet understood. In these severely affected litters we find decreases of greater than 95% in the norepinephrine levels of heart and spleen and about 80% in small intestine. Thin sections of a mixed nerve (sciatic) showed an almost complete absence of small unmyelinated fibers. Despite the massive effects on sympathetic and DRG neurons, no adverse affects are seen in the developing adrenal medulla by either light microscopic or biochemical criteria. The severity of effects on the offspring of different female guinea pigs is highly variable. Effects as severe as those described above have been seen in the offspring of only 4 of over 25 guinea pigs immunized. The major source of variability appears to be the degree of cross reactivity of antibodies raised against mouse NGF with g.p. NGF. It is possible to produce guinea pigs with lesser degrees of sensory deficit and these animals will survive. These results demonstrate the potential usefulness of this approach. However, variability represents an impediment to the use of this experimental approach in the study of the biology of NGF. Possible approaches to resolving the problem of variability will be discussed. (This work was supported by the March of Dime Birth Defects Fdn., The Familial Dysautonomia Fdn, and NIH grant HL-20604. EMJ is an Established Investigator of the AHA.

49.1 COMPARATIVE MORPHOMETRIC ANALYSIS OF THE BASILAR MEMBRANE IN SELECTED MAMMALS AND ITS IMPLICATIONS TO AUDITION. G. Fred Ramprashad*, Jack P. Landolt, and Kenneth E. Money*. Dept. Zool., Univer. Guelph, Guelph, Ontario, Canada N1G 2W1 and D.C.I.E.M., Downsview, Ontario, Canada M3M 3B9

Serial sections and graphic reconstruction of the cochleae of the rabbit (Oryctulagus cuniculus), gerbil (Meriones unguiculatus), chinchilla (Chinchilla laniger), little brown bat (Myotis lucifugus), two-toed sloth (Choloepus sp.), and harp seal (Pagophilus groenlandicus) were used to study changes in width, thickness and cross-sectional area, and to derive an approximation of the "stiffness gradient" along the basilar membrane of these mammals. Moreover, a plot of the theoretical frequency distribution along the basilar membrane of these six species using Greenwood's formula (J. Acoust. Soc. Am. 33: 1344-1356 (1961)), showed that the frequencies to which these mammals were most sensitive tended to coincide with regions of morphological discontinuity as demonstrated by the stiffness gradient. Moreover, the density of afferent VIII-nerve fibers (as indicated by the density of the bipolar ganglion cells as a function of their location along the length of the cochlea), also coincided within this same region. These findings suggest that the morphological discontinuities, that are present within the basilar membrane, may alter its mechanical and vibrating properties, thereby facilitating a peripheral auditory fine-tuning-mechanism within the basilar membrane. (Supported by Defence and Civil Institute of Environmental Medicine Research Contracts 3278008/2SU78-00209 and 3279027/8SU79-00311.)

49.2 DIFFERENTIAL LABELING IDENTIFIES SUBPOPULATIONS OF SPIRAL GANGLION NEURONS AND COCHLEAR EFFERENTS AFTER AMINO ACID INCUBATIONS. Ilsa R. Schwartz and Allen F. Ryan*, Division of Head and Neck Surgery, UCLA School of Medicine, Los Angeles, CA 90024 and Otolaryngology Research Laboratory, UCSD School of Medicine, San Diego, CA 92103.

Label localization has been compared autoradiographically in various structures in the gerbil cochlea following in vivo perfusion with artificial perilymph containing micromolar concentrations of one of eight tritiated amino acids: l- and d-aspartic acid, l-glutamic acid, glycine, GABA, taurine, alanine, glycine and muscimol. Major findings include differential labeling of efferents under the inner hair cells as compared to the outer hair cell efferents with d-aspartic acid. Also, after incubation with taurine, about 5% of spiral ganglion neurons including the least myelinated cells were labeled much more heavily than the remaining 95%.

At the light microscopic level, efferents beneath the inner hair cells showed heaviest labeling after incubations with d-aspartic acid and GABA. These fibers were more modestly labeled after incubations with l-aspartic acid and l-glutamic acid. After GABA incubations, efferent terminals beneath the outer hair cells were also labeled. Tunnel crossing fibers were most heavily labeled after GABA incubations. Labeling of inner and outer hair cells was slight with all of the amino acids tested, but after alanine and glycine incubations the labeling was slightly greater in inner than in outer hair cells. After taurine incubation there was noticeably less labeling of hair cells than of surrounding supporting cells.

The glial cells around spiral ganglion neurons were most heavily labeled after incubations with GABA and showed a similar but lighter labeling after taurine. The glial cells around spiral ganglion neurons were also moderately labeled after d- and l-aspartic acid, l-glutamic acid and alanine. The spiral ganglion neurons themselves were modestly labeled after d-aspartic acid, l-glutamic acid, glycine, alanine and taurine, and even more lightly after GABA, l-aspartic acid and muscimol. Only after taurine incubations were any differences noted in the labeling of cells within the spiral ganglion neuronal population.

Electron microscopic studies are under way to confirm the identity of the labeled elements.

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49.3 CELLULAR UPTAKE OF 2-DEOXYGLUCOSE IN THE COCHLEA. Allen F. Ryan* and Nigel K. Woolf (SPON: B. Pfingst). Otolarynology Research Laboratory, University of California at San Diego Medical School and San Diego VA Medical Center, San Diego, CA 92103.

The uptake of ^{3}H-2-deoxyglucose (2-DG) was examined at the single cell level in the cochlea of the mongolian gerbil. ^{3}H-2-deoxyglucose (New England Nuclear) was administered by intracardiac injection (167 µCi/100gm in 0.1 ml) to unanesthetized gerbils. The animals were immediately placed in a double-walled, sound-attenuated chamber for 1 hr, either in silence or exposed to wide band noise at 85 dB SPL. The cochleas were then removed, frozen intact in Freon 12 at -159 °C, lyophilized at -40 °C for 72 hr, vapor-fixed with osmium and acrolein, and embedded in plastic. Single-turn segments were sectioned at 1 µm and coated with dry emulsion (NBT2, Kodak) by a loop technique. Sections were exposed for periods varying from 3 to 6 weeks.

In the spiral ganglion, uptake of 2-DG was observed in both the neurons and their associated Schwann cells. In general, uptake of 2-DG was appreciably higher in the glial cell than in the associated neuron. Much less uptake was observed in the myelinated axons within the spiral ganglion. Wide band noise exposure increased the relative uptake of 2-DG in both neurons and glial cells of the spiral ganglion, when compared to other cochlear structures.

In the lateral cochlear wall, grains were spread uniformly through the cellular portion of the spiral ligament, but not in connective tissue elements. Uptake of 2-DG appeared to be uniform in the spiral prominence as well. However, the basal cells of the stria vascularis appeared to incorporate 2-DG at a higher rate than marginal cells.

In the organ of Corti, 2-DG uptake was subtantially lower than in the lateral wall structures or in the spiral ganglion. There appeared to be a slightly higher level of uptake in hair cells than in structural cells in some sections. However, longer exposure times will be necessary to confirm this observation.

It is concluded that the uptake of 2-DG in spiral ganglion reflects both neural and glial metabolism. Also, the metabolic requirements of both neurons and glial cells appear to increase during functional activation of spiral ganglion neurons by acoustic stimulation. The relatively low rate of 2-DG uptake in the organ of Corti is consistent with previous reports of a low metabolic rate at this site.

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49.4 EVIDENCE FOR THE PROJECTION OF TYPE II SPIRAL GANGLION NEURONS TO THE COCHLEAR NUCLEUS IN THE CHINCHILLA. M. A. Ruggero and P. A. Santi. Dept. of Otolaryngology, Univ. of Minnesota, Minneapolis, MN

Two types of neurons have been identified in the cochlear ganglia of several mammalian species. The overwhelming majority of neurons (Type I) innervate the inner hair cells and degenerate upon transection of their central axons. The minority population (Type II) resists retrograde degeneration and apparently innervates only the outer hair cells. Because of their peculiar degeneration behavior, and because it has not been possible to trace their central axons, Spoendlin (Acta Otolaryngol., 1979, 87:381-387) has suggested that Type II ganglion cells do not project to the brain. In order to test this hypothesis we have studied the transport of horseradish peroxidase (HRP) from the cochlear nucleus to the cochlea. Two groups of chinchillas were used: the experimental group received HRP injections (30%, 0.3-0.8 µl) in the left cochlear nucleus; the second group, which served as a control, was identically treated, except that the HRP was purposefully spilled over the cochlear nucleus and at the entrance of the internal auditory meatus. After 24 hr survival, the animals were intracardially perfused with glutaraldehyde and the brains and cochleae were removed. After decalcification in EDTA and glutaraldehyde (18-72 hr) each cochlea was dissected so as to open Rosenthal's canal widely. Incubation in diaminobenzidine and H_2O_2 was followed by Epon embedding, sectioning (2 µm) and light staining with toluidine blue. Type I neurons are characterized by their pale nuclei and by a distinct perinuclear investment of Nissl substance. Type II neurons - less than 5% of the ganglion population - are smaller, their nuclei are relatively dark and have little perinuclear Nissl substance. They are almost exclusively located at the periphery of the ganglion, particularly near the intraganglionic spiral bundle. In the control animals HRP could be demonstrated in only a few ganglion neurons, apparently corresponding to labelling of a few axons in the cochlear nuclei. In the experimental group, there was substantial labelling of both Type I and Type II neurons. HRP reaction product was usually present in the form of granules scattered through the cytoplasm; granules tended to be smaller and fewer in Type II neurons. No HRP could be demonstrated in some Type II neurons, even when surrounded by well-labelled Type I neurons. We conclude that Type II ganglion cells do project to the brain, although their termination may not be identical to that of Type I cells. (Supported by NIH Grant NS12125).

49.5 A STUDY OF THE MECHANISM UNDERLYING THE DIRECTIONAL SENSITIVITY OF THE ANURAN EAR. Walter Wilczynski, Carl Resler*, and Robert R. Capranica. Sect. Neurobiol. and Behav., Cornell University, Ithaca, NY, 14853.

Recent work (Feng, J. Acoust. Soc. Am., 68:1107, 1980) has verified that auditory nerve fibers in frogs are highly sensitive to direction, demonstrating that the anuran has directionally sensitive ears despite lacking pinnae and a large, boney head. As the initial phase of a study to elucidate the mechanism of this directionality, we recorded single eighth nerve units in the leopard frog, Rana pipiens, via a ventral approach to the nerve. A movable speaker presented tones (200 to 1800 Hz) from different azimuthal positions in the free field while sound intensity at the eardrum was monitored with a condensor microphone. Unit responses as a function of sound direction were determined under two conditions. In one, the frog's mouth was opened to expose the Eustachian tubes, maximizing a pressure gradient across the tympanic membranes. In this condition, units were directionally sensitive at all frequencies such that a null, or area of least response, was present in the frontal field. In the second condition, the mouth remained open, but a clay and plastic cap molded to mimic the natural mouth spaces interconnecting the middle ear and nasal cavities was inserted and sealed with grease. This changed the directional characteristics of the units' response such that stimulation at low frequencies still yielded a null pattern, but stimulation above 600 Hz in general yielded a gradually decreasing response as the speaker was moved from the ipsilateral to the contralateral side of the animal. This phenomenon depended on the frequency of stimulation, regardless of the actual characteristic frequency of the unit, implicating a mechanical mechanism independent of hair cell physiology or position in the inner ear. The results also suggest that while a pure pressure gradient mechanism could account for low frequency directionality, other factors contribute to high frequency directionality. In order to determine what these factors are, and whether a pressure gradient is in fact involved in low frequency directionality, experimental manipulations of these two conditions, such as plugging the nares or Eustachian tubes, coupled with eighth nerve recording and concurrent laser interference measurements of tympanic membrane movement are now in progress. Preliminary results from these experiments suggest that while the anuran ear is directionally sensitive, if a pressure gradient mechanism is involved, it is not a dominant factor in determining the directional sensitivity of the anuran's peripheral auditory system.

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49.6 POSSIBLE FACTORS AFFECTING THE RESPONSE PHASE OF AUDITORY NERVE FIBERS. W.G. Sokolich*, D.R. Maceri*, and D. Strelioff. Div. Head & Neck Surgery, UCLA School of Medicine, Los Angeles, CA 90024.

In a previous comparative study in our laboratory, the time-locked responses of auditory-nerve fibers to acoustic sinusoids having frequencies between 100 Hz and 400 Hz were studied in gerbils and guinea pigs. It was found that the preferred response phase of fibers with CFs between 1 and 10 kHz varies in a systematic way with fiber CF in gerbils, but is relatively independent of fiber CF in guinea pigs. In the present study, preliminary data obtained from chinchillas indicates that a CF-independent response phase is atypical of preparations having the most sensitive whole-nerve and single-fiber thresholds. This observation suggests that the CF-independent response phase typical of less-sensitive chinchillas and of guinea pigs used in the previous study may be indicative of a traumatized preparation. The suggestion that even the slightest trauma could substantially affect the operation of the cochlea has rather unappealing implications concerning the validity of routinely-used procedures for making measurements of cochlear microphonics and basilar membrane displacement. Refinements in surgical procedures and controlled studies of the effects of various cochlear manipulations on the response phase of fibers in the most sensitive preparations will be required to place the issue of the sensitivity of the cochlea to insult on a more firm basis.

49.7 "^{3}H-GABA UPTAKE IN ISOLATED VESTIBULLARY CRISTAE OF CHICK INNER EAR". °G. Meza, C. Hernández$^+$ and M. Ruíz$^+$. °Dept. Neurociencias, CIFIC, UNAM and $^+$Dept. Histologia, Facultad de Medicina, UNAM, México 20, D.F.

Afferent neurotransmission in the vestibule of vertebrates is chemical in nature as indicated by considerable evidence. Although the identification of the neurotransmitter has been controversial, strong indication exists favoring GABA as the afferent neurotransmitter in the accoustico-lateralis system of amphibians and fish (Flock and Lam, Nature 249: 142-144, 1974). As this system is fairly constant in vertebrates, we would like to postulate that some similarities must exist amongst them, concerning the neurochemical correlates of neurotransmission. In a previous report (Meza, G. et al, Trans.Am.Soc.Neurochem. 12 (1): 256, 1981) we presented our findings of GABA synthesis as evidence for postulating its participation in the avian vestibule neurotransmission. As further support, in this communication we report the results of some experiments in which ^{3}H GABA uptake was investigated in isolated vestibule cristae of chick inner ear, since Na^+ and energy dependent high affinity transport has been shown to be the inactivating mechanism for GABA in SNC and it is proven to be a suitable criterion when pointing out a transmitter candidate.

For this purpose, ampullary cristae were dissected out from 1 day-old chicks and incubated with 0.5 uM ^{3}H GABA in a Krebs-Ringer-bicarbonate buffer (KRBB), pH 7.3, at various times at 37°C. Sodium-free experiments were performed in the same conditions, incubation buffer was Krebs-Ringer-HEPES, pH 7.3, in which Na^+ was substituted by choline. Temperature dependence was studied by incubation of the tissue in KRBB pH 7.3 at 4°C. Uptake was stopped by centrifugation and tissue was digested in NCS and the radioactivity counted in a Packard TriCarb Scintillation Spectrometer.

We found that 0.5 uM ^{3}H GABA can be accumulated twenty fold in an hour in a saturable manner by isolated cristae of chick inner ear; when incubation took place in the absence of Na^+ or at 4°C, it was inhibited by 90%.

These results indicate that GABA uptake found in isolated vestibullary cristae of chick inner ear shows the characteristics presented by the GABA transporting mechanisms in vertebrate CNS and although the cell responsible for this activity has not been yet identified, this transport might represent the inactivating mechanism for GABA and supports its participation in neurotransmission in the vertebrate vestibule.

49.8 ANATOMICAL AND PHYSIOLOGICAL CHARACTERISTICS OF HRP-LABELED PRIMARY VESTIBULAR NEURONS IN THE BULLFROG. S. Sitko,* V. Honrubia,* J. Kimm and I. Schwartz (SPON: J.M. Miller). Div. of Head and Neck Surgery, UCLA School of Medicine. Los Angeles, CA 90024.

Intracellular recordings were made in the VIIIth nerve of the bullfrog (Rana catesbiana) to measure the membrane characteristics and obtain records of spontaneous and evoked spike activity of primary semicircular canal afferents. Physiological stimulation of the canals was achieved by rotating the preparation on a servo-motor driven turntable with the animals' head centered in the rotational axis. The responses of each neuron to sinusoidal rotations at frequencies of 0.05 Hz, 0.5 Hz and 2 Hz and for impulsive accelerations of 400 deg/sec^2 were obtained. Membrane characteristics measured included the cell resting and action potential amplitude, cell input resistance and spike-activation threshold for applied currents. Physiologically characterized neurons were injected with HRP by applying pneumatic pressure and/or inotophoretic currents to the micropipettes containing 5% horseradish peroxidase in 1 m KCl. Following survival times of 12-48 hours, the VIIIth nerve and attached vestibular end organ was removed for histochemical processing using a diaminobenzidine procedure to visualize the HRP reaction product. Light microscopy was used to discern the anatomical features of the neurons and to trace their peripheral dendritic trajectories from the ganglion to their terminations in the crista. Our studies have revealed that the bullfrog's primary vestibular afferents are characterized by a broad range of soma and axon diameters which correspond to an equally broad range of spontaneous and evoked activity characteristics. The largest neurons had more irregular spontaneous firing rates and consistently exhibited the greatest gain and smallest phase shifts with respect to head acceleration. These neurons consistently terminated at or near the central region of the crista. On the other hand, the smallest neurons were characterized by having the most regular spontaneous discharge patterns the lowest gains and greatest phase shifts with respect to head acceleration. Our findings are thus consistent with the view that the anatomical features of the primary vestibular neurons are important in determining the neuron's physiological characteristics. In terms of response dynamics our observations indicate that the receptors in the frog's crista ampullaris are heterogeneous and differentially sensitive to a wide range of stimulus frequencies.

49.9 RESPONSES OF SEMICIRCULAR CANAL AND OTOLITH AFFERENTS TO LINEAR ACCELERATION. A.A. Perachio,[1,2] M.J. Correia,[1,2] T. Clegg*[1]
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The traditional view that the semicircular canals are insensitive to linear acceleration has been challenged by evidence that canal afferent activity can be modified by static repositioning of the head with respect to the gravity vector (e.g., Estes, et al., J. Neurophysiol. 38: 1232-1249, 1975; Goldberg and Fernandez, Acta Otolaryng. 80: 101-110, 1975). This evidence was questioned as potentially artifactual since the internal auditory meatus was exposed by removal of overlying tissue allowing thermal gradients across the labyrinth to act as a caloric stimulus to the canals. Stereotaxic methods were used in urethane/ketamine anesthetized gerbils in which the brain and tissue surrounding the labyrinth were left intact. Five conditions of head tilt relative to the cardinal head axes were used. 0° tilt (lateral canal coplanar to the earth horizontal), ± 10° pitch angle, and ± 10° roll angle. Sixty vestibular afferents have been tested to date in 9 male gerbils. Of these, 41 were in ampullary nerves innervating the semicircular canals (16 lateral/25 anterior). A change of no less than ±10% in firing rate was considered to be significant. Among neurons with a coefficient of variation (C.V.) of less than 0.20 (n=24), thirty-three per cent of afferents from the semicircular canals exhibited a statistically significant change in firing rate as a result of head tilt. Neurons with a C.V. of greater than 0.20 also responded to tilt. Statistically significant changes in average firing rate occurred in a large percentage of this type of afferent. 91.1% responded to pitch tilt; 72.7% responded to roll tilt. Responses of canal afferents to head tilts were recorded in each of the 9 preparations. Bi-directionality of responses was consistently observed only for lateral canal afferents; firing rate increased when the nose was pitched up and decreased when the nose was pitched down. In contrast, no general characteristics could be identified for anterior canal afferents in terms of response directionality. For purposes of comparison, the responses of identified macular afferents to the same test paradigm were recorded. These afferents were so classified by their lack of response to angular acceleration about the vertical head axis. Macular afferents with a regular firing rate (C.V. = less than 0.2) were sensitive to the small angle head tilts; 88% responded. 83% of irregularly firing otolith afferents also responded to linear acceleration.

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49.10 SEISMIC SENSITIVITY IN VIIIth NERVE AFFERENT FIBERS OF THE WHITE-LIPPED FROG. E. R. Lewis and P. M. Narins. Electronics Research Laboratory, University of California, Berkeley, CA 94720 and Department of Biology, University of California, Los Angeles, CA 90024.

Seismic sensitivity (sensitivity to substrate-borne vibration) is known to reside in the inner ears of salamanders and frogs (D. W. Ashcroft & C.S. Hallpike, J. Physiol., 81:23P, 1934; R.J. Ross & J.J.B. Smith, Comp. Biochem. Physiol., 65A:167, 1980). The most acute seismic sensitivity reported for amphibian inner-ear afferents was found in saccular fibers of *Rana catesbeiana* (E.R. Lewis & R.A. Baird, Abstr. ARO Res. Mtg. 4:11, 1981). Those fibers exhibited thresholds in the neighborhood of 0.0001 g substrate acceleration, with best excitatory frequencies between 40 and 100 Hz; and their responses typically saturated at 0.0005 to 0.001 g. Field observations in the Luquillo Mountains of eastern Puerto Rico suggested that *Leptodactylus albilabris* (Günther), a terrestrial-breeding frog, might have even greater seismic sensitivity. Adults of this species cease calling at the slightest disturbance caused by a would-be observer, even when that observer apparently is not visible to the frog. To date we have recorded the responses of seven seismic afferents from the inner ear of *L. albilabris*, and each of those fibers exhibited sensitivity approximately ten-fold that of the *R. catesbeiana* saccular fibers. All seven exhibited thresholds in the neighborhood of 0.00001 g (with best frequencies between 50 and 100 Hz); and all seven exhibited saturated response at 0.00005 to 0.0001 g. On the basis of their position in the VIIIth nerve, we believe the seven fibers originated at the saccule; but confirmation of this awaits dye-injection studies.

Recordings were made in the VIIIth nerve between the brain and the intact otic capsule. To facilitate access to the nerve (via the roof of the mouth), the frog was mounted upside down, firmly attached to a small platform connected to an electromechanical vibration excitor. This apparatus was placed on an acoustically isolated vibration table, the surface of which exhibited ambient vibration with peak acceleration less than 0.000005 g. Sinusoidal vibrations were applied along the dorsoventral axis and measured by means of an accelerometer (B&K 4370) mounted to the surface of the small platform, immediately adjacent to the frog's head.

At 100 Hz, the peak-to-peak sinusoidal displacement was approximately 0.25 nm at the threshold for *L. albilabris* seismic fibers. This sensitivity is very nearly the same as the most acute displacement sensitivity determined behaviorally in fish (R. R. Fay & M.L. Patricoski, in Abnormal Animal Behavior Prior to Earthquakes II, U.S. Geol. Surv., Menlo Park, CA., p.63, 1980).

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49.11 A MORPHOLOGICAL COMPARISON AMONG THE VESTIBULAR SEMICIRCULAR CANALS IN GUITARFISHES AND SKATE. R.F. Dunn and D.P. O'Leary. Univ. of Pittsburgh Sch. of Med., Pittsburgh, PA 15213.

Striking morphological differences were present when the three semicircular canals were considered in terms of their: overall shape; major radii; length percentages of the canal segments (i.e., large diameter canal, ampulla, small diameter canal); and the canal lumen diameters. Three species were used, Raja eglanteria (Clear-Nosed skate); Rhinobatos lentiginosus (Spotted Guitarfish), and Rhinobatos productus (Shovel-Nosed Guitarfish). The dimensional relationships were generally similar in all three species with only minor variations noted.

Both the horizontal and anterior canals were oval-shaped, having major/minor axes of 14 mm/8 mm, and 12 mm/7 mm, respectively. The posterior canal was nearly circular-shaped having axes of 15 mm/13 mm. Anatomically all three canals were divided into three segments; thick-walled, small diameter segment (SDS); ampulla (AS); and thin-walled, large diameter segment (LDS). The length percentage values of these three segments to the circumference were approximately:

	HOR	ANT	POST
SDS	65	59	55
AS	7	9	8
LDS	28	32	37

All three canals appeared to be largely separated from either the saccular or utricular chambers such that these chambers were not a direct segment of any canal. The posterior canal appeared to be complete and independent with a possible connection near the endolymphatic duct. The LDS of the horizontal canal consisted of two portions: a short LDS communicating with the anterior canal LDS. Hence the LDS of both the horizontal and anterior canals formed a common duct along the majority of its length.

Particular attention was directed to a comparison of the lumen diameters of the small diameter thick-walled canal segments. In cross-section, the horizontal SDS was approximately triangular in shape, while that of the vertical SDS's were circular to oval-shaped. The lumen dimensional ratios were: HOR-ANT 1.5; HOR-POST 1.5; and ANT-POST 1.0. Hence, the lumen diameter of the horizontal canal SDS was 33% less than that of the vertical canals SDS's.

This anatomical information in this study defines the necessary boundary conditions to determine accurate hydrodynamic models of these receptors.

49.12 LINEAR PREDICTION OF SEMICIRCULAR CANAL AFFERENT RESPONSES FROM SWIMMING MOVEMENTS IN THE GUITARFISH. D.P. O'Leary and R.F. Dunn. Univ. of Pittsburgh Sch. of Med., Pittsburgh, PA 15213.

Initiation of swimming movements is controlled by muscular contractions which result in relatively brief rotational acceleration pulses at the guitarfish's head. We investigated the afferent response profiles predicted from these brief acceleration pulses when the latter were considered as stimuli for the semicircular canals.

Signal processing techniques derived from linear systems theory provided the basis for predicted neural responses, in accord with the apparent linearity of semicircular canal afferents. Linear system transfer function parameters were first determined from single units in afferent nerves innervating each of the three canals, via cross-spectral analysis of experimental spike train responses resulting from pseudo-random ("white noise") rotational acceleration stimuli. The transfer functions were then Laplace transformed into time-domain impulse response functions, which were convolved with swimming motion acceleration trajectories to produce predicted afferent responses.

High speed cinematography was used to record swimming trajectories in a sea water tank. Film segments were digitized frame-by-frame to determine time series of sequential fish positions, which were twice differentiated to obtain acceleration trajectories.

Results showed that parameters determining the earliest epochs of the afferent linear system impulse responses dominated the profile of the predicted responses during the first 1 to 3 seconds following initiation of swimming movements. Moreover, these "dominant" parameters corresponded to an initial early time constant as opposed to the later "cupular" time constant often described as a system integrator. Consistent difference were found among predicted responses from vertical and horizontal canals, corresponding to differences in swimming movement dynamics in the respective planes stimulating these canals.

These results are consistent with a parametric control model in which afferent nerves with specific response parameters project information to the brain from specific receptor regions that are maximally stimulated by swimming movements that match the response parameters.

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50.1 STUDIES ON PRIMARY CELL CULTURES OF DISSOCIATED HUMAN FETAL BRAIN. J.W. Walsh*, G. Goranflo* and J. Oeltgen* (SPON: E.T. Iwamoto). Div. of Neurosurgery, Univ. of Ky. Med. Ctr., Lexington, KY 40536.

Tissue from the brain of human fetuses of 11 to 21 weeks gestation was established in primary cell culture to provide a substrate for specific morphological and virological investigations. The fragments of tissue obtained from each specimen were mechanically disaggregated by repeated aspiration through large bore capillary pipettes and then seeded into tissue culture flasks or onto collagen-coated glass coverslips. Approximately 1000 flask and 200 coverslip cultures have been studied. Tissues of each gestational age were cultured separately so that the relative survival and maturation of their constituent cells could be investigated. All cultures were maintained in RPMI salt solution, 10% fetal calf serum, 600 mg% glucose and antibiotics. At days 3, 5, 9 and 12, the overlying nutrient medium was removed and the unattached cells that it contained were separated by centrifugation, resuspended in fresh nutrient medium, and inoculated back into the same culture flasks or dishes. After 12 days, the nutrient medium was changed to one containing 3% fetal calf serum. The cultures were then maintained and studied for periods of up to six months.

Each culture was examined with phase contrast microscopy throughout its period of cultivation and serial photomicrographs were taken. Cultures were also stained with hematoxylin and eosin, and phosphotungstic acid hematoxylin and by the Bodian's and Holtzer methods. Acetylcholinesterase, glial fibrillary acidic acid, galactocerebroside and tetanus-toxin like neuronal cell surface marker were localized in specific cell types and electron microscope and time lapse cinematography studies were done.

At first, a monolayer of immature astrocytes was formed. After successive reintroduction of unattached cells, neuroblasts and immature oligodendroglia were seen. Numerous long neurites grew from small tissue clumps. Myelin formation was not seen. When gestational age was considered, tissue obtained from 14 week fetuses contained numerous bipolar neuroblasts that retained their configuration through their period in culture. After 16 weeks gestation, maturation into multipolar neurons took place over a 3 to 4 week period. If the tissue were of gestational age of more than 18 weeks, neuroblasts and oligodendroglial cells could not successfully be maintained. The results suggest that there is a critical period in human neuronal development at about 18 weeks gestation and confirm that before this stage their maturation and growth can be studied by cell culture techniques.

50.2 HOMOTYPIC EXTRACELLULAR MATRICES ENHANCE THE PROLIFERATION OF HUMAN NEUROBLASTOMA CELLS. Angelika Michler-Stuke* and Jane E. Bottenstein. Department of Pediatrics, University of California, Los Angeles, CA 90024.

The extracellular matrix produced by cells in vitro corresponds in part to the intercellular material on which cells in the organism proliferate, aggregate, migrate, and/or differentiate. Some of the components of in vivo and in vitro extracellular matrices have been identified. These may include different types of collagen, elastin, glycosaminoglycans, proteoglycans, hyaluronate-proteoglycan aggregates, and several glycoproteins (e.g., fibronectin and laminin). Although in vitro studies have described the biochemical composition of mouse and rat neuroblastoma extracellular matrices, no functional role for these neuronal matrices has been reported.

We have investigated the relationship between LA-N-1 human neuroblastoma-derived extracellular matrices and the proliferation of autologous LA-N-1 cells in serum-free defined media. Cell-free extracellular matrices were prepared from high density cultures by treatment with 0.02% EGTA. These homologous extracellular matrices increased the incorporation of tritiated thymidine into TCA-precipitable material three-fold in cultures of moderate density and nine-fold in low density cultures. Enzymatic treatments of the matrices indicated that trypsin> neuraminidase>hyaluronidase diminished their growth enhancing effect. DNase, RNase and collagenase treatment had no effect. The sialoglycoprotein fibronectin alone or in combination with a polylysine coated substratum could not mimic the effect of the extracellular matrices. The specificity of extracellular matrices derived from other donors, including different cell types, will be discussed as well as the influence of neuronal-glial matrices. The significance of these results may lie in their relevance to normal neuroblast proliferation.

50.3 CHICK EMBRYO SPINAL CORD CELLS CULTURED IN DEFINED MEDIUM AND ALTERATIONS IN HIGH AFFINITY GABA UPTAKE. C.R. Walker, C.A. Glass* and R.C. Strohman*. Dept. Zoology, U.C.Berkeley, Berkeley, CA 94720

We have successfully cultured chick embryo spinal cord cells in a completely defined medium. Seven day old chick embryo spinal cords were dissociated with 0.1% trypsin and plated on polylysine coated 35mm dishes(1ml of 0.01mg/ml polylysine, Sigma II, in H_2O overnight, 3x rinse before use). The medium is MEM with Eagles salts with added amino acids to conform to the DM-153 medium of H. Katsuda & F. Takaoka(1976) in Methods of Cell Biology ed. O.M. Prescott, v. 14, pp. 145-158. 10μM HEPES, 0.5μg/ml insulin, and 0.1μM dexamethasone were also added.

The neuronal cells exhibit extensive neurite development and survive at least two weeks. Non-neuronal cells do not survive and cultures appear to be completely neuronal. The cells can be refed with a serum containing medium after several days and few non-neuronal cells are seen after a week. This procedure appears to be a very effective way to obtain pure neurons for biochemical studies without toxic drugs.

Chick spinal cord cells have a high affinity uptake mechanism for GABA and this mechanism was investigated in the defined medium. Autoradiography revealed that a majority of the neurons are GABAnergic and may suggest that these neurons survive preferentially in this medium. A kinetic analysis of ^{3}H-GABA uptake was done using spinal cord cells plated in the defined medium at 0.2 spinal cords per dish. One half of the cultures were refed with a medium containing 10% horse serum and 1.5% embryo extract after 24 hrs. The high affinity uptake at 4 days was the same for both groups, Km=3μM. The Vmax or uptake capacity of the two groups was different, defined medium, Vmax= 2.6pmo/dish and serum medium, Vmax=8.0pmol/dish. This difference probably represents the more extensive neurite development that is seen with neurons in serum.

In a separate experiment, cells were refed serum at day 5 then analysed at day 8 for GABA uptake. This experiment showed that the older neurons in defined medium had a lower affinity, Km=12μM than the neurons in serum, Km=2.5μM. Others have reported that the affinity for GABA uptake decreases with the age of neurons in culture. This decrease maybe due to changes in membrane structure and culturing in defined medium may speed up this process. It is known that cells cultured in defined media have different lipid composition of membranes than cells cultured with serum. These changes we have found in the kinetic properties of GABA uptake may represent an example of regulation of a cell property by membrane lipids.

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50.4 COMPONENTS OF THE PERIPHERAL NERVOUS SYSTEM DEFINED BY MONOCLONAL ANTIBODIES. Carson J. Cornbrooks, Richard P. Bunge, and David I. Gottlieb. Dept. Anat. & Neurobiol., Wash. Univ. Sch. of Med., St. Louis, MO 63110.

The interaction between three cell types (Schwann cells, neurons and fibroblasts) is essential for the development and maintenance of the PNS. In an attempt to define cell surface or secreted molecules which play a role in these processes, monoclonal antibodies were made against components of the PNS. Balb/c mice were immunized with preparations of Schwann cells and neurons grown in culture for 5 weeks and one month later received a second immunization of sciatic nerve obtained from a 6 day postnatal rat. Four days after the second injection, the mouse spleen cells were fused with SP2/0 myeloma cells and hybridomas were obtained by standard techniques (Galfré et al., Nature 266:550-552, 1977). The binding of hybridoma-secreted antibodies was initially screened using ^{125}I-RaM FAB'2 secondary (2°) antibody and cells cultured from rat dorsal root ganglion (DRG). These DRG preparations were housed in plastic, multiwell dishes which provide reproducible cultures of neurons with proliferating Schwann cells in defined medium (Bottenstein and Sato, PNAS 76:514-517, 1979) or fully differentiated (myelinating) Schwann cells in serum-embryo extract containing media. Wells containing hybridomas which demonstrated high binding to PNS cells and poor binding to lung preparations were cloned and ascites tumors were produced. An antibody designated B3 demonstrated saturable binding to cultures containing neurons and Schwann cells and considerably lower binding to preparations of sciatic nerve fibroblasts, and DRG neurons after 8 and 23 days in culture. Visualization of B3 binding with 2° antibody conjugated to fluorescein or HRP revealed a ring of staining surrounding myelin sheaths in 10λg, frozen sections of adult sciatic nerve. Myelin sheaths and axons were not stained. Immunohistochemical staining with HRP conjugated 2° antibody of cultures containing both neurons and Schwann cells without fibroblasts revealed staining over the surface of axon-related Schwann cells. At the electron microscopic level a flocculant precipitate of the reaction product was observed associated with the Schwann cell basal lamina surrounding Schwann cells related to both myelinated and unmyelinated axons. We believe this monoclonal antibody is directed against an antigenic component of the basal lamina which is secreted by the differentiating Schwann cell. Further studies will be directed to determine the role of this antigen during the neuron-Schwann cell interaction. (Supported by NIH Grant GM28002.)

50.5 MAINTAINED MITOTIC ACTIVITY IN MORPHOLOGICALLY DIFFERENTIATED RAT PHEOCHROMOCYTOMA CELLS TREATED WITH NERVE GROWTH FACTOR. M.J. Ignatius*, C.E. Chandler* and E.M. Shooter, Dept. of Neurobiology, Stanford Univ. School of Medicine Stanford, CA 94305.

Cultures of rat pheochromocytoma (PC12) cells continue to synthesize DNA for up to 14 days in the presence of nerve growth factor (NGF), (Gunning et al., in press). In cultures treated with NGF for eight days, both process-bearing and non-process-bearing PC12 cells have labelled nuclei following a brief incubation with ^{3}H-thymidine. The present study, in addition to further characterizing the continued DNA synthesis in NGF treated PC12 cells, looks at whether the presence of ^{3}H-thymidine uptake actually reflects cellular division in morphologically differentiated neurons.

PC12 cells (courtesy of D. Schubert) were grown on polylysine coated plastic cover slips in DMEM with 10% horse serum. Some cultures were given 50 ng/ml β-NGF every other day while control cultures lacked NGF. DNA synthesis in individual cells was determined by adding ^{3}H-thymidine (1μCi/ml) for 2 hrs, followed by fixing and processing for autoradiography. In all instances nuclear labelling was reduced by 95% with 10 μM cytosine arabinoside, a DNA polymerase inhibitor. In 8 day old control cultures (no NGF) 30% of the cells had labelled nuclei, after 2 hr ^{3}H-thymidine pulse. In cultures treated with NGF for 8 days, 7% of the cells were still synthesizing DNA. The fraction of process bearing and non-process bearing cells which had labelled nuclei were identical; thus these labelled cells (7%) were not a subpopulation of NGF non-responsive cells. Even after 14 days in culture with NGF, 7% of the cells with neurites were still synthesizing DNA during a two hr pulse. With continuous ^{3}H-thymidine labelling in the presence of NGF from 8 to 13 days in culture, 65% of the process bearing cells were labelled, indicating again that labelled cells were not a minority population of abnormal or rapidly dividing cells. Thus both morphologically differentiated and undifferentiated PC12 cells respond to NGF by slowing down but not stopping their synthesis of DNA.

The DNA synthesis observed was accompanied by normal cellular division and did not result in polyploid or cells with or multinucleate. This conclusion is supported by two findings. Fewer than 2% of the cells were multinucleate and the amount of DNA per nuclei remained constant, as determined by flow micro fluorimetry. Preliminary time lapse cinematography has shown a number of morphologically differentiated cells dividing in culture.

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50.6 EVIDENCE FOR VASOPRESSIN-LIKE NEURONS IN PRIMARY CULTURES OF DISSOCIATED FETAL MOUSE HYPOTHALAMUS. D.T. Theodosis *, P. Legendre*, I. Cooke and J.D. Vincent. INSERM, Rue Camille Saint-Saëns, 33077 - BORDEAUX-CEDEX (France)

Cells cultured from fetal mouse hypothalamus have been studied by light and electronmicroscopy to correlate their morphology and immunoreactivity with electrophysiology. Following techniques described by Faivre-Bauman et al. (Brain Res., 185 : 289, 1980), hypothalami were dissected from 14 day fetal mice, mechanically dissociated, plated on 35 mm Lux dishes and grown in Ham's F 12 supplemented with 10 % fetal calf serum and 1 % glucose at 37°C, 5 % CO_2. No antibiotics were present after the first week. After 21 days, the cultures are composed of a continuous basal layer of glial and mesenchymal cells over which neurons (identified by morphology and electrophysiology) are growing in discontinuous patches. Phase contrast, intracellular injection of horseradish peroxidase or lucifer yellow demonstrates neurons whose extent and complexity of processes increase with age. Three types predominate: (a) the largest, pericarya 10-20 μm, have 2 or 3 stout dendrite-like processes bearing numerous spines, and a long thin axon-like process, occassionally branching, often with dilatations; (b) neurons with a stellate arborization of thin, spine-bearing processes and a single long axon-like process; (c) neurons from which a single process emerges and bifurcates close to the soma. In EM, many features typical of neurosecretory neurons are found including dense-cored secretory granules (100-200 nm) which occur in small numbers in perikarya and more frequently in processes and dilatations. Synapses, identified by the presence of pre- and post-synaptic densities ans synaptic vesicles, are seen to impinge onto these elements. Immunocytochemical staining (using immunofluorescence and the unlabeled antibody enzyme method with PAP) shows that a number of neurons of type (a) react positively to vasopressin antisera. The immunoprecipitate is localized mainly in processes and dilatations and less abundantly in perikarya. It is at no time evident within the basal layer. All neurons displayed action potentials and complex synaptic-like responses. Plateau depolarizations of 1/2-5 min duration were recorded from type (a) neurons (see Legendre, et al., this vol.).

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51.1 CLONIDINE DECREASES SEROTONIN SYNTHESIS AND METABOLISM IN RAT BRAIN CORTEX, John F. Reinhard, Jr. and Robert H. Roth. Depts. of Pharmacology & Psychiatry, Yale University School of Medicine New Haven, CT 06510

Considerable electrophysiological evidence suggests that serotoninergic neurons are modulated by a facilitory noradrenergic input. Pharmacologic studies have shown that the alpha noradrenergic agonist clonidine affects brain serotonin metabolism. Unfortunately, there is little agreement as to the direction or the mechanism of these effects. Thus, the present studies were undertaken to resolve the nature of clonidine's serotoninergic interactions.

Tissue indoles and catechols were measured using liquid chromatography with electrochemical detection. MHPG was measured by gas chromatography-mass spectrometry.

Time-course studies revealed a maximal decrease of both 5-HIAA and MHPG at 3 hours; the decrease was evident at 2 hours, persisted until 4 hours, and was no longer present at 6 hours.

Dose-response studies, conducted at 3 hours, demonstrated changes in MHPG with as little as 30 ug/kg (i.p.), while significant changes in 5-HIAA were not observed below 300 ug/kg.

Administration of clonidine prior to decarboxylase inhibition with Ro4-4602, antagonized the Ro4-4602-induced accumulation of both DOPA and 5-HTP by 33%.

The possibility that clonidine acts by decreasing noradrenaline release was tested by administering L-amphetamine along with clonidine. Amphetamine completely antagonized the clonidine-induced decrease in 5-HIAA, at a dose which when given alone did not alter either 5-HIAA or MHPG.

The present studies reveal that clonidine decreases cortical serotonin synthesis and metabolism. The correlation between the changes in 5-HIAA with those of MHPG, and the antagonism of the clonidine-induced decrease in 5-HIAA by L-amphetamine, suggest that clonidine decreases serotonin metabolism by reducing the release of noradrenaline molecules into brain synapses. This effect may be due to clonidine's interaction with autoreceptors of noradrenergic cell bodies or nerve terminals. (These studies were supported by NIH grant MH-14092; J.F.R. was supported by NRSA #1 F32 MH08358-01.)

51.2 CHOLINERGIC DENERVATION AND BLOCKADE INCREASE α ADRENERGIC RECEPTOR BINDING IN THE RAT BRAIN. A. Leslie Morrow*, Charles C. Wurtz*, Rebekah Loy and Ian Creese, Dept. Neurosciences, Univ. Calif. San Diego, La Jolla, CA 92093.

Many studies have suggested an intricate relationship between adrenergic and cholinergic activity. Recently, cholinergic activity has been implicated in the regulation of non-cholinergic receptor systems. The present studies were undertaken to determine if alterations in muscarinic activity through surgical denervation of the septo-hippocampal pathway or by pharmacological blockade using the muscarinic antagonist atropine directly affect α adrenergic binding in the CNS.

Electrolytic or aspiration lesions of the cholinergic septo-hippocampal projection were performed on adult female albino rats. After 9-30 days the binding of [^{3}H]WB4101 to hippocampal homogenates was assayed in parallel for lesioned and sham-operated animals at three concentrations: 1 nM, .5 nM and .2 nM. For each pair (n=16) an increase in binding was observed. Scatchard plots of the data indicated there was no change in affinity but that cholinergic denervation produced about a 39% ($p<.005$, 1-tailed Student's t-test) increase in the number of binding sites for the α_1 adrenergic ligand. No alterations in muscarinic, α_2 or β adrenergic binding were detected.

Atropine sulfate (2 mg/kg) or physiological saline (.2 cc) was administered s.c. to female albino rats every 12 hrs for 21 days. Adrenergic binding using [^{3}H]prazosin (PRAZ), Kd=.12 ± .01 nM, and muscarinic binding using [^{3}H]QNB, Kd=.05±.01 nM were measured in the thalamus and limbic cortex (including hippocampus, dentate gyrus, subiculum, entorhinal cortex and cingulate gyrus). Assays and analyses of saline and atropine treated tissue were performed in pairs and differences are expressed as percent increase over control.

Chronic atropine treatment resulted in an up-regulation of PRAZ binding in the limbic cortex (X=15.1±4.5%, $p<.05$) and in the thalamus (X=11.9±3.5, $p<.025$). This increase was present in animals sacrificed 6 hrs, but not 48 hrs, following the last injection. In addition there was a simultaneous increase in [^{3}H]QNB binding in the limbic cortex (X=35.9±4.9, $p<.001$) which had diminished but was still significant after 48 hrs (X=16.3 ±2.2, $p<.05$). Affinities were unchanged for both ligands.

Considered together these results strongly suggest cholinergic regulation of α_1 adrenergic receptors in the CNS. It is possible that the PRAZ up-regulation following atropine treatment was due to the antagonist action of atropine at the receptor (Ki=750 nM for [^{3}H]PRAZ binding). We are presently investigating this possibility.

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51.3 STIMULATION OF DOPAMINE RECEPTORS INHIBITS VIP SENSITIVE ADENYLATE CYCLASE ACTIVITY IN MALE RAT ANTERIOR PITUITARY. P. Onali*, J. P. Schwartz and E. Costa. Lab. Preclin. Pharmacol., NIMH, St. Elizabeths Hosp., Washington, D.C. 20032.

Vasoactive intestinal peptide (VIP) stimulates adenylate cyclase (a.c.) activity of pituitary gland (Borghi et al., FEBS Lett. 108:403, 1979) and selectively increases prolactin release in vitro (Enjalbert A. et al., Neuroendocrinology 31:200, 1980). Since dopamine (DA) inhibits prolactin release by acting on D-2 receptors, we used the activation of a.c. by VIP as a model to investigate the possible linkage of DA receptors with a.c. in male rats anterior pituitary. DA failed to affect the basal activity of pituitary a.c. at concentrations as high as 10^{-4}M, but inhibited the stimulation of a.c. elicited by different concentrations of VIP (10^{-8} to 5×10^{-7}M). This inhibition occurred without an apparent lag phase, was dose-dependent and reached a maximum (50-60% reduction of the VIP stimulation) at 10^{-5}M. The IC_{50} of DA, was approx. 4×10^{-7}M. DA receptor agonists such as apomorphine, A-6,7-DTN and bromocryptine mimicked the effect of DA. Classical neuroleptic drugs and (-)sulpiride, a selective DA D-2 receptor blocker, antagonized the inhibitory effect of DA stereospecifically. Phentolamine (10^{-8} to 10^{-5}M) did not block DA inhibition. The VIP sensitive a.c. was not affected by l-phenylephrine and l-isoproterenol, whereas it was significantly inhibited by noradrenaline (NA) at 10^{-5}M. The effect of NA was antagonized by 1 μM (-)sulpiride but not by 1 μM phentolamine. Dopamine failed to affect the activation of a.c. by prostaglandin E_1, a hormone which does not stimulate prolactin release. Muscimol, a GABA agonist which inhibits prolactin release in vitro, did not change the magnitude of a.c. stimulation by VIP. The results show that stimulation of D-2 receptors exerts an inhibitory effect on the activation of a.c. by VIP and indicate that these DA receptors are linked in an inhibitory manner to a.c. in anterior pituitary.

51.4 THE EFFECTS OF TRH ON THE IN VIVO RELEASE OF NE FROM THE MEDIAL PREOPTIC/ANTERIOR HYPOTHALAMUS OF THE RAT. S.K. Salzman*, M.S. Sellers*, and A.L. Beckman. Research Dept., Alfred I. duPont Inst., P.O.Box 269, Wilmington, DE 19899.

It is now widely accepted that thyrotropin releasing hormone (TRH) is a neurotransmitter or neuromodulator in the CNS. This tripeptide has been implicated as a mediator of arousal. One important question that must be addressed is what portion of TRH action is mediated by other neurotransmitter systems? We are investigating the possibility that the effects of TRH are mediated by an ability to augment the release of norepinephrine (NE) in the medial preoptic/anterior hypothalamus (POAH), as has been indirectly demonstrated in other brain regions.

Ten male Sprague-Dawley rats have been implanted with push-pull cannulae in the medial POAH. After postoperative recovery and habituation to the testing chamber, rats were perfused with an artificial CSF at a rate of 20.6 μl/min with a Harvard syringe pump. After a 30 min. washout period, 3 serial 20 min. samples were collected under baseline conditions where-upon an artificial CSF containing TRH (1.0, 3.0, 10.0 or 100.0 μg/ml) was remotely switched into the push flow, and 3 additional 20 min. samples collected. NE, and its major metabolite, free MHPG, were detected in all samples by liquid chromatography with electrochemical detection using DHBA as the internal standard.

Addition of TRH to the inflow resulted in behavioral and motor activation at all dose levels. At the higher dose levels (10.0 and 100.0 μg/ml), TRH produced a series of behaviors reminiscient of the opiate abstinence syndrome. The release of NE and MHPG correlated with arousal state (NE and MHPG in pmol/20 min ± SEM): waking with movement - 1.83 ± 0.33 (N=12), 3.59 ± 0.45 (N=6); waking, no movement - 0.72 ± 0.14 (N=11), 0.84 ± 0.13 (N=3); behavioral sleep - 0.70 ± 0.20 (N=6), 0.36 ± 0.10 (N=4).
In the pre-TRH baseline samples, NE was released at a rate of 0.89 ± 0.12 pmol/20 min (N=23) and MHPG at 0.70 ± 0.09 pmol/20 min (N=11). TRH augmented the release of one or both compounds; NE and MHPG in pmol/20 min: TRH (1.0 μg/ml) - 2.22 ± 0.32 (N=4), 0.44 ± 0.06 (N=4); TRH (3.0 μg/ml) - 2.62 ± 1.51 (N=3), 3.17 ± 1.81 (N=2); TRH (10.0 μg/ml) - 2.54 ± 1.13 (N=3), MHPG not determined; TRH (100.0 μg/ml) - 0.29 ± 0.19 (N=3), 3.00 ± 0.62 (N=4).

These results indicate that pharmacological doses of TRH can augment NE release in the medial POAH, and suggest that this may in part be responsible for the arousing effects of TRH.
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51.5 PRESYNAPTIC INTERACTIONS OF CHOLINERGIC SYSTEM WITH AMINERGIC AND PEPTIDERGIC SYSTEMS CONCERNING RELEASE OF PEPTIDE HORMONES. Y. Yukitake*, and N. Hagino, (SPON: V. Williams) Department of Anatomy, Univ. of Texas Health Sci. Ctr., San Antonio, TX 78284.

The presynaptic receptors and uptake of blood borne materials were investigated electronmicroscopically in the posterior pituitary gland of the rat since peptidergic, aminergic and cholinergic axon terminals in the posterior pituitary have no synaptic connections. There are three types of vesicles in axon terminals in the posterior pituitary: Type 1 vesicles are 120-150 nm in diaeter and contain dense droplets; Type 2 vesicles are 40-50 nm in diameter and appear as clear vesicles; Type 3 vesicles are 50-70 nm in diameter and contain small dense granules. Cholinergic axon terminals contain only type 2 vesicles, however, peptidergic axon terminals contain type 1 vesicles together with type 2 vesicles and aminergic axon terminals contain both type 2 and 3 vesicles. In addition, coated pits are found on the membrane surface of the three kinds of axon terminals. Horseradish peroxidase (16% HRP) was infused intravenously under urethane anesthesia. Fifteen minutes later the animals were perfused with fixative and the brains were removed and processed for visualizing the HRP in the posterior pituitary by electronmicroscopy. Tetramethylbenzidine was used as a substrate for the HRP. Coated pits and vesicles associated with all three types of axon terminals were found to contain the HRP. Intravenous infusion of acetylcholine (Ach, Carbachol 120 μg/ml/hr in 2.5% dextrose in 0.5% saline, 286 mosmoles/kg volume) under urethane anesthesia increased the diameter and quantity of type 2 vesicles in all three types of axon terminals, and further, Ach tended to suppress the release of type 1 vesicles from peptidergic axon terminals caused by urethane anesthesia. It suggests that peptidergic axon terminals may accept Ach through coated pits and the release of peptide hormones may be suppressed at the level of axon terminals. Furthermore, cytochemical reaction of dopamine dependent adenylate cyclase (DD-AC) as an indication of dopamine receptors was investigated in the posterior pituitary. All three kinds of axon terminals demonstrated positive reaction for DD-AC; DD-AC was found within axon terminals and sometimes in between type 1 vesicles. This suggests that dopamine receptors are present in all three kinds of axon terminals and may be involved in the uptake of the dopamine. It is concluded that the uptake of neurotransmitters by the axon terminals of the posterior pituitary may be involved in the regulation of peptide hormone release. (Supported by NIH Grant NICHD 10071).

51.6 INTERACTIONS OF ESTROGEN WITH CHOLINERGIC AND DOPAMINERGIC SYSTEMS IN THE REGULATION OF PROLACTIN SECRETION. N. Hagino, D. Liu*, A.T. Modak and W.B. Stavinoha, Departments of Anatomy and Pharmacology, Univ. of Texas Health Sci. Ctr., San Antonio, TX 78284.

Acetylcholine (Ach) in blood is associated with rat estrous cycles, and further, ovarian estrogen is one of the factors controlling Ach in blood and CNS. Estrogen elevates serum levels of prolactin (PRL), therefore if ovarian estrogen interacts with Ach in blood and CNS to regulate PRL secretion, intravenous or intracerebroventricular application of Ach could alter blood levels of PRL. In 6 intact female rats under urethane anesthesia intravenous infusion of 2.5% dextrose in 0.5% saline maintained 125.7 ± 38.0 ng/ml of serum PRL. Following this Ach (Carbachol 120 μg/ml/hr in 2.5% in 0.5% saline) was infused and this resulted in an increase of serum PRL at 15 min (738.0 ng/ml); however, serum PRL was reduced at 60 min (345.6 ng/ml) and 120 min (175.0 ng/ml) during Ach infusion. Intracerebroventricular infusion of Ach (7.5 μg/μl/hr) using Alzet minipump increased serum PRL (139.1 vs 28.9 ng/ml) in 6 unanesthetized freely moving rats.

These results suggest the hypothesis that Ach may act directly on the tuberoinfundibular dopaminergic neurons (TIDA) to suppress their activity and in turn facilitate PRL secretion.

In order to elucidate the problem, Ach (120 μg/ml/hr) was infused intravenously under urethane anesthesia in 10 ovariectomized rats (ovex) and 11 estradiol benzoate (5 μg/rat for 3 days) primed ovex rats and serum PRL was assayed by RIA. Ovex rats exhibited Ach concentration in plasma (0.32 nmol/ml) and in cellular compartments (cc 0.37 nmol/ml), and serum PRL (65.5 ng/ml) persistently. Infusion of Ach for 2 hrs following infusion of vehicle in ovex rats increased serum PRL during the infusion period, and no reduction of serum PRL was observed. Intact rats infused with Ach under similar conditions showed a reduction in serum PRL. Injections of estrogen in ovex rats increased Ach in plasma (1.04 nmol/ml) and cc (0.93 nmol/ml). Ach infusion in estrogen primed ovex rats suppressed serum levels of PRL.

The results illustrate the possible involvement of acetylcholine as a neurohormone in the regulation of prolactin secretion and since the dopaminergic system is involved in the regulation of prolactin secretion, an interaction of estrogen with cholinergic and dopaminergic systems is indicated. (Supported by NIH Grant NICHD-10071).

52.1 ABERRANT NEURITE AND MEGANEURITE DEVELOPMENT IN FELINE SPHINGOMYELIN LIPIDOSIS AS REVEALED BY THE GOLGI METHOD. Steven U. Walkley, Henry J. Baker* and Dominick P. Purpura. Dept. Neuroscience, Rose F. Kennedy Center for Research in Mental Retardation and Human Development, Albert Einstein College of Medicine, Bronx, N.Y. 10461.

Golgi studies of human and feline gangliosidoses have revealed that certain types of neurons in these disorders develop conspicuous enlargements interposed between somata and axon hillocks (meganeurites) and additionally sprout secondary neurites from these same areas. Ultrastructurally, both meganeurites and secondary neurites have been shown to be the site of synapse formation, these contacts being associated with afferents of unknown origin. Golgi-demonstrable morphological changes which were qualitatively similar but quantitatively less severe also have been described in feline mannosidosis. It has been proposed that the insidious development of these abnormal structures and their associated synaptic input are primary contributors to the neurobehavioral deterioration evident in these disorders.

A 7-month old Balinese cat with a history of progressive neurobehavioral deterioration was killed humanely and tissues taken for biochemical and morphological assessment. Light microscopic, ultrastructural, and biochemical findings, including the demonstration of a 20-fold elevation of sphingomyelin in liver, were consistent with a diagnosis of sphingomyelin lipidosis or Niemann-Pick disease. Golgi studies were performed on a wide variety of CNS regions and successful impregnations revealed an array of morphological changes which appeared to be specific for given types of neurons. Neocortical pyramidal neurons demonstrated axon hillock-associated neurite growth and occasional meganeurite development. Other pyramidal neurons were normal looking or demonstrated dendritic spine decrement. Significant somatic enlargement was not a conspicuous feature. Occasional neocortical intrinsic neurons also were observed that possessed meganeurites but not secondary neurites. Meganeurite development was found to be of greater prominence in the amygdala and claustrum, and secondary neurite growth also was observed on many neurons of these areas. In the caudate, medium spiny cells generally lacked meganeurite and neurite growth, although axon hillock enlargement sometimes was observed. Rarely, medium spiny cells were found which displayed prominent meganeurite development. In the cerebellum, basket, stellate and Purkinje cells were found which had focal dendritic enlargements but no meganeurite or neurite growth.

Thus sphingomyelin storage disease in the cat represents another lysosomal disorder in which aberrant neurite and meganeurite growth occurs at axon hillock regions on select types of neurons. (Supported by NS-07512 and NS-10967).

52.2 PERTURBED MICROTUBULAR ARRAYS WITHIN CORTICAL NEURONS IN MENTAL RETARDATION. N. Bodick*, J. K. Stevens, and D. P. Purpura. Albert Einstein College of Medicine, Rose F. Kennedy Center for Research in Mental Retardation, Bronx, NY 10461, and Playfair Neuroscience Unit, University of Toronto, Toronto, Ontario, Canada.

Golgi impregnated biopsy tissue from the frontal cortex of developmentally retarded infants and children reveals an alteration in the dendritic structure of pyramidal cells. In contrast to the normal spine-studded cylinder, these processes are varicose: pronounced swellings occur along the length of the dendrite.

The microtubular array within these varicose dendrites has been reconstructed in 3-space, using a computer-graphic representation generated from serial electron micrographs. A new tracing strategy, microalignment (Stevens, Brain Res. Reviews, v. 203,No. 3, p. 265, 1980), facilitates data collection from small structures, over a large field.

Microtubules, within the constricted regions of the varicose dendrite occur in close parallel array. Within the varicosities, the relatively few microtubules frequently spiral. Notably, these expanded regions correspond to discontinuities in the microtubular array. Although synapses persist on these dendrites, the occurrence of spines is reduced.

The microtubular structure of the varicose dendrite is contrasted to that of the normal cortical dendrite in the mouse. In the normal dendrite, segmented tubules occur in parallel array. The tubules occupy the process in a space-filling fashion i.e., the shape of the array mimics the shape of the cell. It is suggested that, in the varicose dendrite the perturbed microtubular array underlies the perturbed cell shape. Quantitative description will be given.

52.3 TRIMETHYLTIN (TMT) INDUCED HIPPOCAMPAL LESIONS IN MICE. L. W. Chang, T. Tiemeyer*, G. R. Wenger* and D. E. McMillan*. Department of Pathology and Department of Pharmacology, University of Arkansas for Medical Sciences, Little Rock, AR 72205.

Alkyltin compounds are used extensively in plastic industries and in agriculture. Among alkyltin compounds, trimethyltin (TMT) is found to be extremely neurotoxic and has a selective toxic impact on the hippocampal formation. Mice were found to be very sensitive to TMT neurotoxicity and may serve as an animal model for the study of this potent neurotoxiciant. BALB/c and C57BL/6 mice, in groups of 6,were given a single intraperitoneal injection of trimethyltin (TMT) chloride at a dosage of 3.0 mg/kg body weight. Control animals were injected with equal volumes of saline solution. Whole-body tremor was observed in all TMT-treated animals within 24 hours. All animals were sacrificed at 48 hours post-injection. Animals were anesthetized with an intraperitoneal injection of Nembutal, chest opened surgically, and perfused intracardially with saline solution followed by 2.5% buffered glutaraldehyde. Brains were removed surgically and sliced before fixation. Half of the brains (right hemisphere) were fixed in Bouin's solution followed by 10% buffered formalin for light microscopy study. The other half of the brains (left hemisphere) were fixed in 2.5% buffered glutaraldehyde followed by 1% buffered osmium tetroxide for electron microscopy investigation. Extensive neuronal necrosis which appeared as cells with eosinophilic cytoplasm and pyknotic nuclei were observed in the granule cells of the fascia dentata in the hippocampal formation. Vacuolar changes in the fascia dentata as a result of neuronal loss were also evident in some animals. Necrotic changes in the pyramidal cells of the hippocampal formation were only minimal under these toxic conditions. With electron microscopy, it was revealed that significant accumulation of lysosomes and extensive degenerative changes occurred in the neuronal cytoplasm of the granule cells in the fascia dentata. Some pyramidal cells were also affected but most of these cells were spared. The present study demonstrated not only a sensitive model for the investigation of TMT neurotoxicity but also a system for chemical induction of a specific lesion in the granule cell layer of the hippocampus and should allow future behavioral studies to elucidate the toxic impacts of TMT as well as the function of the granule cells in the hippocampal formation. (Partially supported by an Institutional Research Grant from the University of Arkansas for Medical Sciences.)

52.4 MEMBRANE AND CYTOPLASMIC FACTORS IN NITROSOUREA SENSITIVITY OF HUMAN GLIOMA CELL LINES IN VITRO. B. H. Smith, M. Vaughan*, P. L. Kornblith*, M. A. Greenwood*, A. Robinson*, N. Shitara*, and P. E. McKeever*, SPON: (J. Kebabian). Surgical Neurology Branch, NINCDS, NIH, Bethesda, MD 20205.

Nitrosourea-sensitive and resistant glioma cell populations have been defined utilizing a microtiter chemotherapeutic agent assay (Kornblith, Smith, and Leonard, 1981). The basis of this clinically significant difference in sensitivity is not known. The mechanism by which nitrosoureas act to kill tumor cells has been thought to be alkylation and DNA-strand crosslinking. In an effort to determine whether non-nuclear factors may be involved in the sensitivity or resistance of human glioma-derived cell lines to nitrosoureas, we have used a combination of microcinematographic and scanning and transmission electron microscopic and electrophoretic methods to study the interaction of 1,3-bis(2 chloroethyl)-1-nitrosourea (BCNU) with neoplastic glial cell lines LM (sensitive) and NN (resistant) derived and maintained in our laboratory.

Marked bleb formation and cell retraction were observed to occur in a dose-dependent relationship (at 15μg/ml BCNU) within minutes of BCNU addition in sensitive cells (90%) but not resistant cells. The surface changes reflect major cytoskeletal rearrangement at a time too early to be accounted for by known interstrand DNA crosslink mechanisms and suggest that cytoplasmic and/or membrane events may be significant in the cytotoxic action of nitrosoureas. Sensitive glioma cells show more rapid uptake and slower release of ^{14}C-BCNU and its metabolites as well. Seven and one half percent SDS-polyacrylamide gel electrophoretic studies of ^{14}C-BCNU binding are now being utilized to study cytoplasmic binding in resistant and sensitive cells. Such studies may be useful in determining and modifying abnormal glial growth control mechanisms.

52.5 AMYLOID PLAQUES IN EXPLANTS OF SCRAPIE MOUSE BRAIN. G.S. Merz* P.A. Merz* and H.M. Wisniewski (Spon: G.Y. Wen) Dept. of Pathological Neurobiol., Inst. for Basic Res. in Mental Retard., Staten Island, N.Y. 10314

To unravel the origin of amyloid plaques of the type associated with dementia and aging we examined similar plaques in IM mice infected with scrapie strain 87V. The plaques are a prominent pathological feature especially in the needletrack left by intracerebral inoculation. Our first step has been to explant the needletrack into tissue culture and follow the fate of the plaques in vitro. Needletrack with adjacent cortical and subcortical tissue was aseptically removed from the brain, embedded in 3% agarose in culture medium and cut into 200 μ slices roughly perpendicular to the needletrack. Slices were mounted in a Maximov slide assembly on collagen coated coverslips. Extensive neuronal autolysis and tissue necrosis was evident for the first few days in vitro. Later, there was extensive outgrowth of fibroblasts, macrophages and other, unidentified cell types. Numerous cell types also populated the tissue slice. Addition of congo red to the culture medium revealed (within 24 hr.) amyloid plaques by their characteristic green-red-birefringence in polarized light. By electron microscopy the overall appearance of the plaques was that of a loose deposit of amyloid with no discernable core structure. The fibers were typically unbranched, straight and 10 nm in diameter. They were arranged in clusters and interspersed among cellular processes. Ultrastructural features suggested the cells associated with the plaque were predominately macrophages and microglia. Occasionally small numbers of amyloid-like fibers were seen in the cytoplasm of microglia. None were seen in macrophages. These features are essentially the same as those seen in vivo even though the plaques were cultured for over a month before fixation. Two observations suggest the in vitro deposition of amyloid. Two cultures devoid of birefringence for 7 days after addition of congo red subsequently developed areas of red/green birefringence. Second, a few cultures showed an increase in the mass of birefringence. Furthermore, in no case were plaques lost from a culture even in cases when they were followed for up to 80 days in vitro. Taken as a whole these observations suggest in vitro amyloid plaques and their associated cells represent a useful approximation of in vivo circumstance and should provide a useful approach to the origins and evolution of CNS amyloid.

52.6 NEURAL DIFFERENTIATION AND REGULATION OF MEASLES VIRUS REPLICATION. Carol A. Miller* and Michael C. Graves.* University of Southern California School of Medicine, Department of Pathology and University of California, Los Angeles, Department of Neurology. Los Angeles, CA 90033

The state of development and differentiation of the CNS has been shown to affect replication of many neutropic viruses. Subacute sclerosing panencephalitis, a persistent virus infection of the CNS in children, has been directly correlated with measles virus infection prior to age two. To examine the role of neural differentiation in measles virus replication, N_2A cells, a clonal murine neuroblastoma line, and TE671 cells, a human medulloblastoma culture, were incubated for 3 days with medium containing 2.5 ug/ml papavarine, an inhibitor of cyclic AMP phosphodiesterase, infected with measles virus, and refed with the agent. Neural-specific differentiation was assessed by quantifying neurite formation, and either acetylcholine esterase or choline acetyltransferase activity. Intracellular cyclic AMP levels were determined by radioimmunoassay. Papavarine-treated cells manifested a marked reduction ($>10^5$ pfu/ml) in release of infectious virus compared to control cultures. Treated cells manifested elevated levels of cyclic AMP, over a 15-20% increased numbers of neurites, and in TE671 cells, a ten-fold elevation of neural specific enzyme activity. Removal of papavarine two to seven days post-infection resulted in release of infectious virus, reaching maximal control levels within 48 hours. Changes in viral replication are neural specific, and did not occur in companion treated monkey kidney cells (CV-1, or Vero). Using immunofluorescent staining, markedly decreased amounts specifically of the viral Matrix (M) protein were seen in treated cultures. The role of cyclic AMP in mediating regulation or modification of viral proteins will be discussed. These results suggest that host cell factors, possibly including cyclic AMP may be significant both in converting an acute infection in the CNS into a persistent one, as well as the reactivation of a latent infection.

52.7 OPIATE RECEPTORS ON CULTURED OAT-CELL CARCINOMA CELLS REGULATE ADENYLATE CYCLASE. C.B. Pert, S. Gentleman*, U.K. Schumacher*, D. Carney*, A. Gazdar* and J. Minna*. National Institute of Mental Health and National Cancer Institute, Bethesda, MD 20205

About 25% of all lung cancers are of the small cell (SCCL) type, a rapidly growing lung tumor which metastasises early and widely, often to the brain. Recent studies of established lines which retain a constant genetic composition have revealed the presence of high dopa-decarboxylase levels and neurosecretory-like vesicles. Like other APUD cells, small cells or oat cells, as they are often called, manufacture hormones and neuropeptides. For example, the tetradecapeptide bombesin appears to be a highly selective marker for oat-cell carcinoma since it was present in 17 out of 17 oat-cell lines examined but was not present in 8 non-oat-cell lung tumors of various types (Moody et al., in preparation). In membranes derived from at least one cell line (NCI-H69), the β-receptor agonist, isoproterenol (5 μM) caused a two-fold stimulation of adenylate cyclase activity, an effect completely reversible by 10 μM propranolol. A significant reversal of the isoproterenol-induced stimulation of adenylate cyclase was produced by the opiate receptor ligands morphine (1 μM), D-Ala^2-D-Leu^5-enkephalin (50 nM), β-endorphin (0.5 μM), ethylketocyclazocine (10 nM) and etorphine (10 nM). These opiate effects could be reversed by opiate antagonists. [^{125}I]D-Ala^2-D-Leu^5-enkephalin bound specifically to oat-cell membranes. The binding could be displaced by the following opiate receptor ligands, in order from the most to the least potent: etorphine, leu-enkephalin, met-enkephalin, D-Ala^2-D-Leu^5-enkephalin, ethylketocyclazocine, β-endorphin and morphine. While specific [^{3}H]dihydromorphine binding on oat-cell membranes was undetectable, selective ion effects on specific [^{3}H]D-Ala^2-D-Leu^5-enkephalin were clearly measurable. Oat cells appear to have the "primitive" opiate receptor, which, "fixed" in the adenylate-cyclase-coupled "δ" conformation, is unable to assume the μ state. Therapeutic strategies to exploit the presence of high-affinity opiate peptide binding sites on oat cells might include the use of [^{131}I]-labeled opiate peptides or the use of opiate peptides coupled to other cell poisons for precise target delivery.

53.1 DIFFERENTIAL ACTIVATION OF LIMBIC AND CORTICAL DOPAMINERGIC SYSTEMS TO STRESS AND ANTICIPATION OF STRESS. J.P. Herman*, D. Guillonneau‡ R. Dantzer‡ P. Mormède‡ H.Simon *and M. Le Moal. Lab. Neurobiol. des comportements, Univ. Bordeaux, Bordeaux France.

Dopaminergic (DA) terminals of the mediofrontal and cingulate cortices and of the nucleus accumbens (AC) has been described to be activated by severe stress, such as electrical foot-shocks or immobilization. In the present study we extended these investigations to other terminal areas of the mesolimbic and mesocortical systems using two sort of stress : electrical shocks and the placing of the animal in a new environment. Also in view of the participation of some of these structures in cognitive functions, we tested whether the anticipation of a stress would affect their DA activity. Dopaminergic activity was followed in the mediofrontal cortices, olfactory tubercle, AC, striatum, septum and amygdaloid complex of the rat by measuring their DOPAC content. Activation of the pituitary-adrenal axis was evaluated by measuring plasma corticosterone levels. Placing the rats in a new environment for 20 min augmented the DOPAC level of the AC accumbens by 34 %, while no change could be detected in the other structures tested. Plasma corticosterone level went up to 447 ng/ml. On the other hand a 20 min electrical foot-shock session (650 shocks, 1.5 mA, 180 msec) activated the dopaminergic terminals of the mediofrontal and sulcal cortices, nucleus accumbens and amygdaloid complex (DOPAC levels 168, 148, 150 and 175 % resp. compared to controls) in rats previously habituated to the shock-chamber, while plasma corticosterone level augmented by 270 % (control : 159 pg/ml) The same changes were observed in the rats shocked on two consecutive days and sacrified after ten second shock session. On the other hand, in rats placed without shock in the chamber in which they were shocked on the previous day only the mediofrontal cortex reacted by significant elevation of its DOPAC content. This elevation was less than that seen in the rats shocked twice + 35 vs 65 % ; the increase in plasma corticosterone level was diminished (+ 149 vs. + 265 %).

53.2 EFFECTS OF 6-OHDA LESIONS TO THE FRONTAL CORTEX, NUCLEUS ACCUMBENS OR CORPUS STRIATUM ON AMPHETAMINE-INDUCED BEHAVIOR AND ACQUISITION OF THE CONDITIONED AVOIDANCE RESPONSE. H. Simon*, M. Le Moal and G.F. Koob. Lab. Neurobiologie des comportements, Univ. Bordeaux, Bordeaux France and A.V. Davis ctr. Behavioral Neurobiology, Salk Inst., San Diego, Calif. 92138.

Male, Sprague-Dawley rats were subjected to 6-hydroxydopamine (6-OHDA) lesions of three terminal areas of the midbrain dopamine (DA) system : frontal cortex (F.C.), N=8, Region of the Nucleus Accumbens (N.Acc) N=10, and the Corpus Striatum (C.S.), N=10,in order to determine the specific DA terminal region responsible for deficits in acquisition of the conditioned avoidance response (CAR) observed following treatment with DA receptor blockers. Lesions were made by infusing 8 µg (base) of 6-OHDA dissolved in 2 µl of vehicle (0.1 mg/ml ascorbic acid in saline) through a 30 gauge needle over a 8 minute period. Sham operated rats, N=10, received vehicle alone. Rats were allowed 10 days to recover from surgery and then were tested in photocell cages for motor activation induced by d-Amphetamine (AMPH), 1.0 and 4.0 mg/kg, S.C., Photocell beam breaks were counted every 10 min. and frequency observations of stereotyped behavior (sniffing, head down, rearing, locomotion, grooming, licking) were made every 20 min. As previously reported (Kelly, Seviour and Iverson, BRAIN RES. 94,1975), lesions to the N. Acc differentially blocked the locomotion induced by AMPH, but not the more intense stereotyped behavior. In contrast, C.S. lesions blocked the more intense stereotyped behavior, but not the locomotor activity. F.C. lesions had no effect on locomotion or stereotyped behavior. Subsequently (days 18-24 post-lesion), the rats were trained in a one-way CAR in a shuttle box as described by Beninger et al.,(JPET 213, 1980). The rats with N. Acc. lesions showed a significant, but weak impairment of acquisition of the CAR. The F.C. and C.S. rats showed no impairment relative to the shams. A fifth group of rats subjected to a combined 6-OHDA lesion to both the region of the N. Acc. and C.S. were transiently aphagic and adipsic, but regained feeding within 10 days post-lesion following daily intubations and access to a sweetened diet. These rats showed no response to either dose of AMPH and were more severely impaired in the acquisition of the CAR. These results suggest that a severe deficit in the CAR requires the destruction of the whole midbrain DA system, and that both the mesolimbic and nigrostriatal DA systems act in concert to produce response initiation to important environmental events.

53.3 MONOAMINE CHANGES FOLLOWING CONDITIONED EMOTIONAL RESPONSE. P.K. Burns*, M.P. Sands*, D.R. Cherek, J.E. Smith and J.D. Lane (SPON: J.L. Steinberg). Psychiatry Research Unit, Department of Psychiatry, Louisiana State University Medical Center, Shreveport, LA 71130.

Conditioned emotional response (CER) is thought to be an animal model for anxiety. CER has frequently been used to evaluate behavioral-drug effects, with anxiolytics clearly attenuating the emotional response. To evaluate the neurochemical changes associated with this paradigm, groups of three littermate Fisher F-344 rats were food deprived and shaped to lever press in a standard operant chamber. Lever pressing was maintained by a variable interval (VI) 1 min schedule of food presentation. Following stabilization of responding, one hour classical conditioning sessions were initiated. Classical conditioning consisted of presenting conditioned stimulus (tone) of varying lengths (2 min-10 min) and unconditioned stimulus (foot shock, 1mA, 500 msec) approximately 8 times during each session. UCS was presented at the end of CS. One rat of each group received this classical conditioning for 5 sessions. To isolate the conditioning component from the shock history and animal activity, the other two littermates received yoked CS (tone) or USC (shock) only. On test day, the CS was presented to the triads for 15 minutes while lever pressing was maintained by the food presentation schedule, after which the animals were totally frozen and stored at -70°C until analysis. The pre-tone and post-tone responding on the VI1 schedule were compared. The CER animal responded very little (<<1 res/min) after tone presentation, while both controls continued at pre-stimulus rates (circa 15 res/min). Brains were dissected into discrete cortical and sub-cortical areas and the content and utilization of biogenic amines and amino acid neurotransmitters determined. There were very few changes in content of neurotransmitters or their metabolites, suggesting that small functional pools were being utilized. Comparison of shock history (shock only versus tone only) revealed predominantly decreases in turnover of DA, NE and 5-HT in multiple areas. Comparisons of the conditioning/emotional component (CER versus shock only) revealed a general increase in turnover of DA and NE in multiple areas and mixed changes in turnover of DA and 5-HT in limbic versus motor areas. These data are consistent with CER being a model for studying emotional behavior in the presence of an aversive stimulus, and suggest roles for biogenic monoamines in modulating the behavior. (supported by USPHS Grant MH-31835).

53.4 STRIATAL DOPAMINE RELEASE IN FREELY MOVING RATS DURING COLD STRESS. Richard W. Keller, Jr., Michael J. Zigmond, and Edward M. Stricker, Department of Biological Science, University of Pittsburgh, Pittsburgh, PA 15260

Animals sustaining dopamine (DA)-depleting brain lesions show impaired behavioral responses to homeostatic challenges. Nonetheless, most previous attempts to measure increases in striatal DA turnover during such stressors have failed, possibly because the changes are too transient to be detected by conventional biochemical means. We have employed voltammetric electrodes implanted in striatum to monitor extracellular DA concentration during cold stress. Carbon paste-epoxy electrodes (150 um) were prepared according to the method of Conti et al. (Life Sci. 23: 2705, 1978) except that dodecyl sodium sulfate was added to the carbon paste mixture. As a result of this treatment, and the choice of oxidizing voltage (+0.5V), the electrodes were observed in vitro to be 4 times more sensitive to DA than to two other potentially interfering compounds, dihydroxyphenylacetic acid and ascorbic acid. Electrodes were stereotaxically implanted in 200-300 gm male, Sprague-Dawley rats. Four carbon paste-epoxy electrodes were placed bilaterally in striatum. A Ag/AgCl reference electrode and an auxiliary electrode were placed in contact with cortex. Animals were allowed at least 48 hr to recover and then tested for up to 3 months. During testing electrical connection was made via a cable and commutator. Chronoamperometric measurements were made on each electrode every 4 min by applying a +0.5V pulse of 500 msec duration and averaging the current during the last 30 msec. This average current was proportional to DA concentration in vitro and was used for calibration and measurement. The ability of electrodes to monitor endogenous DA release was further suggested by their responsiveness to amphetamine (5 mg/kg, i.p.), a drug which releases DA but is not itself detected by our electrode. Following a 1-hr period of stabilization, animals were placed in a 3-cm ice water bath. In 15 of 20 cases, cold stress produced an increase in current which was maximal by 1-4 min (the initial measurement). This signal had usually decayed significantly by the second or third measurement (4-8 min later) but did not return to baseline during the 45-min cold exposure. We observed a briefer increase in signal when animals were removed from the ice bath, and still smaller increases when rats were transfered from one cage to another. All environmentally-induced increases in current were completely blocked by the administration of α-methyl-p-tyrosine (125 mg/kg, i.p.) 4 hr before testing. These results suggest that DA is released in striatum soon after exposure to external stimulation. (Supported by NIMH grant 29670.)

53.5 SHORT- AND LONG-TERM EFFECTS OF UNCONTROLLABLE STRESS ON EXPLORATORY PATTERNS. V. Bruto* and H. Anisman. Department of Psychology, Carleton University, Ottawa, Ontario, Canada.

Unlike the behavior of animals that had been exposed to escapable shock, animals that had received yoked inescapable shock exhibit severe deficits of later escape behavior. We similarly observed that only the latter treatment disrupted the pattern of exploration in a free-running 8-arm radial maze. Specifically, naive mice or mice that had received 60 escapable shocks (150 μA) exhibited a systematic pattern of exploration in which they tended to visit those arms that had least recently been explored (spontaneous alternation). Moreover, a prominent response sequence consisted of successive visits to immediately adjacent arms. Exploratory patterns did not appear to be related to, or influenced by, motor activity. In contrast, in mice that had received inescapable shock, the pattern of exploration was altered, and appeared to progress in a haphazard fashion. Although the altered exploratory pattern was transient, being absent 24 hours after inescapable stress, it could be re-induced if mice were re-exposed to just 15 shocks prior to test. Among naive mice the 15 shock treatment was without effect. The inescapable shock treatment was found to provoke a transient reduction of NE in several brain regions, and like the behavioral deficits could be re-induced by limited stress re-exposure. Finally, pharmacological manipulations that depleted DA and/or NE successfully mimicked the effects of inescapable shock. It is suggested that in addition to deficits of response initiation and maintenance, uncontrollable stress provokes a short-term alteration in the way in which animals attend to or respond to environmental stimuli. These behavioral changes might be subserved by the effects of stress on catecholamine activity, and long-term behavioral deficits might be due to conditioning or sensitization of the mechanisms governing catecholamine activity.

53.6 HABITUATION OF AGGRESSION IN MICE: EFFECT OF BLOCKING SEROTONIN TRANSMISSION. J.T. Winslow* and K.A. Miczek (SPON: P.B. Dews). Dept. Psychology, Tufts Univ. Medford, MA 02155.

The decline of aggression during consecutive episodes of fighting appears to be a habituation phenomenon. Previous experiments showed that the frequency of biting and threats by male mice on conspecific intruders declined in the course of ten 5-minute confrontations distributed over 2 h. Aggression returned to about 50% of original levels when a new intruder was introduced, indicating dishabituation, and fully recovered within about 1 h. We reported earlier that d-amphetamine selectively blocked the decline of aggression suggesting an activating role for catecholamines on aggression, (Neurosci. Abstr.,1980, 112, 42.5). Serotonin systems may mediate inhibition of behavior by inhibiting catecholamine activation. The objective of our experiments was to describe the effect of interfering with serotonin transmission on the decrement of aggression in repeated confrontations between mice. We investigated the effect of blocking serotonin transmission with methysergide, p-chloroamphetamine, and lisuride on agonistic and non-agonistic behavior in mice. Aggressive behavior was generated in a male mouse, housed with a female, by introducing an unfamiliar group-housed male mouse into the home cage.

Methysergide (1,3 10mg/kg, IP), a serotonin receptor blocker, produced a dose dependent reduction of all behaviors measured and did not selectively affect aggressive behavior. Parachloroamphetamine (10mg/kg, IP), a serotonin synthesis inhibitor, increased attack, threat, and locomotion only 24 hrs. after injection when its neurochemical effects were producing a non-specific increase of endogenous levels of 5HT and CA; all behaviors returned to baseline levels 1 and 2 wks later, when PCA is selectively blocking serotonin synthesis. Lisuride (0.03, 0.3, 1.0 mg/kg, IP), like LSD, blocks indices of central and peripheral serotonin activity, but may also have properties of a dopamine agonist; this drug dose-dependently reduced both agonistic and non-agonistic behavior. Residents became hyperreactive: frequently assuming defensive postures and squealing at the approach of the intruder. A significant increase in the number of aggressive intruders was observed during treatment of the resident mice.

The data indicate that aggression in mice is not modulated by a dual system of catecholamine activation and serotonin inhibition. Although catecholamine does appear to be involved in the activation of aggression, blocking serotonin transmission did not result in release from inhibition. Suppression of behavior was observed, possibly as a consequence of peripheral effects of serotonin blockade.

54.1 POSTNATAL BEHAVIORAL EFFECTS OF ETHANOL CONTINUOUSLY ADMINISTERED TO MICE IN UTERO. D. M. Gilliam and A. C. Collins* (SPON: W. Proctor). Inst. for Behavioral Genetics, Univ. of Colorado, Boulder, CO 80309.

There have been few previous reports of behavioral and developmental measures taken on animals that were continuously exposed to ethanol in utero. The method used in the present study was devised to permit continuous chronic exposure of gestating mice to specific ethanol concentrations.

In the first experiment, gestating C3H mice received continuous infusions of 16.25±.82 g/kg/day ethanol via a surgically implanted jugular cannula for 48±6 hr starting at day 15±.5 of gestation. Fetal whole body ethanol concentrations (FBEC) and amniotic fluid ethanol concentrations were highly correlated with the mothers' blood ethanol concentrations. Fetal brain ethanol concentrations were 76% of FBEC.

In the second experiment, gestating C3H mice received continuous iv infusion of either ethanol (12.6±.7 g/kg/day) or saline during the final 8 days of gestation. Offspring were assessed neonatally for developmental and behavioral measures as described by Wahlsten (Brain Res., 72:251, 1974) or for open-field activity and ethanol preference as adults. Offspring of mice that received saline during gestation did not differ on any measure from offspring of mice that served as operated or unoperated controls. Offspring of ethanol-treated mothers showed hyperexcitability, muscular weakness, and evidence of delayed parturition. Prenatal ethanol treatment did not alter ethanol preference, but offspring of ethanol-treated mothers showed significantly higher open-field activity scores than did offspring of saline-treated mice.

54.2 EFFECTS OF CHRONIC ALCOHOL CONSUMPTION ON RAT MATERNAL BEHAVIOR. Freya A. Weizenbaum and William Hartigan, Jr.*. Dept. Psychology, Vir. Polytech. Inst. and S.U., Blacksburg, VA 24061.

Chronic alcohol treatment during pregnancy and continuing through lactation adversly affects rat pup growth and survivorship. Since pup development is dependent upon the integrity of maternal behaviors, it was hypothesized that the pup impairments would be associated with decrements in the maternal behavior of Alcohol-dams. Therefore, the purpose of the present study was to investigate the effects of chronic alcohol consumption on rat maternal behaviors. Alcohol treatment was initiated 40 days prior to pregnancy and continued till day 14 postpartum, when the experiment was terminated. On day 1 postpartum, litters were culled to 6 pups and the litters were cross-fostered either across treatment conditions, or within a treatment condition across dams. The offspring of Alcohol-dams that were paired with Alcohol-dams postpartally, showed a significant retardation in body weight and a higher mortality rate than Water-pups paired with Water-dams. As predicted, there were significant decrements in Alcohol-dam maternal behavior, measured in 15-minute tests on day 3 or 4, and on day 7 or 8 post-partum. Alcohol-dams showed significant increases in latency to contact and in latency to retrive Alcohol-pups. Also the number of Alcohol-pups retrived and the time in contact with the Alcohol-pups was significantly reduced. Furthermore retrival behavior, in the day 3 - 4 maternal test, was positively related to subsequent pup mortality. The poor performance of the Alcohol-dam in the maternal test was not due solely to alcohol consumption. Alcohol-dams that consumed equivalent amounts of alcohol, but which were paired with Water-pups postpartally showed a level of maternal behavior that was similar to that of Water-dams paired with Water-pups. Moreover, pup prenatal alcohol exposure was not a sufficient condition for impairing maternal-young interaction. Maternal behavior pup growth and pup surviorship in the Alcohol-pup/Water-dam group was similar to that of the Water-pup/Water-dam group. Thus the results of this experiment indicate that the post-partum deficits induced by alcohol are significantly influenced by both the mother and the pups, and that their contributions are synergistic rather than additive.

54.3 EFFECTS OF EARLY POSTNATAL ETHANOL CONSUMPTION IN THE RAT. T. Sonderegger, H. Calmes*, D. Colbern*, S. Corbitt*, and E. Zimmermann. Dept. Psychol., U.Nebr.-Lincoln, Lincoln, NE. 68588; Dept. Anat. and BRI, Sch. Med., UCLA, Los Angeles, CA. 90024

Separation of the effects of malnutrition from those of ethanol present a serious methodological problem in animal studies of the fetal alcohol syndrome. This problem has been studied in the rat during the gestation period using low doses of ethanol but not in the postnatal period while the brain is still developing.Thirteen litters of Charles Rivers CD albino rats were adjusted to sizes of 8 pups on Day 1 after birth. Pups were randomly assigned to one of three groups: ethanol (E); Sustagen (S), or handled (H). From Days 2-9, E pups were fed doses of 20% ethanol, with doses increasing from 0.8 to 1.2 mg/g, twice daily. The ethanol was in a 20% Sustagen (Mead Johnson) solution; S animals received equal volumes of isocaloric Sustagen; H animals were handled. (A study on another group of rat pups similarly treated showed high levels of blood alcohol (75% of original feeding level) 4 hours after the feeding.) Of the original 101 pups, 99 survived. Same sex body weights did not differ among postnatal treatment groups during treatment nor at subsequent weighings on Days 21,49, 147, or Day 180, showing that malnutrition, as indicated by body weight deficits, was not present. Activity measures, obtained beginning on Day 49, did not differentiate among postnatal treatment groups although females were more active than male counterparts. Between Days 116-147, some animals from each group were tested for the pituitary-adrenal stimulating effects of ethanol. Plasma samples obtained 30 min. after an injection of ethanol (2 mg/kg/ip) were assayed fluorometrically. Steroid levels were elevated and within the expected ranges for the sex of an animal similarly treated with ethanol but same sex postnatal treatment group levels did not differ. On Day 180, females from each group were anesthetized and implanted with chronic electrodes aimed at the medial forebrain bundle. Animals from the E,S,and H groups learned to bar-press for rewarding electrical brain stimulation. Current thresholds and bar-ressing rates were comparable, suggesting unimpaired neural substrates of reinforcement. In this study, low doses of ethanol did not cause behavioral, neuroendocrine, or growth impairment when malnutrition effects (body weight deficits) were controlled.

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54.4 THE EFFECTS OF ADRENOCORTICOTROPIN AND ADRENALECTOMY ON VOLUNTARY ETHANOL INTAKE IN MICE. M. J. Kassam* and J. N. Pasley* (SPON: R. E. Stull). Dept. of Physiology and Biophysics, Univ. Ar. Med. Sci., Little Rock, AR 72205.

Recently, it has been suggested that adrenal glucocorticoids play a permissive role in the development of both tolerance to and dependence on ethanol (Sze, Drug and Alcohol Dependence 2:318, 1977; Wood, Psychopharmacol 52:67, 1977; Tabakoff and Yanai, Psychopharmacol 64:123, 1979). This study was designed to examine the effect of adrenocorticotropin (ACTH) treatment on voluntary ethanol ingestion in intact and adrenalectomized C57/BL6 mice, a high ethanol preferring strain. All mice were adult males, singly caged and maintained at 25° C on a 12:12 LD cycle with Purina Laboratory Chow *ad libitum*. Animals were divided into eight groups of eight mice each. Four groups remained adrenally intact and received either 4 units ACTH or gelatin vehicle injections. Within a given injection protocol, mice were further divided to be tested for selection of 5% or 10% ethanol. The treatments for the four groups of adrenalectomized mice were arranged in the same manner. The adrenalectomized mice were allowed a one week recovery period before testing and were maintained on daily i.m. injections of 50 µg cortisol acetate (Upjohn). Drinking bottles fitted with ball bearings were presented in a three bottle-two choice paradigm; one calibrated drinking bottle contained tap water, another contained ethanol in tap water; and the third bottle was empty. Individual ethanol and water consumption were measured daily between 0900 and 1100 hrs with bottle position also being changed daily. Daily subcutaneous injections of either 4 units ACTH (Cortigel-40, Savage) or the gelatin vehicle also occurred between 0900 and 1100 hrs. At the end of the 21 day injection period, all animals were killed by cervical dislocation. Thymus and adrenal glands were weighed to the nearest 0.0001 gram. Trunk blood was collected for later determination of corticosterone levels in serum by RIA. Adrenalectomized mice, whether or not treated with ACTH,demonstrated a decrease in ethanol selection during the injection period, as opposed to intact mice, in which case an increase in ethanol selection was observed, independent of ACTH treatment ($p<0.05$). Food intake did not differ with treatment. Adrenal and thymus weights reflected the various treatments to which the animals were exposed. The results argue against a direct role of ACTH in ethanol drinking but support the notion of adrenal gland involvement in ethanol preference in mice.

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54.5 P3 AMPLITUDE DIFFERENTIATES SUBJECTS WITH AND WITHOUT A FAMILY HISTORY OF ALCOHOLISM. R. Elmasian, H. Neville, D. Woods*, M. Schuckit*, and F. Bloom*. Salk Institute, San Diego, CA 92138.

Genetic background has long been suspected as a factor in alcoholism (Goodwin, D.W., Arch Gen Psychiatry, 36: 57-61, 1979). Only recently, however, has the correlation between family background and the development of alcoholism been supported by physiological findings (e.g. Schuckit, M.A. and Rayses, V., Science, 203: 54-55, 1979). We studied event related potentials (ERP's) to further test the hypothesis that ethanol might have different effects on neural processes associated with cognitive functioning in subjects with and without a family history of alcoholism (Schuckit et al, in preparation). Family history positive subjects (FHP - those with alcoholic parents or siblings) were matched on height-weight ratio and personal drinking history with family history negative (FHN) subjects. None of the subjects were themselves alcoholic so that the effects of past alcohol abuse would not be confused with possible risk factors. Before receiving ethanol, the P3 response generated when subjects detected a rare 75 msec tone burst in a background of 300 msec tone bursts was similar in amplitude (about 13 microvolts; recorded vertex to mastoid) and latency (about 360 msec) for the two groups. After .56 g/kg ethanol P3 latency increased to 490 msec in both groups. Although P3 amplitude was essentially unchanged in the FHN group, it was reduced to less than 60% of baseline amplitude in the FHP group. The different effects of ethanol on P3 in the two groups can be observed in individual subjects and have been replicated in another experiment (Neville and Synder, in preparation). The P3 differentiation of FHP subjects from FHN subjects is theoretically important since it is the first direct physiological evidence that ethanol differentially affects cognitive and related neural functions in those at high risk for developing alcoholism (FHP subjects) and those at low risk (FHN subjects). This research is supported by PHS AA 07273, NIAAA 03504, and the Medical Research Service (Special Biology of Alcoholism Grant) of the Veterans Administration.

54.6 AN INVESTIGATION OF THE INTERACTION BETWEEN THE REINFORCING PROPERTIES OF FOOD AND ETHANOL USING THE CONDITIONED PLACE PREFERENCE PARADIGM. R.B. Stewart* and L.A. Grupp, Dept. Pharmacology, Univ. of Toronto, Toronto, Canada M5S 1A8.

Ethanol can maintain self-administration behaviour (e.g., Smith & Davis, Physiol. Psychol. 4: 91, 1974) and therefore has positive reinforcing properties and also produces a conditioned taste aversion (e.g., Lester et al., Quart. J. Stud. Alc. 31: 578, 1970) and therefore has aversive properties. Thus, different environmental factors and contingencies may interact in very different ways with ethanol to determine how its administration affects behaviour. In order to assess one such possible interaction, we used the conditioned place preference paradigm and compared the behavioural effects of ethanol alone to those when it was combined with the availability of food.

Rats were maintained at 85% of their free feeding weights. In the Ethanol only condition every second day was an ethanol trial and three groups of rats were given intraperitoneal (i.p.) injections of 250, 500 or 1000 mg/kg ethanol and placed in a distinctive box for 30 min - half of each group in the white box and half in the black box. Intervening days were saline trials and the animals received i.p. saline injections and put in the opposite coloured box. In the Ethanol and Food condition a second set of three groups were treated identically to the Ethanol only groups with the addition that food was available in both boxes during the ethanol and saline trials. After four ethanol and four saline trials a test trial without injections or food was given where the animals shuttled freely for 15 min between a black and a white compartment. Relative preference was assessed by comparing the time spent in the two compartments.

In the Ethanol only condition the 250 and 500 mg/kg groups spent equal time in both compartments (t_7=1.6, N.S.; t_7=0.32, N.S.) while the 1000 mg/kg group showed a significant aversion to the side paired with ethanol (t_7=6.5, $p<.01$). In the Ethanol and Food condition the 250 mg/kg group again showed no preference or aversion for either compartment (t_7=1.05, N.S.), but, in contrast to the Ethanol only condition, the 500 mg/kg group showed a significant preference for the side paired with ethanol and food (t_7 =2.95, $p<.05$), and the 1000 mg/kg group spent equal time in both compartments (t_7=0.24, N.S.).

These findings indicate that ethanol does interact with other reinforcing stimuli in its environment in a way that cannot be predicted from its effects when presented alone. Thus the observed interaction with food at the 500 and 1000 mg/kg doses appeared to be one that could not be predicted by simply adding the individual effects of food, ethanol and saline.

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54.7 LOW LEVEL HYPERBARIC ETHANOL ANTAGONISM: TIME COURSE. R.D. Malcolm*, D.A. Finn* and R.L. Alkana. Insitute for Toxicology, School of Pharmacy, University of Southern California, Los Angeles, CA 90033.

Our recent studies have demonstrated that exposure to environments of 4-12 atmospheres absolute (ATA) of helium-oxygen (He-Ox) beginning immediately after ethanol injection causes a pressure and dose-related antagonism of ethanol narcosis in mice. The present investigation examined the effect on hyperbaric ethanol antagonism of pressurizing at different time points after ethanol administration. Drug-naive, male C57 mice were injected i.p. with 3.6 g/kg ethanol (20% w/v). Following loss of their righting reflex, they were placed in hyperbaric chambers. Time course mice were kept in a chamber atmosphere of 1 ATA air for 0, 5, 10 or 20 minutes before pressurization to 12 ATA He-Ox. Other groups of mice were kept at 1 ATA air throughout testing (controls) or switched to 1 ATA He-Ox at times paralleling pressurization of the time course mice. Upon wake-up, the chamber was decompressed and whole brains were removed and prepared for subsequent gas chromatographic determination of brain ethanol concentration (BEC). Immediate post-ethanol exposure to 12 ATA He-Ox significantly reduced sleep-time and increased wake-up BECs. Delaying the onset of hyperbaric exposure did not reduce the antagonistic effect. Similar significant reductions in sleep-time and increases in wake-up BECs were seen in mice pressurized 5-20 minutes after ethanol administration. The 1 ATA He-Ox mice did not display a reduced sleep-time or increase in wake-up BECs. The similar antagonistic efficacy of immediate and delayed pressurization indicates that there is not a critical time period during which hyperbaric pressure must be applied in order to induce antagonism and suggests that hyperbaric induced alteration of ethanol distribution to the brain does not represent an important factor in mediating the antagonism. These findings are in accordance with membrane expansion/fluidization theories of anesthesia. (Supported by NIAAA Research Grant AA03972).

54.7 ACUTE EFFECTS OF ETHANOL ON LIMBIC AFTERDISCHARGES. Henry Lesse. Department of Psychiatry and Brain Research Institute, School of Medicine, University of California, Los Angeles, Calif. 90024.

Acute effects of ethanol on the initiation and maintenance of electrically induced limbic afterdischarges (AD) were studied in cats performing a bar pressing task. Current thresholds for evoking AD and AD durations in the amygdala, hippocampus and septal region were determined following alternating saline and ethanol administrations. Three doses (0.4, 0.8 & 1.6 Gm/kg; I.V.) were tested at 96 hr. intervals.

Ethanol-induced changes in limbic afterdischarges were found following low doses which produced neither observable changes in arousal state nor altered task performance. Elevations of hippocampal AD threshold followed the high dose and a biphasic effect was evident in some subjects after the low dose. By contrast, increases in amygdalar and septal AD threshold were induced by all test doses. This effect proved dose-related and greater in the amygdala than in the hippocampus (90% vs 12% following the high dose). In addition, ethanol induced dose-related reductions in AD duration which were greater in the amygdala and septal region than in the hippocampus. These effects were found when limbic AD were localized and also when fully developed motor convulsions were evoked. Restrictions in AD propagation to distant sites occurred during both early and late stages of seizure development. Time-course experiments at post-infusion intervals of 10 min. to 24 hrs. indicate that single doses of ethanol result in protracted changes in AD threshold and duration with biphasic effects beginning 8 - 16 hrs. after drug administration. These results suggest that ethanol has potent but differential acute effects in modifying the excitability of closely related limbic structures.

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54.9 PROMOTION OF STEREOTYPY BY ALCOHOL. L. D. Devenport, V. J. Merriman*, and F. A. Holloway. Dept. Psychiat. & Behav. Sci., Univ. Okla. Health Sci. Ctr., Oklahoma City, OK 73190.

Even at moderate doses, ethanol seems to have as one of its principal actions the narrowing of behavioral variability (Devenport et al., Alcoholism: Clin. and Exp. Res., in press). This is apart from any stimulant or depressant property it may possess. In order to exhibit this action more plainly and to assess its boundaries, the behavior of rats in an 8-arm radial maze (after Olton & Samuelson, J. Exp. Psychol.: Anim. Behav. Process. 2:97, 1976) was observed across alcohol doses of 0, .75, 1.5 and 2.0 g/kg (injected i.p. as a 10% w/v solution). The experiment was conducted with a view toward assessing 1) topographic variability, 2) spatial variability, and 3) sequential variability.

The four groups of male rats were food-deprived and placed individually in the central compartment of the radial maze with access available to all arms. Subjects were permitted 10 min to run from arm to arm or until they obtained 8 rewards, (90 mg Noyes pellets). This experiment was conducted with reward replacement. That is to say, rewards were replaced as the animal returned to the central compartment. In principle, a subject could obtain its allotted pellets by returning 7 times to the same arm. Three 10 min (or 8 pellet) trials were afforded in daily sessions. Throughout each trial, the number of separate arms visited (spatial variability), the order of visitation (sequential variability), and the number of alternative non-goal directed behaviors emitted (e.g., rearing, sniffing, etc; topographic variability) were recorded.

Except at the lowest dose, alcohol exerted a dose-related attenuation of all forms of behavioral variability: Fewer arms were visited, the sequence of visitation was more stereotyped, and the number of behavioral topographies was fewer.

Phase 2 of the experiment imposed a win-shift rule (pellets were not replaced) in an effort to bring stereotypy into conflict with formal task requirements. Not surprisingly, alcohol groups performed inefficiently, returning to previously visited arms. We conclude that alcohol broadly attenuates behavioral diversity. Performance on any particular task is likely to depend upon whether that task rewards stereotypy or variability.

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54.10 PRINCIPAL COMPONENT ANALYSIS OF THE EFFECTS OF ALCOHOL ON VISUAL EVOKED RESPONSES IN THE GENICULO-STRIATE SYSTEM. T.J. Willey, K.R. Erickson*, D.M. Riley*, A.F. Lawrence and J.M. Fuster. Department of Psychiatry and Brain Research Institute, School of Medicine, University of California, Los Angeles, Calif. 90024.

Flash evoked potentials were recorded with implanted electrodes from the lateral geniculate nucleus and striate cortex of two monkeys performing a delayed matching-to-sample task. The flash served on every trial as an alerting signal preceding the cue (sample). Electrical and behavioral records were taken before and after intravenous injection of control saline or ethyl alcohol (0.25, 0.5, 1.0, or 2.0 g/kg) with a remotely controlled Sage pump. A PDP-12 computer was used to process the data. Potential records following each flash were digitized and stored on disk for each of the 200 trials of an experiment. A principal component analysis was done on data from each electrode site and animal. Templates of evoked response were obtained by grouping data under all experimental conditions and by using potential measures in successive time points after flash as variables. The first five eigenvectors were computed and plotted. Correlations with sub-averages from the original data (in successive groups of ten events) were also computed and plotted for control, low dose (0.25 and 0.5 g/kg), and high dose (1.0 and 2.0 g/kg). An analysis of variance was performed comparing pre- and post-drug correlation coefficients. Control and low-dose categories showed little or no shift in correlation coefficients. Significant deviations from pre-drug correlation were seen at high drug levels in each site and monkey. One of the first two eigenvectors showed shifts in lateral geniculate nucleus, indicating action of alcohol in that nucleus or at a previous stage of the visual pathway. By contrast, the first three eigenvectors showed correlation shifts in striate cortex. This difference from geniculate data may have at least two possible explanations: a) separate populations of cortical neurons differing in onset of response to and recovery from alcohol, or b) alcohol effects on subcortical structures (e.g., brain-stem reticular formation) that, in addition to the lateral geniculate body, provide input to the striate cortex. (Supported by grant NIAAA 03513).

54.11 EFFECTS OF NALOXONE ON ETHANOL TOLERANCE AND INDUCED TASTE AVERSION IN MICE. D. Miceli, M. Ptito, P. Marfaing-Jallat and J. LeMagnen (SPON: P. Pépin). Lab. de Neuropsychologie Expérimentale, Univ. du Québec, C.P. 500, Trois-Rivières, Québec, G9A 5H7, Canada.

It has been suggested that alcohol and opiates share a common mechanism of action in the CNS. Some evidence in favor of or against the latter hypothesis has been based on the ability of naloxone, a specific opiate receptor antagonist, to counteract various ethanol-induced behavioral alterations. The aim of the present study was to examine 1) naloxone interaction with regard to initial and acquired tolerance to ethanol in mice using the rotarod test 2) the effect of pairing ethanol and naloxone on subsequent spontaneous ethanol consumption. The study was performed on 44 male C57BL mice aged 6-7 wks assigned to 4 groups (N=11). Each received starting at 10 a.m. and over 10 consecutive days (Days 1-10), paired IP injections of either ethanol (3 g/kg: E), saline (S) or naloxone (10 mg/kg: N); groups SS, SN, ES, EN followed 20 min later by rotarod tests. Nervous tolerance was measured by the number of falls at 2 and 3 min intervals. At 10 a.m. on the day following the last day of behavioral tests, mice were offered a two-bottle choice between ethanol (5% V/V) and water over the next 10 days (Days 11-20). Throughout Days 1-10, SS and SN animals showed virtually perfect scores indicating no effect of naloxone per se. An effect of alcohol was observed in groups ES and EN accompanied by significant development of tolerance. However, the number of falls recorded during Days 1-4 were significantly higher in EN compared to ES mice (respectively $\bar{X}\pm$S.E. 14.3±0.8, 14.2±1.1 and 9.8±1.8, 10.0±1.8 for Days 1-2 and 3-4; 2 min values). Such differences were no longer apparent thereafter, attaining respectively 6.2±2.0 and 5.7±2.0 on Days 9-10. During the ethanol-water choice period, no differences were observed between groups SS, SN and ES; (respectively 78±4, 77±3 and 71±2 % ethanol/total fluid intake: Days 11-12; 92±1, 95±2 and 93±4 %: Days 19-20) but significantly smaller quantities of ethanol were drunk by the group EN (respectively 34±10 and 43±13 %. Rotarod tests conducted on Days 22-23 after 3 g/kg ethanol showed a maintenance of tolerance enhancement in groups ES (5.1±1.7) and EN (5.6±1.9) compared to SS (12.3±1.4) and SN (12.9±0.9). The results demonstrated that naloxone produced 1) an initial reduction in nervous tolerance towards ethanol, which attenuated with increasing tolerance acquisition and 2) a strong and persistent aversion to ethanol flavor following IP pairings with the drug. Naloxone's initial potentiation of ethanol-induced behavioral deficits is consistent with its effect on ethanol-induced taste aversion and its non-specific and hypernociceptive action on aversive or stressful stimuli.

55.1 MECHANISMS OF BEHAVIORAL RECOVERY FROM LESIONS IN COCKROACHES: A TEST OF SUFFICIENCY. Noga Vardi and Jeffrey M. Camhi. Sect. Neurobiology & Behavior, Cornell Univ., Ithaca, NY 14850

Cockroaches turn away from wind gusts such as those produced by a predator's strike. The wind is detected by means of filiform hairs located on the cerci — two posterior appendages. If one ablates the left cercus, on most trials performed the next day, the cockroaches respond to wind from the left by turning incorrectly toward the left. After 30 days, however, the cockroaches make significantly more correct turns toward the right (Vardi and Camhi, Neurosci. Abst., 1979). In correlation with this behavioral recovery, we found physiological recovery among a group of individually identified cells, the giant interneurons (GI's), which are known to mediate in part the directional escape behavior. These GI's receive most of their input from the ipsilateral cercus. Thus, ablation of the left cercus renders most of the left GI's silent, whereas the right GI's remain responsive. However, after a 30-day period of behavioral recovery, the left GI's give somewhat enhanced responses to wind, specifically, they regain about 1/4 of their normal number of action potentials, which show normal directional selectivity.

We asked whether this partial GI enhancement was sufficient to account for behavioral recovery, or whether it was necessary to postulate further neuronal changes in the thoracic ganglia, where the GI's evoke responses in the leg motor neurons. We attacked this question by producing a situation in which the left GI's gave the same number of action potentials as after 30 days of recovery from left cercal ablation, but without having any recovery period. Specifically, we ablated only part of the left cercus such that the number of action potentials in GI's 1, 2, and 3, which are thought to be important in setting the initial direction of turning, would be comparable to the number observed in these GI's after behavioral recovery from full left cercal ablation. The turning responses of the partially ablated cockroaches were tested the next day. The mean angle of turn, in response to wind from 140 to 180° left (180° = head-on wind), was 22° to the right and the percentage of correct turns (i.e., toward the right) was 71%. This behavior is remarkably similar to that of the behaviorally recovered animals whose mean angle of turn is 23° right and whose percentage of correct turns is 73%. Thus we conclude that the number of action potentials in the left GI's 1, 2, and 3, or other parameters correlated with it in these or other ascending neurons, is sufficient to account for the behavioral correction which occurred during the 30-day recovery period. No change in the readout of the ascending information by thoracic motor centers need be postulated to account for behavioral recovery.

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55.2 ADJUSTMENT OF ESCAPE TURNING BEHAVIOR FOLLOWING UNILATERAL CERCAL ROTATION IN THE COCKROACH. Christopher M. Comer and Jeffrey M. Camhi. Sect. Neurobiology and Behavior, Cornell University, Ithaca, NY 14850

Cockroaches (Periplaneta americana) turn away from small puffs of wind, such as those generated during the strike of a natural predator. These turning responses are believed to be mediated by giant interneurons (GIs) in the abdominal nerve cord. The wind receptors are filiform hairs oriented along the cerci — a pair of abdominal appendages.

The behavioral plasticity exhibited by this system (Vardi & Camhi, Neurosci. Abs. 1980; Volman, Camhi & Vardi, Neurosci. Abs. 1980) indicates that a cockroach can compensate for a gross imbalance of activity in the two cercal sensory nerves. Within 30 days, nymphal cockroaches correct turning mistakes induced by either ablating or merely covering up one cercus. Behavioral recovery in both instances is corelated with changes in GI wind responsiveness.

We were interested in determining if cockroaches could also adjust to changes in the pattern of activity in the cercal sensory nerves. To investigate this question, we filmed the wind-mediated escape responses of animals which had one cercus misaligned. Each cercus normally extends caudally from the abdomen at an angle of about 60° from the long axis of the body. In a group of nymphs, we rotated the right cercus medially and glued it in a position nearly parallel to the body axis. This causes directionally specific mistakes: most winds from the right elicit appropriate turns to the left, however, frontal winds within 30° right of midline elicit inappropriate turns to the right. Two and 3 weeks later the animals behaved similarly. At 4 weeks, though, these insects made a significantly greater percentage of left (correct) turns in response to right frontal winds (χ^2 test, $p < .02$) than they had initially. Our analysis suggests that this 'correction' corresponds to a directional shifting of escape behavior such that the angular extent of the frontal region from which winds elicit wrong turns becomes smaller.

Preliminary electrophysiological studies indicate that unilateral cercal displacement causes a rotation of the directional wind-receptive fields of some GI's which parallels the deviation of the cercus. Studies of the GI's in 'corrected' animals are now in progress.

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55.3 DIRECTIONALITY OF ACOUSTIC ORIENTATION IN FLYING CRICKETS. G.S. Pollack and N. Plourde*. Dept. of Biology, McGill University, Montreal, Que. H3A 1B1

Tethered flying crickets attempt to steer towards a loudspeaker broadcasting conspecific calling song. A component of steering behavior is lateral flexion of the abdomen. We measured the effects of sound location and intensity on the amplitude of steering behavior, as indicated by the extent of abdominal flexion.

Teleogryllus oceanicus females performing stationary flight were stimulated with an electronically synthesized calling song model with a carrier frequency of 5 kHz. The song was played sequentially from two loudspeakers located in the cricket's horizontal plane, at equal angles to the left and right of the midline. The song was first played from the left for 12 seconds, and then was switched to the right for an additional 12 seconds. Amplitude of the steering attempt was taken as the angular difference in abdominal position (measured with a capacitative position transducer) just before and 12 seconds after the song was switched from the left to the right.

For all speaker locations, steering amplitude showed a characteristic intensity function. Abdominal flexions were small at threshold intensity (50-60 dB SPL), increased to a plateau which was maintained over an intensity range of 70-90 dB, and then decreased at higher intensities. This function is the result of binaural processing; when crickets were unilaterally deafened abdominal flexion increased monotonically with intensity.

The plateau level of abdominal flexion varied systematically with the azimuth of the sound source. Flexions were small when the speakers were located close to the midline and increased regularly to a maximum when the speakers were at 90° to the midline. This relationship held for posterior as well as anterior speaker locations. The minimum deviation of the speakers from the midline needed to elicit steering behavior was 2.5-5°.

Thus, flight phonotaxis behavior is graded; its amplitude varies both with sound intensity and sound location. These findings suggest that the amplitude of steering behavior is determined by the apparent difference in sound intensity at the two ears.

55.4 MODULATION OF A SENSORY-MOTOR SYNAPSE BY NEUROACTIVE PEPTIDES IN APLYSIA CALIFORNICA. Jeff Goldberg* and Ken Lukowiak* (SPON: G. Mpitsos). Dept. of Medical Physiology, Faculty of Medicine, University of Calgary, Calgary, Alberta T2N 1N4.

The gill withdrawal reflex of Aplysia californica has proven to be an excellent model system for investigating the neurophysiological mechanisms underlying behavioral state. Gill reflex behavior is mediated by the peripheral nervous system and modulated by the central nervous system according to the animal's biological needs. The three behavioral states that have been described are the facilitated, normal and suppressed states. These classifications are based upon the comparison of gill reflex amplitude to the amplitude of centrally mediated, spontaneous gill movements (SGMs). Our present concern centers around the suppressed state, which is characterized by: 1) reflex amplitudes less than 35% of SGM amplitudes; 2) increased rate of habituation in comparison to preparations displaying the normal state. Although suppressed behavior is associated with food satiation or sexual activity, it can also be induced upon superfusion of methionine enkephalin, arginine vasopressin or arginine vasotocin over the abdominal ganglion. Concomitant to the behavioral effects, these bioactive peptides bring about a reduction in the number of action potentials evoked in central gill motor neurons upon tactile stimulation of the siphon. To determine the site of action of these peptides, we obtained simultaneous intracellular recordings from a siphon mechanoreceptor (LE) and one of its follower motor neurons (L_7, L_9, LB_{S1}). Single presynaptic action potentials evoked short latency EPSPs in the postsynaptic motor neuron. In preparations displaying the normal behavioral state, superfusion of arginine vasotocin over the abdominal ganglion reduced the evoked EPSP amplitude by 50%. In addition, the rate of decrement of EPSP amplitude upon repeated excitation of the LE neuron (one action potential per 30 seconds) was increased during arginine vasotocin superfusion.

In behaviorally suppressed preparations, superfusion of the enkephalin antagonist naloxone over the abdominal ganglion increased the evoked - EPSP amplitude by more than 100%. Since conductance changes were not observed in the postsynaptic motor neurons upon application of the drugs, a presynaptic site of action is postulated. The presynaptic gate may be located at the LE terminal for the modulation of transmitter release. Alternatively, there is evidence that the number of functional synapses between the sensory and motor neuron is modulated through a gating of neuronal transmission at the branch points of LE neurites.

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55.5 MULTIMODAL SENSORY INTERNEURONS IN THE BRAIN OF THE FLATWORM, ALLOEOPLANA CALIFORNICA. Michael H. Solon and Harold Koopowitz. Developmental and Cell Biology, Univ. of California at Irvine, Irvine, CA 92717.

Arousal is of considerable interest in the study of complex behavior. In polyclad flatworms, vibration is an effective arousal stimulus. While investigating the neural basis of this phenomenon, we found cells in the brain of Alloeoplana which are excited by water-borne vibration and by light offset. Lucifer Yellow fills of these cells showed them to fall into two categories. One group consists of single somata which send neurites across the midline, the other of pairs of dye-coupled somata located symmetrically across the midline. Both types are multipolar, and send processes to the lateral and posterior nerves leading into the ventral submuscular plexus.

These are not primary sensory cells. Excitatory input is compound, and was reversibly blocked by 200 mM Mg^{++} or 4 mM Cd^{++}. Cells habituated to repetitive stimuli of either modality, and we found clear instances of dishabituation of one modality through stimulation via the other.

Under normal conditions, activity in these cells, regardless of the frequency or duration, did not evoke obvious muscular activity. Intracellular injection of tetraethylammonium (TEA) increased spike duration forty-fold, and each such spike was followed by a contraction of the anterior margin of the animal. These contractions were blocked by 200 mM Mg^{++} or 4 mM Cd^{++}. Those cations did not, however, abolish the plateau of the TEA spike, but rather prolonged it. Tetrodotoxin (TTX) abolished both the spike and the plateau, and no activity was seen in its presence. This suggests that the plateau is not due to an inward calcium current. There may, however, be an inward calcium current which activates a calcium-sensitive potassium current, since repolarization of TEA spikes is further prolonged by high Mg^{++} or Cd^{++}.

These cells do not elicit motor activity in the absence of TEA. Further, they do not appear to send processes into those areas that contracted after the cells had been filled with TEA, suggesting that they are not motor cells. It seems more likely that they are interneurons which receive multimodal input, and affect motor pathways through weak synaptic connections. This is what one might expect in a modulatory pathway such as might mediate arousal.

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55.6 PHYSIOLOGICAL AND MORPHOLOGICAL CHARACTERISTICS OF VIBRATION SENSITIVE NEURONS IN THE BRAIN OF THE POLYCLAD FLATWORM, NOTOPLANA ACTICOLA. C. Larry Keenan* and Harold Koopowitz, Dept. of Developmental and Cell Biology, Univ. of California, Irvine, CA 92717.

Intracellular recordings of vibration evoked activity in ventrally and dorsally located brain cells of the marine flatworm, Notoplana acticola, reveal both similarities and differences between these two classes of cells. Ventrally located cells send major processes contralaterally across the midline of the bilaterally symmetrical brain. These cells have never been observed dye-coupled to other cells. Dorsally located neurons are of two general morphological types. One group has cells that are rarely dye-coupled to other cells and never with bilateral symmetry. The processes of these cells project contralaterally across the brain midline. The other type is normally strongly dye-coupled to a mirror image counterpart across the brain midline. These cells send major processes ipsilaterally. The physiological parameters that were compared include latencies, amplitudes and rise times of synaptic potentials, and the decrement of response to repeated vibration stimuli. High Mg^{++} and high Cd^{++} concentrations, or low Ca^{++} concentrations result in abolishment of the vibration responses. Under the same conditions, however, the cells could be driven to spike by injecting depolarizing current. Tetrodotoxin (TTX), tetraethylammonium (TEA), low Na^{+}, and low Ca^{++} all had separate effects on the various components of the vibration responses. The morphologies of the cells were analyzed after they had been iontophoretically filled with either the fluorescent dye Lucifer Yellow CH or horseradish peroxidase (HRP). The morphology and electrophysiology of these vibration-responding cells suggest they function as sensory interneurons. Furthermore, although these brain cells are found in a group of animals at the base of the phylogenetic tree, they nevertheless exhibit morphological and physiological complexities similar to those found in the neurons of both higher invertebrates and vertebrates.

(Supported by a grant from NIH - NS13713)

56.1

WITHDRAWN

56.2 INTERACTION OF SYNTHETIC PYRETHROID INSECTICIDES WITH KAINIC ACID AND THEIR EFFECT ON KAINIC ACID BINDING TO MOUSE FOREBRAIN MEMBRANES. Christina G. Staatz*, John J. Lech* and Alan S. Bloom (Spon: L.F. Tseng). Dept. of Pharmacology and Toxicology, Medical College of Wisconsin, Milwaukee, WI 53226.

Permethrin (NRDC 143 3-phenoxybenzyl [1R,1S]-cis,trans-3-[2,2-dichlorovinyl]-2,2-dimethylcyclopropane carboxylate), a synthetic derivative of the pyrethin-based insecticides, produces neurotoxic symptoms in mice consisting of hyperactivity and tremor. At high doses this can progress into a state of sustained clonic seizures. This syndrome has been demonstrated to be predominantly central in origin (Staatz, C.G., Bloom, A.S. and Lech, J.J., Fed. Proc. 39:624, 1980) as a 200 fold increase in potency occurs following central (icv) as opposed to peripheral (iv) injection. On gross observation the toxic syndrome produced by permethrin is similar to that seen following either cerebral or peripheral injection of kainic acid, a dicarboxylic acid containing pyrrolidine which is a rigid analog of glutamate and a potent neuro-excitant. Kainic acid is able to potentiate the convulsive action of permethrin; mice injected with a subconvulsive dose of kainic acid, i.p. and subsequently injected with a subconvulsive dose of permethrin, i.v., show a 50% incidence of convulsions. Also, following pretreatment with kainic acid (icv 60 ng/mouse), 3 days prior to permethrin injection, the ED50 for permethrin-induced convulsions was reduced more than 4-fold. In light of these observations the effect of pyrethroids was investigated on the in vitro binding of [^{3}H] kainic acid (^{3}HKA) to membranes prepared from mouse brain. In these studies decamethrin [(S)-α-cyano-3-phenoxybenzyl-(1R,3R)-cis-3-(2,2-dibromovinyl)-2,2-dimethyl-cyclopropane carboxylate)], a representative member of the cyano-class of pyrethroid insecticides, was used because of its greater potency. The specific binding of ^{3}HKA to mouse forebrain membranes was measured at 4°C and was found to be saturable with a dissociation constant (K_D) of 16 nm and an apparent maximal density of binding sites (Bmax) of 120 fmol/mg protein. Decamethrin in doses ranging from 1 nm to 20 μm caused a dose-dependent decrease in the amount of ^{3}HKA specific binding, the maximal effect being a 40% reduction when compared to vehicle. These results suggest a possible interaction of this pyrethroid with a kainic acid binding site. (Supported by USPHS grants ES-01080 and ES-01985).

56.3 EFFECTS OF PERMETHRIN, A SYNTHETIC PYRETHROID INSECTICIDE ON OPERANT BEHAVIOR AND FEEDING. Alan S. Bloom and Christina G. Staatz*. Dept. of Pharmacology and Toxicology, Medical College of Wisconsin, Milwaukee, WI 53226.

The synthetic pyrethroids are a group of potent insecticides that exhibit a very favorable insect:mammal toxicity ratio and a desirable rate of biodegradability. They are believed to kill insects by an action on the nervous system. High doses (~500 mg/kg, IP) of permethrin (NRDC 143, 3-phenoxybenzyl [1R, 1S]-cis, trans,3-[2,2-dichlorovinyl]-2,2-dimethylcyclopropane carboxylate) produced a syndrome in rodents that consists of increased responsiveness to external stimuli, hyperexcitability, and tremors and convulsions that may result in death. To investigate the toxicity of doses of permethrin below the threshold for this syndrome, operant conditioning techniques were used. Male Sprague-Dawley rats, maintained on a 23 hour food deprivation schedule, were trained to respond on a VI-20 second schedule for food reinforcement. After stable response rates were achieved, each animal was tested 20 minutes after the IP injection of 15, 30 and 60 mg/kg of technical grade (40% cis-60% trans) permethrin or its emulphor-ethanol vehicle. Treatment order followed a latin square design. These doses of permethrin lowered response rates by 12%, 25% and 60%, respectively. The effects of the 30 and 60 mg/kg doses were statistically significant. The vehicle itself was without effect. In spite of the significant effects on operant behavior, by gross observation these animals did not appear to be affected by the drug.

In order to determine if the decrease in operant response rate was due to drug induced anorexia, the effects of permethrin on food intake were also studied. Food intake was measured for both 1.5 and 24 hour periods after treatment with permethrin using the same design as in the operant studies. Significant decreases in food intake were observed only after the 60 mg/kg dose (44% in the 1.5 hour study and 16% in the 24 hour study). These data indicate that subconvulsive doses of permethrin can have significant effects on learned behavior and that these effects may be due in part to drug induced anorexia.

56.4 EFFECT OF REPEATED ADMINISTRATION OF DESMETHYLIMIPRAMINE ON THE NIGHT-TIME RISE IN MELATONIN OF RAT PINEAL GLAND. W.E. HEYDORN, D. BRUNSWICK* AND A. FRAZER. Depts. of Psychiat. and Pharmacol., Univ. of Penn. and Vet. Admin. Hospital, Phila., Pa. 19104.

It has been shown that repeated administration of antidepressants to rats diminishes responses elicited by catecholamine-induced activation of β-adrenergic receptors. In most studies, the response measured was the production of adenosine 3',5'-monophosphate (cyclic AMP). In view of such observations, we examined whether repeated treatment of rats with desmethylimipramine (DMI) would diminish an adrenergic response mediated via cyclic AMP. Male rats, kept in a 12 hour light/dark cycle (lights out at 1900 hours), were injected for seven days with either saline or DMI (10mg/kg, twice daily). In one experiment, twenty-four hours after the final injection of saline or DMI, the rats were killed and the specific binding of ^{3}H-dihydroalprenolol (^{3}H-DHA) to pineal gland homogenates was measured. In another experiment, rats were decapitated at the following times (always 24 hours after the final saline or DMI injection): 1000, 1600, 1930, 2200, 2400, 0200, 0400 or 0600 hours. The pineal glands were removed and frozen for subsequent analysis of melatonin by radioimmunoassay. In animals killed in the middle of the light cycle, repeated administration of DMI lowered the binding of ^{3}H-DHA significantly, from a control value of 711±49 fmoles/mg protein (n=7) to a value of 480±50 fmoles/mg protein (n=6) in DMI-treated rats ($p<0.01$). Similar results were obtained if animals were killed in the middle of the dark cycle. The content of melatonin in the pineal gland rose markedly (over twenty-fold) during the night. Treatment of rats with DMI decreased significantly the content of melatonin in the pineal gland ($F=8.88$; $p<0.01$). However, the magnitude of the inhibitory effect of DMI treatment on the night-time rise of pineal gland melatonin was rather small (about 20%) and significant decreases in melatonin content of the pineal gland in DMI-treated rats were not seen at all time points. Our results indicate that melatonin formation may not be compromised as markedly as cyclic AMP production by the DMI-induced development of β-adrenergic receptor subsensitivity. (Supported by Research Funds from the Vet. Admin., MH 29094 and the AFPE).

56.5 BEHAVIOR AND ELECTROPHYSIOLOGY OF CATS MAINTAINED CHRONICALLY ON A NEUROLEPTIC. R. B. Glassman, H. N. Glassman* and B. M. Baltrus*
Dept. of Psychology, Lake Forest College, Lake Forest, IL 60045.

To survey the brain for changes that might be relevant to tardive dyskinesia cats, having chronically implanted arrays of 30 gross electrodes, were observed during 1-3 periods of 1 to 11.5 months on chlorpromazine (25-50mg/day, orally) and during .5 to 3 months following withdrawal. A preliminary report was presented in 1977 (Abstract #1405). Histologically verified electrode placements were in frontal cortical areas, basal ganglia, amygdala, thalamus, substantia nigra, and other points. While behavioral results were positive, electrophysiological observations gave only weak clues to possible underlying factors:

Behavior. Six of 9 cats showed increased incidence of licking during maintenance and all showed a further increase on withdrawal. In a typical case, the average number of licks during a 5-min. observation period was 11 before drug, 8 during early maintenance, and 89 after 3.5 months maintenance. During the first week of withdrawal there were 230 licks per 5-min. period, decreasing to 63 licks by the 10th week. In varying degrees, withdrawal also caused increased paw or body shakes and "fly-chasing movements" of the skin of the back.

Potentials evoked by forelimb stimulation. Cortical potentials meeting our criterion of .16 mV were observed in 8 cats before drug. Potentials at some points increased in amplitude during drug in all cats (up to threefold) and decreased to baseline or below on withdrawal.

Potentials evoked in striatum by substantia nigra stimulation. There was a widespread progressive decrease in amplitude during drug and withdrawal in 2 of 4 cats. In one cat, amplitude increased on drug and decreased below baseline on withdrawal. In the 4th cat a variable record showed no net change. Occasional testing with stimulation of other points also usually showed EPs decreasing with time but in one cat stimulation of some points was associated with increased cortical EPs during drug, which returned to baseline on withdrawal.

Cortical spindling evoked by stimulation of caudate and other subcortical points. Spindles increased in duration and amplitude, in 3 of 4 cats, during early drug maintenance. A decrease in amplitude by the end of the experiment was not clearly related to treatment conditions.

Spontaneous EEG. Slowing during early drug maintenance appeared greater at more rostral points, greatest in cortex.

Terminal acute behavioral probes. Behaviors elicited by high doses of apomorphine, haloperidol, physostigmine or atropine included licking; PCPA and 5-HTP did not elicit licking.

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56.6 CHRONIC LITHIUM ADMINISTRATION: PARTIAL REDUCTION OF HALOPERIDOL-INDUCED BEHAVIORAL SUPERSENSITIVITY WITHOUT AN INFLUENCE ON DOPAMINE RECEPTOR ELEVATION. D.A. Staunton, P.J. Magistretti, S.N. Deyo*, W.J. Shoemaker, and F.E. Bloom. A.V. Davis Center for Behavioral Neurobiology, The Salk Institute, La Jolla, CA 92037.

Spontaneous locomotor activity and dopamine receptor responsivity were assessed after 4 weeks of dietary treatment with the anti-manic drug, Li. Prolonged dietary Li administration was not accompanied by overt toxicities and led to growth rates approximately 60% of those observed in animals fed the control diet. Moreover, the diet consistently yielded serum and brain Li values in the prophylactic range for manic-depressive disease (0.8-1.2 mEq/L). Four weeks of Li administration led to diminished spontaneous locomotor activity compared to subjects on the control diet, with or without pair-feeding. The depression of locomotor activity following an injection of apomorphine was less severe in subjects that ingested Li compared to those with free access to the control diet. As previously reported (Pert et al, Science 201: 171), chronic Li administration partially attenuated apomorphine-evoked stereotyped behaviors in animals rendered supersensitive to the drug by daily injections of haloperidol (HAL) for three weeks.

In a series of parallel experiments, the influence of chronic dietary Li adminstration was evaluated on dopamine receptor supersensitivity in the rat corpus striatum. In contrast to previous data (Pert et al., ibid) the attenuation of HAL-induced behavioral supersensitivity following long-term dietary Li administration was not accompanied by prevention of increased ^{3}H-spiroperidol binding sites in the corpus striatum. For example, in the subjects used for behavioral analysis, Scatchard analysis revealed that three weeks of treatment with HAL resulted in a 27% elevation of neostriatal ^{3}H-spiroperidol binding sites in animals fed the control diet versus a 29% elevation in those given Li. Similar results were obtained when the withdrawal period from chronic HAL was varied (range 1-14 d). In these experiments, HAL-pretreatment did not affect the affinity of ^{3}H-spiroperidol for its binding site and long-term Li exposure affected neither the K_D or the B_{max} values. In addition, neostriatal dopamine-sensitive adenylate cyclase was not affected by either long-term dietary Li or chronic neuroleptic treatment. As a group, these results are incompatible with the hypothesis that the anti-manic action of Li is related to its ability to prevent dopamine receptor supersensitivity. An influence of the drug at other receptor sites or a non-receptor-mediated action of the drug can not be excluded. Supported by NIMH 08080, 29466, and NIAAA 07273. PJM supported by Swiss NSF.

56.7 INFLUENCE OF AGE ON THE EFFECT OF CHRONIC FLUPHENAZINE ON RECEPTOR BINDING IN RAT BRAIN. H. Shelat, C. H. Misra, R. C. Smith. Department of Biological Psychiatry, Texas Research Institute of Mental Sciences, Houston, Texas 77030.

Some of the side-effects of chronic administration of neuroleptic to man, such as tardive dyskinesia, have increased prevalence in the geriatric patient treated with neuroleptics. Recently, we have shown (Misra et al, 1980) that aging rat brain has a reduced number of dopaminergic of adrenergic receptors. The present work has been done to keep in mind, that changes in receptor function after chronic administration of neuroleptics in the aging brain may provide clues to some of the underlying pathophysiology of tardive dyskinesia and other side-effects of neuroleptics. The effects of age on alterations in brain dopaminergic (spiperone), β-adrenergic (DHA), α-adrenergic (WB-4101) and cholinergic (QNB) binding induced by chronic administration of fluphenazine was studied in the rat. Compared to age-matched saline controls, older-age (25 month) fluphenazine rats showed: (a) a similar increase in specific spiperone binding (at .1 nM) but a slightly smaller increase in the B_{max} of spiperone binding in the striatum than younger fluphenazine-treated rats; and, (b) a substantially greater increase in the B_{max} of DHA binding and slightly greater increase in B_{max} of WB-4101 binding in the cerebral cortex than younger fluphenazine-treated rats. There was no significant interaction of age with the effect of chronic fluphenazine on QNB binding in rat striatum.

56.8 THE ACUTE AND CHRONIC EFFECTS OF LITHIUM ON THRESHOLD OF INTRACRANIAL REINFORCEMENT. G. P. Cassens, V. W. Slayton* and D. Manigold* Dept. of Psychiatry, Harvard Med.Sch. Boston,MA 02115

Although it is generally recognized that chronic administration of lithium is required for its clinically therapeutic effects in the treatment of mania and bipolar depressions, relatively few investigators, heretofore, have systematically explored the effects of chronic administration of lithium on intracranial self-stimulation (ICSS) behavior. Moreover, previous studies have measured lithium's effects on rates of response for intracranial reinforcement (ICR), but these procedures do not discriminate between drug-induced alterations in motor response and changes in threshold of reward. In these studies, we found that acute administration of lithium (0.5,1.0 and 2.0 mEq/kg) produced significant dose-related increases in threshold of intracranial reinforcement, using a rate-independent measure previously described (Cassens,G.P. and Mills,A.W.,Psychopharmacologia, 30:283,1973). After chronic injection of lithium (1.0 mEq /kg given once daily for 23 days), significant increases in threshold were observed during the first 1-5 days of lithium administration; however, by Day 7 these threshold elevations were not significantly different from pre-drug thresholds despite continued treatment with lithium.

It has previously been proposed that withdrawal from chronic administration of amphetamine may serve as an animal model of drug-induced or naturally occurring bipolar depressions. Amphetamine withdrawal in the rat responding for ICSS is characterized by significant increases in threshold of ICR, i.e. decreased sensitivity to intracranial reward(Leith, N. and Barrett,R. Psychopharmacology , 46:19,1976; Cassens,G. in press). In order to explore the prophylactic effects of lithium on the"depression" induced by amphetamine withdrawal, on Days 13-17, lithium and saline pretreated animals received chronic escalating doses of d-amphetamine (1-12 mg/kg)according to the method of Leith and Barrett (1976). While lithium, at the dose used in this study (1 mEq/kg) tended to attenuate the threshold-decreasing effects of 1 mg/kg of d-amphetamine, lithium did not block or attenuate the increases in threshold of ICR observed during amphetamine withdrawal. These results will be discussed in relation to lithium's clinical effects in the treatment of mania and bipolar depressions.

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56.9 TOLERANCE DEVELOPMENT DURING DIFFERENTIAL PAVLOVIAN CONDITIONING WITH MORPHINE. Richard W. Lambert*, and Michael Gabriel (SPON: E.B. Gardner). Dept. Psych., Univ. Texas, Austin, TX 78712.

It has been suggested (eg. Siegel, J. Comp. Physiol. Psych., 89:498,1975) that the tolerance that develops to many drugs including morphine is due, in part, to associative processes. Specifically, it is held that stimuli reliably paired with drug administration acquire the capacity to elicit a conditioned response (CR) that is in opposition to the physiological effects of the drug. When the drug is administered in the presence of stimuli with such capacity, the CR that they elicit competes with the effects of the drug, producing an attenuated response (ie. tolerance).

The present study attempted to assess the development of the postulated conditioned response during trials, and its moment by moment within-trial time course, by recording continususly the physiological response measures of heart rate and peripheral ear temperature during differential Pavlovian conditioning. The conditional stimula (CSs) were pure tones (1kHz and 8kHz), and the unconditional stimulus (UCS) was a 10 mg/kg dose of morphine sulfate solution injected through a chronic indwelling jugular cannula. The rabbits alternately received one or two conditioning trials per day, the trials spaced not less than 10 hours apart. On each conditioning trial the tone CS was presented for a total duration of 11 min. Three min. after CS onset the rabbit was injected with morphine, on CS+ trials, or saline, on CS- trials. Following 24 conditioning trials (12 with each CS), each subject received a test trial in which the CS+ was paired with saline, and another in which the CS- was paired with morphine. Interspersed between the last few conditioning trials the subjects received two additional test trials in which morphine or saline were administered in the absence of any CS.

Tolerance was manifested in the form of a progressive reduction over trials in the magnitude of the hyperthermic and bradycardic responses to the morphine UCS. However, no significant heart rate or temperature responses occurred to the CSs in anticipation of the morphine UCS during conditioning. Nevertheless evidence for a role of conditioning in tolerance development was obtained form the test trial data: the morphine UCS produced its original (large) hyperthermic effect in tolerant subjects, on test trials in which the drug was not preceded by the CS+. These results suggest that the conditioned response contributing to tolerance development in the present study was not manifested by the thermal and cardiovascular measures during CS presentations. Rather, the response in these measures was withheld until the time of UCS delivery.

56.10 BRAIN OPIATE LEVELS AND MORPHINE TOLERANCE TO THE NOCICEPTIVE TAIL FLICK TEST. C. Advokat and L. Isaac. Pharmacol. Dept., Univ. Ill. Coll. Med., Chicago, IL 60680

Previous work demonstrated that the acquisition of tolerance following morphine pellet implantation was facilitated by experience with the tail flick assessment (TF) procedure, and that, as long as the pellet remained intact, tolerance was retained for at least seven days. The purpose of the present work was to determine the relationship between brain opiate levels and the acquisition of tolerance and to determine whether retention of tolerance is influenced by "extinction" trials or electroconvulsive shock (ECS).

For each study, male, albino rats (200-225 gm) were implanted with a pellet containing 75 mg of morphine.

To assess the acquisition of tolerance one group of animals received TF tests at 3, 6, 12, 24 and 48 hours post-implant while a second group of rats was not tested until the 48 hour interval. At each time point, several animals from each group were killed and their brains removed, cleared of blood and frozen. Morphine concentration in whole brain was determined by the radioimmunoassay method. Brain opiate levels did not differ between tested and non-tested animals at 48 hrs although tested animals were more tolerant to the TF procedure than non-tested animals at this time.

To examine the retention of tolerance, animals received a TF test at 3, 6, 12, 24 and 48 hours after the morphine implant. Following these tests, pellets were either removed or left intact. Half of the animals in each of these two groups were left undisturbed for one week, the other half received additional TF tests during this period. By the end of the week there was no difference in TF latency among the four groups. However, following a morphine injection (5.0 mg/kg s.c.) animals with intact pellets were more tolerant than animals without pellets and those animals who received extra TF tests were less tolerant than those who did not.

To determine the effect of ECS on retention, another group of animals was tested at 3, 6, 12, 24 and 48 hours post-implant. Immediately thereafter one half received ECS (35 mA, A.C., 0.5 sec duration via ear clips) while the other half was not shocked. In contrast to the results of the preceding experiment these two groups differed in TF latency one week later. Rats who had ECS were less tolerant than controls. However, in response to a morphine injection the groups did not differ in TF latency.

These experiments demonstrate that acquisition and retention of tolerance can be significantly influenced by the environmental context in which they are assessed. More importantly, present work indicates that environmental factors are more critical than opiate levels in the acquisition of tolerance.

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57.1 THE ROLE OF ENDOGENOUS OPIOID SYSTEMS IN ECS-INDUCED POSTICTAL ELECTROGENESIS AND BEHAVIORAL DEPRESSION IN RATS. F.C. Tortella, A. Cowan and J.W. Holaday, Dept. of Pharmacology, Temple Univ. School of Medicine, Philadelphia, PA 19140 and Dept. of Medical Neurosci., Walter Reed Army Institute, Washington, DC 20012

Electroconvulsive shock (ECS) produces postictal opiate-like EEG and behavioral effects in rats which are antagonized by naloxone (NX) (Tortella et al, Soc. Neurosci. Abstr. 6: 318, 1980). Subsequent experiments have demonstrated a selective, naloxone-sensitive action of ECS in attenuating RX 336-M-induced wet-dog shakes and excessive grooming in rats (Tortella et al, Fed. Proc. 40: 287, 1981). In the present study, we assessed the involvement of pituitary endorphins in ECS-induced postictal electrogenesis (EEG-voltage output) and depression in rats.

Sham-hypophysectomized (SHAM) and hypophysectomized (HYPOX) male albino Sprague-Dawley rats (200-250 g; Zivic-Miller) were prepared with cerebrocortical EEG and temporalis muscle EMG electrodes. The animals were divided into 4 groups and injected s.c. with either NX (3 mg/kg) or saline (S) 10 min prior to transauricular ECS (0.2 sec, 60 Hz, 50 mA). In SHAM-S rats, ECS increased electrogenesis 65 ± 18% (s.e.) above control. Duration of the associated postictal depression was 3840 ± 530 sec. NX antagonized the postictal increase in EEG voltage output and behavioral depression. Hypophysectomy (HYPOX-S group) partially attenuated the ECS-induced electrogenesis and decreased the duration of postictal depression. Note, however, that hypophysectomized rats were unresponsive to NX (HYPOX-NX group). These data are summarized in Table I.

Table I. Effects of Hypophysectomy on ECS-induced Voltage Output and Duration of Postictal Depression.

Group	N	Voltage Output (% above controls ± s.e.)	Postictal Depression (mean, sec ± s.e.)
SHAM-S	6	65 ± 18*	3840 ± 530
SHAM-NX	6	9 ± 11^{+}	1117 ± 755^{+}
HYPOX-S	6	31 ± 9*	2360 ± 511
HYPOX-NX	5	36 ± 15*	2004 ± 929

*Signif. diff. from pre-ECS baseline; P<0.05 (paired t-test).
$^{+}$Signif. diff. from SHAM-S group; P<0.05 (t-test).

These results confirm our earlier reports of opiate-like EEG and behavioral effects of ECS in rats and support the hypothesis of an ECS-induced activation of endogenous opioid systems. On the basis of all our data, it would appear that pituitary endorphins are released during ECS and are involved in the mediation of postictal events.

57.2 Effects of Naloxone on Brain Stimulation Reward Threshold in the VNB and MFB. M.J. Lewis, Dept. of Psychology, Howard University Washington, D.C. 20059

The effects of the opioid antagonist naloxone on brain stimulation reward (BSR) threshold were investigated at two brain sites in albino rats. Male albino rats were surgically implanted with platinum bipolar electrodes in either the hypothalamic medial forebrain bundle (MFB) or the mesencephalic ventral noradrenergic bunble (VNB). All were trained to leverpress (L-P) for BSR under a concurrent fixed-ratio/continuous schedule of reinforcement. This schedule was used to determine BSR threshold independent of response rate using a method similar to Huston and Mills (Comm. Behav. Biology 5, 331-340, 1971). After stable performance was established, all rats received a series of 3 intraperitoneal injections of saline, 5 minutes prior to BSR session. They then received injections of naloxone HCl (Endo Lab., Garden City, N.Y.) similarly administered. Threshold was significantly ($p < .05$) increased by 1.0 and 5.0 mg/kg, ip of naloxone in animals with VNB sites, but not MFB sites. The 0.5 mg/kg approached statistically significant ($p < .10$) increase in animals with VNB implants. No effect was seen in MFB animals at this dose. These data are in agreement with those previously reported (Lewis, Soc. Neurosci. Abstr. 6, 367, 1980) showing a selective decrease of BSR response rate by naloxone in animals with implants in the VNB, but not in animals with MFB implants. They are also consistent with our previous data (Lewis, Margules, Costa, and Jacobowitz, Brain Res., 107, 156-167, 1976) indicating the importance of the mesencephalic VNB in opioid effects. (Supported by NIDA grant DA-02176 and NIH grant RPE-1397).

57.3 THE EFFECTS OF ACTH, MORPHINE AND NALOXONE ON LOCOMOTOR ACTIVITY IN THE RAT. R.S. Blair* and Z.H. Galina* (SPON: Z. Brown). Dept. of Psychology, Concordia University, Montréal, Québec.

We have previously reported that ACTH and naltrexone affect the locomotor activity of rats. Our results revealed that low doses of either ACTH or naltrexone enhanced motor activity while high doses of these drugs depressed activity. When we examined the effects of naltrexone on ACTH-induced activity, naltrexone was observed to reverse the depressive effects of ACTH. In the present investigation, we examined the interactions of ACTH, naloxone and morphine in three separate experiments.

In Experiment I, male wistar rats were given a subcutaneous (s.c.) injection of morphine (0,1 or 4 mg/kg) in combination with a s.c. injection of $ACTH_{1-39}$ (0,50 or 200μg/kg). Fifteen minutes after the injections, animals were placed in an open field and activity counts were recorded for 30,60 and 90 minutes. An analysis of variance of the activity counts revealed significant main effects of Morphine, ACTH and Time. All interactions with time were significant. Briefly, ACTH was found to attenuate the excitatory effects of morphine and to potentiate the depressive effects of this opiate.

Experiment II followed the same procedure as Experiment I except that naloxone (0,0.5 or 2 mg/kg) was injected instead of ACTH. An analysis of variance revealed that naloxone produced an overall decrease in activity counts and appeared to attenuate morphine's excitatory effect.

Experiment III followed the same procedure as Experiment I except ACTH and naloxone were injected. An analysis of variance revealed a significant main effect of Naloxone and a significant interaction of ACTH X Time. Naloxone produced an overall decrease in activity counts and did not affect the behavioural effects of ACTH.

The results of the present investigation are in agreement with previous studies showing that ACTH has mixed agonist-antagonist properties. Since naloxone alone produced an overall decrease in activity, it is difficult to determine the extent to which the behavioural effects of ACTH are mediated by naloxone-sensitive, opiate receptors.

57.4 ENHANCED LOCOMOTOR RESPONSE TO β-ENDORPHIN INFUSED[1] INTO THE VENTRAL TEGMENTUM OF MORPHINE PRE-TREATED RATS. J.M. Schwartz*, C. Ksir[2], G.F. Koob, and F.E. Bloom. A.V. Davis Ctr. for Behavioral Neurobiology, The Salk Institute, P.O. 85800, San Diego, California 92138.

The biphasic effect of a high dose of morphine (M) on locomotor activity (catalepsy followed by excitation) is modified selectively by chronic M treatment: tolerance develops to the depressant action while the excitatory effect is enhanced, and the latency to its appearance is decreased (Babbini and Davis, Br. J. Pharm. 1972). This enhancement of excitatory effect after chronic M treatment is extremely persistent, remaining intact eight months after cessation of chronic M administration (Babbini et al., Neuropharm. 1975). A possible locus for this effect has been demonstrated insofar as repeated local application of M to the dopamine-rich A10-ventral tegmental area (VTA) resulted in progressive increases in locomotor activity of rats (Joyce and Iversen, Neurosci. Lett. 1979). Furthermore, intraventricular β-endorphin (β-E) produced an initial excitatory locomotor response in rats pretreated with M, whereas M-naive controls showed a biphasic response of initial depression followed by excitation (Browne and Segal, Neuropharm. 1980).

The present experiment demonstrates that both M-pretreated and M-naive rats respond to β-E stereotaxically infused into the A10-VTA region with only an excitatory locomotor response, and also that the M-pretreated rats show an enhanced locomotor response compared to lactose-pretreated controls. Physical dependence and tolerance to M were induced by s.c. implantation of three 75 mg pellets of morphine sulfate for 6 days. This resulted in a transient hyporesponsiveness to both β-E and M locomotor activating effects at 24 hours following pellet removal (probably secondary to acute abstinence syndrome), followed by a protracted hyperresponsiveness at 72 hours and thereafter. Subsequent to recovery from the abstinence syndrome, both central infusion of β-E into the VTA and peripheral M administration in moderate doses independently caused a sustained increase in locomotor activation as compared to lactose-pretreated controls. This effect is maintained for at least two weeks. These results demonstrate that opiate receptors localized within or near the VTA mediate some of the stimulatory effects of opioid peptides and suggest that tolerance and sensitization can occur at the receptor level.

[1]Supported by USPHS Grant MH 07899.
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57.5 RADIATION-INDUCED HYPERACTIVITY IN THE C57BL/6J MOUSE: EVIDENCE FOR THE RELEASE OF ENDOGENOUS OPIATES. G.A. Mickley, K.E. Stevens*, M.H. Girone*, W.C. Kass*, G.A. White* and G.L. Gibbs* Dept. of Behavioral Sciences and Leadership, USAF Academy, CO 80840 and Penrose Cancer Hospital, Colorado Springs, CO 80903.

Systemic injections of morphine produce a locomotor hyperactivity in the C57BL/6J mouse (McClain, Catravas and Teitelbaum, Soc. Neurosci. Abstr., 3:279, 1977). This hyperactive response is characterized by a sterotypic "stalking" motion and an elevated "straub" tail. Since various stresses have been shown to produce release of endogenous opiates (Katz, Roth, Schmaltz & Sible, Behav. and Neural Biol. 28:366-371, 1980) the present study used hyperactivity of the C57BL/6J mouse as a measure of endogenous opiate release after exposure to the stress of ionizing radiation. In most species irradiation produces hypokinesia and a generalized malaise (Mickley and Teitelbaum, Aviat. Space and Environ. Med. 49:868-873, 1978). However, in this study we predicted that radiation exposure would produce locomotor hyperactivity in the C57BL/6J mouse due to endogenous opiate release.

Baseline measures of locomotor activity were recorded for 2, .5 hour periods before exposure to either 1500 or 2500 Rads (R) of ^{60}Cobalt radiation. After irradiation, mice (1500R: N=6; 2500 R: N=9) were placed back in the activity monitors and locomotion was again recorded for .5 hour. Other mice (1500R: N=6; 2500R: N=9) underwent the same procedure but were sham irradiated. Exposure to ionizing radiation produced a statistically-significant dose-dependant increase in locomotor activity as compared to an average pre-irradiation baseline (Wilcoxon Ranks Test $p < .05$). Irradiated subjects were significantly more active than the sham-irradiated mice (Mann-Whitney U, $p < .05$) and many exposed animals exhibited locomotor "stalking" and elevated "straub" tails.

In a second experiment, the radiogenic hyperactivity was challenged with the opiate antagonist naloxone (2 mg/kg, i.p.). Drug injections were given immediately after irradiation (1500R: N=6; 2500R: N=6) or sham exposure (1500R: N=6; 2500R: N=6). Naloxone attenuated the hyperactivity of irradiated mice to such an extent that the amount of locomotion was indistinguishable from baseline (Wilcoxon Ranks Test, $p > .05$). Irradiated mice injected with naloxone, were actually somewhat less active than sham-exposed subjects which received the drug. None of the naloxone-treated irradiated mice exhibited "straub" tails or "stalking".

Since irradiated C57BL/6J mice exhibit behaviors similar to those observed after injections of morphine, and since these behaviors can be reversed by naloxone, the present studies suggest that some radiogenic changes in behavior may be mediated by release of endogenous opiates.

These studies were supported by the USAF School of Aerospace Medicine.

57.6 PLASMA BETA-ENDORPHIN IN RATS IS INCREASED BY PSYCHOLOGICAL STRESS. J.L. Meyerhoff, B.N. Bunnell and E.H. Mougey*. Dept. Med. Neurosciences, Div. Neuropsychiatry, Walter Reed Army Inst. of Research, Washington, D.C. 20012.

Exposure to physical stressors such as bone fracture (Guillemin, et al, Science 197:1367-1369, 1977) or inescapable electric footshock (Rossier, et al. Nature 270:618-620, 1977) has been reported to cause two- to threefold elevations in plasma beta-endorphin levels in rats. We wished to determine whether significant elevations might also occur following psychological stress such as exposure to stimuli previously paired with inescapable electric footshock. Male Sprague-Dawley rats were adapted to handling for one week and assigned to three groups: Experimental, Shock Control, or No-shock Control. On four consecutive days, all rats were placed in Foringer operant chambers for 15 min sessions. Experimental and Shock Control groups were given footshock during the sessions on a variable time 30 sec schedule. Shock duration was 5 sec and intensity was 0.15 watts. No-shock Controls were placed in the chambers, but no shock was administered. On the fifth day, all rats were placed in the chambers for either 5 or 15 min; Experimental and No-shock Controls did not receive shock, while the Shock Controls were given shock as before.

	Days 1-4	Day 5
Exp	Shock	No Shock
SC	Shock	Shock
N-SC	No Shock	No Shock

(Exp = Experimental, SC = Shock Control, N-SC = No-shock Control)

All rats were then immediately sacrificed by decapitation. Trunk blood was collected in cold, heparinized tubes, and centrifuged for 10 min at 4°C. The plasma was transferred to tubes containing traysylol and stored at -35°C. The samples were extracted to concentrate the immunoreactive fraction and then assayed for beta-endorphin immunoreactivity using antibody produced in rabbits in our laboratory (the antibody is completely cross-reactive with beta-lipotrophic hormone).

The results are given as pg/ml ± S.E.M. beta-endorphin immunoreactivity.

	5 min	15 min
Exp	748.0 ± 102 (N=8)	1049.7 ± 117 (N=7)
SC	1150.8 ± 230 (N=8)	1415.4 ± 70 (N=7)
N-SC	492.5 ± 63 (N=8)	478.6 ± 60 (N=7)

Beta-endorphin immunoreactivity was significantly increased in both Experimental and the Shock Control groups at 5 and 15 minutes. The values for the No-shock Controls remained low at both 5 and 15 minutes. These data indicate that release of beta-endorphin can be elicited by psychological stress.

57.7 OPIOID AND NONOPIOID MECHANISMS OF LONG-TERM ANALGESIA. S. F. Maier*, R. C. Drugan*, J. W. Grau*, R. Hyson* and A. J. J. MacLennan*. University of Colorado, Boulder, CO 80302 USA; J. Madden IV* and J. D. Barchas. Stanford University, Stanford, CA 94305

Rats exposed to an extended series of inescapable shocks (80 5 sec shocks at an average interval of one min) display the well documented short-term stress-induced analgesia which dissipates relatively quickly. However, a pronounced analgesic reaction can be reinstated 24 hrs following the inescapable shock session by a brief reexposure to mild shock, which itself is insuffucient to induce an antinociceptive reaction. This long-term analgesic reaction following inescapable stress is completely reversed by opiate antagonists, completely cross tolerant with morphine, and requires the integrity of the pituitary adrenal axis for its expression. However, a wealth of evidence has recently been reported indicating that stressors produce both opiate sensitive and non-opiate analgesic reactions. Here we report experiments assessing factors which lead to the opioid nature of the long-term analgesia. Evidence regarding the differential environmental factors necessary for the expression of both forms of analgesia will be presented. We report that it is either the number of inescapable shocks or the duration of inescapable shock exposure which determines whether the resulting analgesia is reversible by opiate antagonists and reinstatable 24 hr later by shock reexposure. Both brief and extended exposures produce analgesia, but only the analgesic reaction developing after extended duration is reversible by naltrexone and reinstatable later. Furthermore, these analgesias occur sequentially in the stressed subject as exposure to shock continues. We further report that stress parameters which produce naltrexone-reversible analgesia also produce morphine super-sensitivity and that morphine produces an analgesic reaction reinstatable by stress. Additional characteristics of this reinstatable analgesia will be presented.

57.8 EFFECTS OF LESIONS OF THE BRAIN'S BETA-ENDORPHIN SYSTEM ON STRESS-INDUCED ANALGESIA IN RATS. J. E. Kelsey and Walter A. Hoerman IV*. Dept. Psychology, Bates College, Lewiston, ME 04240.

Although endorphins and enkephalins have been implicated in mediating some forms of analgesia, the role of the brain's Beta-endorphin system in mediating analgesia remains unclear. Presumably, if this system is involved, lesions of this system should reduce the release of Beta-endorphin and reduce analgesia produced in many situations. To examine this hypothesis, the effects of lesions of the arcuate nucleus of the hypothalamus, which contains the cell bodies of the brain's Beta-endorphin system, were examined on analgesia produced by exposure to stress. stress. In contrast to our expectations, we found that exposure to inescapable foot shocks produced significantly more analgesia, as indicated by elevated tail flick latencies, in the rats with lesions of the arcuate nucleus than in sham-operated controls when the rats were tested more than two weeks following surgery. However, when the rats were tested 3-7 days following surgery, the stress tended to produce less analgesia in the rats with arcuate nucleus lesions than in the controls.

There were no differences between any of the groups in pre-stress sensitivity to pain, and the lesions appeared to extensively damage the cell bodies presumed to contain Beta-endorphin. Furthermore, the effects of these lesions did not appear to be due to damage to nearby non-endorphin systems. Therefore, we suggest that our data implicate the brain's Beta-endorphin system in the mediation of stress-induced analgesia and suggest that our results reflect the effects of time-dependent compensatory changes, such as deenervation supersensitivity, occurring within the damaged Beta-endorphin system. To further examine this hypothesis, the magnitude of morphine-induced analgesia is currently being examined on rats with arcuate nucleus lesions.

57.9 ROLE OF NEUROPEPTIDES IN NOCICEPTIVE PROCESSES. M. M. Wallace, R. J. Bodnar, D. Badillo-Martinez*, G. Nilaver and E. A. Zimmerman. Dept. of Psychology, Queens College, CUNY, and Dept. of Neurology, Columbia University CPS, New York, NY.

The anatomical distribution and behavioral effects of the neuropeptides, B-endorphin (BE), met-enkephalin (ME), arginine-vasopressin (VP), substance P (SP) and neurotensin (NT) are suggestive of their respective roles in pain inhibition. To assess further the role of each in nociception, anti-sera raised specifically against each of these peptides plus oxytocin (OXY) were administered intracerebroventricularly (5 ul: 1 ul/15 sec) to rats in determining alterations in nociceptive thresholds, activity and temperature. One hundred and eight animals were tested for tail-flick latencies and subdivided as to anti-sera concentration (100, 50 and 10% dilution) and anti-sera group (two anti-sera plus vehicle). Following each injection, rats were tested over three levels of radiant heat at 15, 30, 60 and 90 min post-injection in an incompletely counterbalanced design. Significant concentration-dependent, intensity-dependent and time-dependent effects were observed. Anti-sera to BE and ME elicited short-lived hyperalgesic responses with intense radiant heat. Anti-sera to SP, VP, NT and OXY elicited biphasic effects with hyperalgesia at intense radiant heat levels and analgesia at moderate radiant heat levels. These effects appeared to be specific to the anti-sera since injections of normal rabbit serum and preimmune serum were without effect across thermal intensities and across time course. Twelve animals tested on the flinch-jump test received microinjections at the high concentration of each anti-sera and vehicle across the same time course in an incomplete counterbalanced design. Yet, no significant differences were found employing flinch-jump thresholds. Since some of these peptides alter thermoregulation and activity, we assessed undiluted anti-sera effects on core body temperature 15, 30, 60 and 90 min after injection. While anti-sera to AVP induced significant hypothermia 60 and 90 min later, anti-sera to ANT and AOT induced hyperthermia across the time course. All other anti-sera were without effect. Finally, activity levels were determined in 15 min blocks for up to 90 min following undiluted anti-sera or vehicle injection. No differences in activity patterns were observed across injections. Given the differential time courses both across anti-sera for a particular behavior and within an anti-sera across behaviors, it is concluded that these observed effects are specific to a particular anti-sera's modulation of that behavior, rather than an epiphenomenological result. (Supported by NIH Grants 14449, 5805RR07064 and AM20337.)

58.1 LESIONS OF NUCLEUS MEDIANUS BUT NOT ORGANUM VASCULOSUM PRODUCE ADIPSIA AND THIRST DEFICITS IN RATS. Thomas W. Gardiner*, Michael L. Mangiapane, and John B. Simpson. (SPON:J.H. Jacoby) Dept. Psychology, U. Washington, Seattle, WA, 98195.

Lesions of various size were made in male Long Evans rats in the area forming the rostral wall of the third cerebral ventricle, from the anterior commissure to the organum vasculosum laminae terminalis (OVLT). Larger lesions produced animals which became adipsic and exhibited inappropriate diuresis, as has been described previously (Johnson and Buggy, AJP 234(3):R122, 1978). However, with discrete lesions , adipsia was observed in some animals that nonetheless conserved fluids normally during dehydration(urine output comparable to water deprived controls), suggesting that the two dysfunctions are neurologically separable. More discrete lesions generally produced animals which showed a very transient adipsia (1-2 days) but in some cases, adipsia was prolonged (up to 7 days). Further, these lesions also reduced the drinking response of several rats to hyperosmotic thirst challenges (2ml of 2M NaCl).

Selective ablation of the ventral portion of nucleus medianus(VNM), with minor subcommissural periventricular damage caudal to VNM, resulted in prolonged adipsia and refractoriness to NaCl drinking in several animals. However, complete ablation of VNM did not result in adipsia or NaCl deficits in some animals including two rats with additional ablation of OVLT and some periventricular tissue. Extensive damage limited to periventricular tissue, or the tissue lying rostral to VNM (dorsal to OVLT) did not result in adipsia or NaCl drinking deficits in any animals unless substantial damage to VNM was also present. Selective ablation of all of OVLT likewise did not produce adipsia or decreased response to hypertonic NaCl. The inconsistencies here suggest that fiber projections terminating in or passing through this region may play an important role in producing behavioral deficits following these lesions. Disruption of drinking could occur following damage to fibers at several locations, despite inconsistent damage to nuclear groups per se.

In summary, selective VNM ablation was observed to result in adipsia and chronic drinking deficits to NaCl in several rats, but was not always sufficient to produce behavioral abnormalities. Destruction of surrounding tissue, including OVLT did not produce these deficits unless VNM was also damaged. Supported by HL 21800.

58.2 SELECTIVE REDUCTION BY IMMOBILIZATION STRESS OF OPIATE-RELATED HYPERPHAGIAS. S. Sangiah[1], M. T. Lowy*, and G. K. W. Yim, Dept. of Pharmacol. & Toxicol., Sch. of Pharmacy & Pharmacal Sci., Purdue Univ., West Lafayette, IN 47907, and Dept. Physiological Sci., Oklahoma State Univ., Stillwater, OK 74074.

Our previous studies with naloxone (Life Sci. 26: 2113, 1980) and dexamethasone (Life Sci. 27: 2553, 1980) have identified nocturnal feeding and the hyperphagias induced by 2-deoxy-D-glucose (2-DG), food deprivation (FD) and tail pinch (TP) as opiate dependent, whereas insulin-induced feeding operates through an opiate-independent mechanism. Since stress can cause subsequent reduction of endorphin release, and block other opiate-related phenomena, the aim of the present study was to determine if acute 1 hr immobilization (IMM) stress might selectively block the opiate-dependent hyperphagias. As anticipated, the stimulation of 4 hr daytime intake of rat chow by 2-DG (400 mg/kg) was decreased following IMM (5.8 $\pm$ 0.4 vs. 3.8 $\pm$ 0.5 g, $p < 0.05$), whereas the hyperphagia following 10 U/kg insulin was not decreased (3.6 $\pm$ 0.3 vs. 3.2 $\pm$ 0.6 g). In addition, 4 hr nocturnal feeding was markedly reduced by IMM (7.4 $\pm$ 0.8 vs. 2.8 $\pm$ 0.4 g, $p < 0.01$) as was FD-induced hyperphagia (10.8 $\pm$ 1.1 vs. 6.8 $\pm$ 0.5 g, $p < 0.01$). However, TP-induced eating of a palatable substance (3 min) was not reduced by prior IMM (3.1 $\pm$ 0.2 vs. 3.6 $\pm$ 0.3 g). Since the TP induced eating is more nonspecific and also dependent on a dopaminergic component, the present results are still consistent with the premise that IMM disrupts an endogenous opiate system, which is important for the full expression of the 2-DG, FD and nocturanal-induced hyperphagias. In contrast, insulin hyperphagia appears to operate through an opiate-independent mechanism. (Supported in part by Pharmacology/Toxicology Training Grant GM-70904, and American Cancer Society Grant CH-194).

58.3 EFFECTS OF BILATERAL SUBSTANTIA NIGRA DAMAGE ON SCHEDULE INDUCED, SCHEDULE DEPENDENT, AND INGESTIVE BEHAVIORS. D. L. Armstrong*, M. J. Wayner, F. C. Barone, T. Orsland*, B. Falk* and J. Sgromo* (SPON: C. S. Weiss). Brain Research Lab., Syracuse Univ., 601 University Ave., Syracuse, NY 13210.

Substantia nigra (SN) damage has been reported to produce changes in motor and ingestive behaviors of rats. Since schedule induced and schedule dependent behaviors have provided a sensitive measure of the severity and duration of lesion induced behavioral effects (Loullis and Wayner, Physiol. Behav. 22: 575-582, 1979; Wayner et al., Physiol. Behav. 18: 503-511, 1977; Physiol. Behav. 20: 427-434, 1978; and Physiol. Behav. 21: 1015-1025, 1978), these behaviors were utilized as a model to study in more detail the effects of various types of damage to the SN. Male rats were reduced to 80% of ad lib feeding body weight and were trained to press a lever for food on a FI-1 min schedule. When schedule dependent lever pressing and schedule induced licking and drinking stabilized during 30 min daily test sessions, the animals were divided into 3 groups, anesthesized with Equi-Thesin, and prepared for surgery. One group consisted of those animals receiving bilateral electrolytic lesions in the SN (LESION group, 1 mA anodal current for 15 sec). Another group consisted of those animals receiving bilateral microiontophoretic ejections of kainic acid in the SN (KA group, 12 µA cathodal current for 5 min through a glass electrode filled with 100 mM kainic acid, pH 7.4 using NaOH). The last group were sham operated controls (CONTROL group). Animals were also examined weekly on a series of behavioral tests frequently used to assess deficits in sensorimotor coordination. When the behavioral data had been collected the animals were perfused intracardially and the brains were removed, sliced into 40 µm sections, and stained with cresyl violet in order to verify histologically the extent of tissue damage. Following surgery, in the LESION group, lever pressing, licking, and water consumption were initially eliminated, then partially recovered, and continued at reduced levels for the remainder of the study. Powdered wet-mash supplements and stomach intubation were required to sustain these animals. In the KA group more transient decreases occurred in lever pressing, licking, and drinking which gradually recovered to pre-surgery baseline levels of responding. The CONTROL group was not affected. Results indicate that SN ascending fibers are critical to the maintenance of schedule induced licking and drinking. Substantia nigra neurons are also important since kainic acid applied in this area disrupts responding under these conditions. (Supported by NIH Grant NINCDS USPHS No. 13543.)

58.4 THE EFFECT OF DOPAMINE-DEPLETING BRAIN LESIONS ON SUCKLING AND WEANING IN RATS. John P. Bruno*, Abigail M. Snyder*, Edward M. Stricker, and Michael J. Zigmond (Spon: D. Kupfer). Department of Psychology, University of Pittsburgh, Pittsburgh, PA 15260.

Large striatal dopamine (DA) depletions, produced by intracerebral injections of the neurotoxin 6-hydroxydopamine (6-HDA), lead to aphagia and adipsia in adult rats, thus suggesting a role for cerebral DA in the control of eating and drinking. We now report the effect of DA-depleting brain lesions on ingestive behavior in neonatal rats. To selectively destroy central DA neurons, rat pups were injected at 3 days of age with desmethylimipramine (25 mg/kg, sc) followed 30 min later with 6-HDA (200 ug/10 ul, ivt). Suckling behavior appeared to be unaffected by 6-HDA although striatal DA was depleted by 99%, which would have produced prolonged aphagia and adipsia in adults. Lesioned pups grew well and were only 20% lighter than controls (39g vs 49g) 2 wk after 6-HDA. Developmental landmarks such as the appearance of fur (at 10-12 days of age) and eye-opening (at 14-15 days) were not affected. By 27 days of age, late in the normal weaning period, these rats weighed 50% less than controls (51g vs 104g), a difference that was maintained through adulthood, yet most rats (17 of 21) survived weaning at this time; during the first 3 days lesioned rats ate chow and drank water and gained 2 g/day, although they consumed much less than controls, which gained 8 g/day. Lesioned rats weaned at 18 days of age, early in the normal weaning period, had much more difficulty. These rats consumed little and lost 3 g/day during their first 3 days on a diet of chow and water, whereas control rats ate and drank and gained 3 g/day. When provided with mixed cereal and milk, the lesioned rats simply maintained their body weight at first and did not begin to grow for 3-7 days. In either case, associated with their low food and fluid intakes were body temperatures which ranged from 31-34 °C. Such marked hypothermia was not observed in lesioned rats weaned at 27 days of age or in control rats weaned at 18 days of age. These results indicate that destruction of central dopaminergic neurons in neonatal rats has little effect on growth until weaning but has a marked effect thereafter, especially when the animals are prematurely weaned. Supported by NIMH Fellowship MH08488 and USPHS Grants NSMH-16359 and MH-29670.

58.5 FUNCTIONAL ROLES OF THE LATERAL HYPOTHALAMUS AND PREFRONTAL CORTEX DURING BAR-PRESS FEEDING BEHAVIOR IN MONKEY. T. Ono, H. Nishino*, M. Fukuda*, K. Sasaki*, and K. Muramoto*, Dept. of Physiol. Fac. of Med., Toyama Med. and Pharmaceu. Univ., Toyama 930-01, Japan.

Unit activity in the lateral hypothalamus (feeding center, LHA) and the prefrontal cortex (FC) was recorded during monkey bar-press feeding behavior which comprised three stages; i) discrimination of food, ii) drive to obtain, iii) real ingestion reward. Their functional roles were deduced from the unitary responses to feeding events. Results were: In the LHA; 1) Of 199 neurons recorded, 69 responded in one or more stages of the feeding task. 2) Firing responses were divided into 3 patterns. Type I responded only in the discrimination stage. Type II responded only in the ingestion stage. Type III responded throughout all 3 stages. 3) Neurons which may be concerned with discrimination of food or non-food were Type I and Type III neurons. Food specific responses were more frequently observed in Type III(80%) than in Type I(15%). 4) Thirteen Type III neurons which seemed to recieve inputs from multiple sources, responded throughout the bar-press stage even in high FR schedule trials. These responses continued more than 10 seconds and seemed to be related with internal and external "drive" to obtain food. 5) Type II responses (mostly inhibition) appeared after food was put into the mouth. This response disappeared when either an aversive food was given, or the monkey became satiated, or glucose or morphine was injected intravenously. In the FC; 1) Of 167 neurons recorded, 77 responded in one or more stages of the feeding task. 2) Neurons in area 8 responded transiently at the sight of food, while neurons in area 10 responded throughout the bar-press stage. 3) In the ingestion stage 40 neurons responded while the monkey ate food. 4) Some neurons responded upon the approach of some significant material(food) toward the monkey. These responses stopped as soon as the monkey took the object in its hand. 5) Six neurons in area 10 responded to the movement of experimenter to manipulate food, or to push the start button, or to the rustle of a bag which contained food. These neurons, however, never responded to the sight of food presented in front of the subject.
The results suggest that the LHA is related to food discrimination, to the drive to obtain it, and to reward perception through situation specific neurons; the FC is related to the drive to obtain food, to reward anticipation, and to reward recognition, also through situation specific neurons.

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58.6 LONG-TERM STABILITY OF ELECTRICALLY INDUCED BEHAVIOR IN GROUND SQUIRREL SUGGESTS NEURAL SPECIFICITY. J.D. Hallonquist. Dept. of Psychiatry, Mount Sinai Hospital, Toronto, Ont., Canada M5G 1X5.

Electrical stimulation of the lateral hypothalamus (LH) in animals can produce behaviors such as carrying of objects, eating and drinking. Such induced behaviors in rats have raised basic questions important for understanding neural substrates of motivation. First, does the stimulation activate the same neural systems involved in normal initiation of the behavior? Second, is the type of induced behavior determined by the specific neural system activated, or by the stimulation of a single functional mechanism which interacts with internal and external cues as well as past experience? The present findings relate to the second (specificity) question.

Sixty-five golden-mantled ground squirrels (Citellus lateralis) with stimulating electrodes bilaterally placed in the LH were screened for electrically induced behavior in the presence of food pellets. During the single 15 min. screening period, 98/130 sites supported 1 of 2 topographically distinct behaviors -- either induced eating (69 sites) or induced holding (29 sites) of the food pellets; no site supported both behaviors. Only 2 squirrels showed neither behavior at both sites. Of particular interest were 9 squirrels which showed different behaviors at bilateral sites, although directed at the same pellets.

Subsequent experiments produced no switching of the induced behavior observed during screening at a particular site. Forced ingestion of pellets via suprathreshold current levels with a consequent rise in threshold for induced eating did not precipitate emergence of induced holding. Furthermore, over a period of 1 year, unilateral sites (8 eating, 4 holding) were tested monthly in 12 squirrels, while bilateral sites (8 eating, 2 holding) were tested alternately approximately every 10 days in 5 squirrels. Despite pronounced circannual cycles of home cage food intake, body weight, body temperature and reproductive state in the squirrels, the behavior induced with threshold current levels at a particular site did not change, even in 2 squirrels which displayed different behaviors towards the pellets at bilateral sites.

The rapid initial appearance and long-term stability of the induced behaviors, coupled with their specificity to the electrode site and not the individual squirrel, suggest that different neural systems subserve the 2 induced behaviors, and argue against plasticity of motivational systems at the level of the LH in these feral animals (Supported by Canadian NRC grants to N. Mrosovsky, Depts. of Psychology & Zoology, U. of Toronto, and the author while at the Dept. of Psychology, U. of Toronto).

58.7 TRAIN LENGTH OF REWARDING STIMULATION AFFECTS FOOD INTAKE DURING FOOD/REWARDING BRAIN STIMULATION COMPETITION. R.A. Frank, R.L. Preshaw* and R.M. Stutz*. Dept. of Psychology, University of Cincinnati, Cincinnati, O. 45221.

It has been demonstrated that increasing the intensity of rewarding electrical stimulation of the brain (RESB) increases the ability of the stimulation to compete with drinking in water-deprived rats (Morgan & Morgenson, Psychon. Sci., 6: 337, 1966). However, it is unclear whether increasing the current intensity of RESB reduces water intake due to an increased field of stimulation (activating fibers that specifically inhibit water intake) or whether increases in intensity produce more effective stimulation of critical fibers (thereby increasing the incentive value of the stimulation).

The relative merits of these two hypotheses were assessed by studying the effects of manipulations of train length of RESB on the ability of RESB to reduce food intake. This manipulation was chosen because changes in train length do not influence the size of the stimulating field about the electrode tip. In addition, it has been demonstrated that changes in train length can modify the incentive value of RESB.

Rats were implanted with medial forebrain bundle electrodes (n=12) and trained to press a lever for RESB. They were then tested for baseline food intake on four consecutive days. Food was available only during 45 min test sessions. Three days of ad lib feeding followed these sessions. Subsequently, subjects were given limited access (45 min/day) to food and a lever that produced RESB on a fixed ratio (FR) 4 schedule for four consecutive weeks. A different train length of RESB was used during each week (250, 500, 750 and 1000 msec). Six subjects received an ascending order and six were exposed to a descending order of train length.

Train length was found to have a significant effect on food intake ($p < .05$), as did days of deprivation ($p < .01$), the week of the study ($p < .05$) and the train length X days of deprivation interaction ($p < .05$). We interpret the significant effect of train length and train length X days of deprivation interaction as evidence that RESB can reduce food intake by virtue of its incentive value rather than by replacing food or inhibiting food intake (see Spies, J. Comp. Physiol. Psychol., 60: 153, 1965).

However, it should be noted that the effect was small and that deprivation, experiential and subject-specific factors appear to play an important role in determining the ability of RESB to compete with food and water intake.

58.8 ORGANIZED BEHAVIOR PATTERNS AND SELF-STIMULATION INDUCED BY INTRAHYPOTHALAMIC ELECTRICAL STIMULATION IN 3-DAY-OLD RATS. Timothy H. Moran*, Mark F. Lew*, and Elliott M. Blass, Johns Hopkins University, Baltimore, MD 21218

When 3-day-old rats are stimulated (40-60uAx500m sec) through electrodes terminating in the medial forebrain bundle, a progressive organized pattern of behavioral recruitment is obtained. The rats first mouth and make slight licking movements. If a paw is contacted by the mouth, it is licked. Then licking becomes vigorous and is no longer directed towards the body or the cage substrate. If passage of current is continued, licking becomes extensive and is integrated with exaggerated gaping behaviors. This pattern intensifies and becomes incorporated into whole-body rolling from side to side. This pattern continues past current termination and often gives way to a stretch response that is remarkably similar to that induced by milk letdown. Occasionally, a lordosis response, similar to that seen in palpated, sexually receptive adult females, is also obtained. These behaviors are reliably induced and invariably occur in the sequence just described (N=15).

Only rat pups exhibiting this sequence push a paddle that produces self-stimulation. Rate of lever pressing is approximately double that of yoked control rats (X298.5vs137). Furthermore, in a more difficult 2-lever discrimination task, the rewarded paddle is also pressed with approximately twice the frequency of the nonrewarded one.

These data show that (a) complex motor patterns are organized centrally in 3-day-old rat pups; (b) these patterns can be fractionated into their component parts; and (c) that a central reward system exists in Day 3 rats that, when activated, can support changes in performance instrumental to self-stimulation.

58.9 OROSENSORY FACTORS IN DIETARY SELECTION AFTER FOOD DEPRIVATION AND SUPPLEMENTATION. M. G. Miller, J. F. TEATES*, and H. E. LIPPA*. Food & Drug Administration, Washington, DC, 20204.

To investigate the role of sensory factors in the adjustment of protein/carbohydrate selection to metabolic requirements,"recovered", partially trigeminally deafferented rats (400-450g) and a surgical control group were subjected to metabolic challenges and their food selection patterns were studied. Animals had access to two isocaloric diets, consisting of crude soy or starch, both with equal amounts of fat, vitamins and minerals. Deafferentation impaired somatosensory input from the lower anterior portion of the oral cavity. The metabolic challenges consisted either of depletion by food deprivation (4 days) or of daily supplementation by isocaloric-isovolemic intragastric protein or carbohydrate loads (10 days).

Food-deprived control rats selectively increased their protein intake on the day of refeeding by 30%, apparently compensating for the negative nitrogen balance during food deprivation. Carbohydrate intake was raised insignificantly. A gavage of protein prior to refeeding suppressed the disproportionate increase of protein. Deafferented rats showed no compensatory change in protein intake, and their total food intake stayed at predeprivation levels.

Daily supplemental protein or carbohydrate loads were compensated for calorically by reducing oral intake in the control and deafferentation groups. In contrast, only control animals compensated for the qualitative (protein or carbohydrate) composition of the intubation. This was most conspicuous after protein gavage. The selection pattern in both intubation control groups gradually returned to the preintubation baseline, resulting in highly aberrant intakes (oral + intragastric) of protein or carbohydrate, respectively. Deafferented rats never compensated selectively for the qualitative aspects of the intubation. Reduction of intake from both diet fractions was proportionate throughout the intubation period. The variability of the selection parameters was increased after food deprivation as well as during supplementation.

Control mechanisms for protein intake appear to be sensitive to the acute metabolic state. However, oral somatosensation has to be intact to allow behavioral adjustment.

58.10 ABOLITION OF GLUCOPRIVIC FEEDING BY FOURTH VENTRICULAR INJECTION OF ALLOXAN. Joan M. Murnane and Sue Ritter, Coll. of Vet. Med., Wash. State Univ., Pullman, WA 99164.

Alloxan is a toxic agent which, when injected peripherally, destroys glucose-sensitive pancreatic β cells. Recent studies have shown that alloxan may also be toxic to glucose-sensitive cells within the brain. We have shown that injection of alloxan directly into the lateral brain ventricles permanently impairs the centrally-mediated feeding response to glucoprivation. This deficit appears to be a specific effect of the drug on gluco-receptors since it can be blocked by co-administration of D-glucose. Thus, alloxan appears to be a useful tool with which to localize and characterize brain glucoreceptor cells.

In order to refine the localization of brain glucoreceptors we have compared the effect of lateral (LV) and fourth ventricular (IV V) infusion of alloxan on glucoprivic feeding. Adult male Sprague-Dawley rats were given either alloxan (40 ug in 5 ul saline), glucose plus alloxan (40 ug alloxan in 5 ul of 3M D-glucose) or saline (5 ul) through LV or IV V cannulae. Feeding and blood glucose responses were measured for 6 hr following injection of 2DG (150 and 350 mg/kg, s.c.) or insulin (2 U/kg, s.c.). Blood glucose responses to 2DG and insulin were not altered by alloxan. Although alloxan at both injection sites impaired glucoprivic feeding, IV V injections were most effective. After LV alloxan, rats ate 37% of control in response to 150 mg/kg 2DG ($p < .01$) and 64% of control in response to insulin ($p < .001$), but did not differ significantly from control in response to the higher dose of 2DG. In comparison, in rats that received alloxan in the IV V, feeding was reduced to 29% of control after 150 mg/kg 2DG ($p < .001$), 46% of control after 350 mg/kg 2DG ($p < .001$) and 37% of control after insulin ($p < .001$). As reported previously, animals injected simultaneously with glucose and alloxan did not differ from control on any test. We also found that alloxan itself elicits feeding when injected in low doses into the IV V. This result suggests that alloxan interacts with a cellular component normally involved in detection of glucoprivation. Alloxan does not impair glucoprivic feeding by damaging catecholamine (CA) neurons or by decreasing CA release since neither regional CA concentrations nor the increase in CA turnover normally observed after insulin or 2DG were altered in alloxan treated rats. Our findings (1) support other work suggesting that glucoreceptor cells controlling glucoprivic feeding reside in the hindbrain, (2) suggest that these cells are probably not CA neurons, and (3) suggest that the adrenal response to glucoprivation may be controlled by a population of cells separate from those controlling glucoprivic feeding.

58.11 FRAGMENTED BEHAVIORAL SEQUENCES DURING FEEDING BEHAVIOR FOLLOWING FIMBRIA-FORNIX TRANSECTIONS IN THE RAT. A. B. Dodek* and B. Osborne. Dept. of Psychology, Middlebury College, Middlebury, VT 05753.

Damage to the hippocampus has been shown to alter feeding and drinking behavior, but the role of the hippocampus has been obscured by inconsistencies -- both increased and normal feeding and drinking behavior have been reported. Some of the differences may be a result of differences in time of measurement or conditions of measurement, as well as the particular behavior measured. In an attempt to gain a more complete description of the effects of hippocampal damage on feeding and drinking behavior and to address the inconsistencies in the literature, detailed analysis of feeding and drinking following fimbria-fornix transection were examined. The behavior of 8 rats with complete fimbria-fornix transection and 10 operated controls was continuously monitored and recorded during the AM and PM and ad lib conditions and also following restricted food access. The rats were housed in large individually enclosed observation cages and the following behaviors recorded: food consumption, water consumption, intermeal intervals, meal duration, total eating time, sleep time, general activity, frequencies of rearing, grooming, carrying shavings, sleeping, drinking, eating, and hoarding. It was found that fornix-transected rats were generally more active and exhibited increased frequencies of rearing, eating, and drinking. In addition, the meals and intermeal intervals of fornix-transected animals were of shorter duration. Food and water consumption were unaffected by fornix-transection as were the frequencies of grooming, sleeping, and carrying shavings. Furthermore, fornix-transected rats hoarded less food pellets under restricted food conditions. These results and those from previous studies may be explained by a basic deficit in the organization of behavior. Although behavior remains adaptable and the components of the behavior and diurnal patterns were not altered, the frequency and sequencing of behavior appears disrupted by hippocampal damage. Moreover, these data are comparable to those from feeding studies following recovery from lateral hypothalamic damage (Rowland, N., J. Comp. Physiol. Psychol., 91:1039, 1977) suggesting a functional similarity.

58.12 DISSECTION OF CORTICAL VS DIENCEPHALIC CONTRIBUTIONS TO MOTOR ACTIVITY, ORIENTING, AND EATING USING A DISCONNECTION PARADIGM. I. Q. Whishaw and B. Kolb. Department of Psychology, University of Lethbridge, Lethbridge, Alberta, Canada, T1K 3M4.

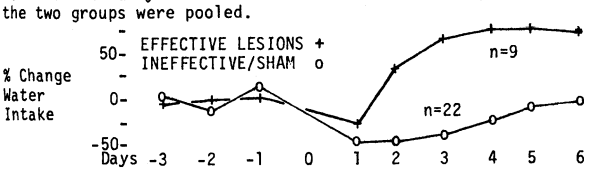
It is well known that lateral hypothalamic (LH) lesions produce aphagia-adipsia, sensorimotor neglect, and catalepsy-akinesia. The rats show a sequence of recovery from each deficit which parallels in some ways the development of movement (see Teitelbaum and Epstein, 1962 and subsequent work). Decortication produces similar types of behavioral changes, but the nature of the abnormalities and the sequence and degree of recovery are partly different (Whishaw, Schallert and Kolb, 1981). In sum, investigations of hypothalamic and cortical function suggest there are at least two systems which partly share control over feeding, sensorimotor and motor activity, and one has a cortical and one has a hypothalamic origin. We are pursuing a line of research intended to dissect the relative contributions of each of these structures. In the present study we report the results obtained using a disconnection paradigm. Unilateral hypothalamic lesions were paired with unilateral-contralateral decortication (control groups consisted of single or combined ipsilateral lesions).

Following unilateral cortex removal, rats displayed profound and chronic contralateral neglect. If the rats also received a contralateral hypothalamic lesion they showed bilateral sensorimotor neglect but recovered orienting contralateral to the LH lesion, in the classic rostrocaudal sequence. Thus, the integrity of the neocortex is necessary for detecting contralateral sensory events. Following unilateral hypothalamic lesions the rats showed a mild turning bias ipsilateral to the lesion; contralateral decortication markedly potentiated this bias. Thus the influence of the intact hypothalamus is released by ipsilateral cortical removal. Rats which received both lesions together showed a syndrome of aphagia-adipsia, sensorimotor neglect, and akinesia-catalepsy. They recovered sensory abilities contralateral to the intact cortex and recovered from a turning bias contralateral to the intact hypothalamus. Recovery from both disabilities occurred in the predictable rostro-caudal sequence. Aphagia and adipsia appeared to be a consequence of these mutually antagonistic disabilities. Thus, the results show that the cortex and the hypothalamus have both facilitory and antagonistic influences in their control of various behaviors.

Teitelbaum, P. and Epstein, A. N. Psych. Rev., 1962, 69, 74-90.
Whishaw, I. Q., Kolb, B. and Schallert, T. JCPP, 1981, 95, 85-103.

58.13 CONTROL OF DRINKING BY A HEMODYNAMIC PONTINE PATHWAY. D. G. Ward and J. H. Kelm* (SPON: M. J. Cronin). Dept. Physiology, Univ. Virginia Sch. of Med., Charlottesville, VA 22908.

There is increasing evidence for a role of vascular volume sensed by atrial receptors in the control of drinking (Zimmerman M. B., et al., Science 211:489, 1981). However the central neural pathways responsible are undefined. We have shown previously that neurons in the anteroventral locus coeruleus and in the locus subcoeruleus respond to changes in atrial filling (Ward, D. G., et al., Brain Res. 181:75, 1980); and that electrical stimulation of the same region inhibits ACTH release by the pituitary (Ward, D. G., et al., Endocrinol. 99:1220, 1976). To define the role of this ascending pathway in the control of drinking, daily water consumption was measured before and after placement of bilateral lesions in the dorsal-rostral pons of 31 cats. Thiopental was used for anesthesia, sterile surgery was performed and 60ml lactated Ringer's was administered intraperitoneally to facilitate recovery. As shown in the Figure, anesthesia alone (SHAM) was associated with decreased water intake. In contrast, lesions that bilaterally included the anteroventral locus coeruleus and the adjacent dorsal subcoeruleus (EFFECTIVE LESIONS) were associated with a marked increase in water intake that was statistically different from the sham animals ($P<0.01$; ANOVA). Lesions that were bilaterally limited to the dorsal or posterior extent of the locus coeruleus, to the dorsal periventricular gray or to the lateral periaqueductal gray (INEFFECTIVE LESIONS) were associated with a pattern of water intake that was not statistically different from the sham animals ($P>0.5$). Thus the two groups were pooled.



The site of highest density of overlap of all the lesions associated with hyperdipsia corresponds precisely to the pontine region shown previously to both inhibit ACTH and contain neurons with input from cardiovascular receptors sensing changes in vascular volume. Accordingly, the present data suggest strongly that the anteroventral locus coeruleus and dorsal subcoeruleus tonically inhibit drinking and is responsible for the control of drinking in response to changes in vascular volume.
Supported by HL26349, RR05431 and HL00827.

58.14 LONGER-TERM ADAPTIVE CHANGES IN AMPHIBIAN NERVOUS SYSTEM. (2) CHANGES IN FEEDING BEHAVIOR DURING OSMOTIC ADAPTATION. Jim W. Dole*, Betty B. Rose*, Ken H. Tachiki*, Roger A. Baldwin* and Claude F. Baxter (SPON: W. Riege). Dept. of Biology, Calif. State University, Northridge, CA 91330, and Neurochemistry Labs, VA Medical Center, Sepulveda, CA 91343.

Feeding behavior of toads is a behavioral paradigm involving a fixed sequence of motor patterns: 1) orienting the body and head toward the prey; 2) extending the tongue and retracting the prey into the mouth; and 3) swallowing the prey. This stimulus-response chain has been extensively studied.

Feeding behavior was assessed in normal, fresh water-adapted (FWA) toads and in toads adapting or adapted to a hyperosmotic (400 mOs) external medium. Toads were placed, one at a time, in an aquarium in the presence of live mealworms. The actions of the toad were observed until six mealworms had been consumed or 15 min had elapsed. Feeding behavior was altered in the hyperosmotically-adapting or -adapted (HOA) toads. We recognize four behavioral states: State A: FWA toads feed rapidly and strike accurately with the tongue. Time elapsed between placement of a toad in the tank and its first attempt to feed (lag time) is short (30±23 sec). Strike accuracy ranged from 67 to 100% ($\bar{X}$ = 89%). State B: Most, but not all, HOA toads showed total disinterest in the mealworms. Instead, they exhibited restlessness and apparent "escape" behavior when placed in the feeding tank. State C: After a variable time period in state B, toads usually showed renewed interest in feeding. In this state, however, lag time was long (173±184 sec) and strike accuracy averaged only 23%; in a 15-min time period, toads often made up to 30-50 tongue flicks, many off-target by a cm or more without successfully capturing a mealworm. State D: Some toads returned to a behavioral state comparable (in strike accuracy and lag time) to that exhibited by FWA toads. The time required to reach a given state was highly variable. Our results show that the HOA toads typically pass through a series of behavioral states returning to a behavior exhibited by FWA toads.

At least two external cues are known to stimulate feeding behavior: visual cues in initiating prey capture are well established and, also, we have found that odors of prey play a role. Toads show evidence of learning the odor of a novel prey after a single experience. Thereafter, the feeding response can often be induced by odor alone. These results suggest that several parts of the brain are involved in the control of feeding behavior.
(Supported in part by a CSUN Foundation Grant (to JWD) and by the Medical Research Service of the Veterans Administration.)

58.15 LONGER-TERM ADAPTIVE CHANGES IN AMPHIBIAN NERVOUS SYSTEM. (1) PUTATIVE NEUROTRANSMITTER AMINO ACID CHANGES DURING HYPEROSMOTIC ADAPTATION. Claude F. Baxter, Roger A. Baldwin*, Ken H. Tachiki*, Jim W. Dole* and Betty B. Rose*. Neurochemistry Labs, VA Medical Center, Sepulveda, CA 91343, and Dept. of Biology, Calif. State University at Northridge, CA 91330.

This is the first of three interrelated abstracts.

It has been observed that when the toad Bufo boreas is adapting to an altered osmotic environment, this adaptation is accompanied by significant changes in the levels of many amino acids in tissues of its nervous system. Specifically, when plasma osmolality is elevated (with NaCl) to 400 mOs or above, the levels of all putative neurotransmitter amino acids (PNTAA), excepting taurine, are elevated in brain tissues within 3 to 5 hrs. Levels of glutamate, aspartate, γ-aminobutyric acid and glycine (expressed as μmol/g tissue wet wt.) are elevated within 24 hrs by from 100% to 200%. Similar elevations of PNTAA are observed after 24 hrs, when the non-ionic osmolyte mannitol is used to elevate plasma osmolality. However, in this latter case, the PNTAA elevations are in part offset by a decreased water content of the brain tissues. Whereas NaCl hyperosmolality resulted in an 8% decrease in cerebral water content, an equivalent hyperosmolality induced with mannitol resulted in a 22% decrease in cerebral water content. Using NaCl as the osmolyte, the peak PNTAA concentrations in brain tissues are reached within 48 to 72 hrs. These levels are maintained for from 72 to 96 hrs and then decline, returning to more normal levels within another 72 to 96 hrs. With mannitol as the osmolyte, the early time sequence of amino acid changes are slower than those described with NaCl but, in both cases, the rates of PNTAA changes are compatible with some inductive mechanisms. All evidence to date indicates that these inductive mechanisms, whether inside or outside of the nervous system, are unrelated to corticosteroids or antidiuretic hormone. A comparison of changes in brain PNTAA induced by lactamide, mannitol and NaCl suggests that the extent of elevation in PNTAA is correlated to the octanol/water partition coefficient (i.e., membrane permeability) of each osmolyte. Thus, the restrictive compartmentation of an osmolyte between blood plasma and brain tissues and/or interstitial fluid and the intracellular compartments, rather than the actual plasma osmolality, may initiate the inductive mechanisms. Other experimental data suggest that elevated PNTAA levels in brain tissues may, in part, be the result of an altered transport of these amino acids across the blood-brain barrier. (Supported by the Medical Research Service of the Veterans Administration.)

58.16 LONGER-TERM ADAPTIVE CHANGES IN AMPHIBIAN NERVOUS SYSTEM. (3) NEUROCHEMICAL CORRELATES OF PUTATIVE NEUROTRANSMITTER AMINO ACID CHANGES AND FEEDING BEHAVIOR. Ken H. Tachiki*, Jim W. Dole*, Betty B. Rose*, Roger A. Baldwin* and Claude F. Baxter (SPON: E. Geller). Neurochemistry Labs, VA Medical Center, Sepulveda, CA 91343, and Dept. of Biology, Calif. State University at Northridge, CA 91330.

The adaptation of toads (Bufo boreas) to a hyperosmotic environment results both in alterations of levels of putative neurotransmitter amino acids (PNTAA) in the brain, and in changes of feeding behavior in response to the presentation of a prey which the toads had learned to identify as food. The brains from toads sacrificed before treatment (fresh water controls) and following initiation of hyperosmotic adaptation were dissected into three areas: cerebral hemispheres, optic lobes and olfactory bulbs. Each sample was analyzed for free amino acids employing an automated amino acid analyzer. Patterns of temporal changes in PNTAA levels of the cerebral hemispheres were compared with changes in patterns of feeding behavior. Results indicated a correlation of the PNTAA glycine, glutamate and γ-aminobutyric acid (GABA) with behavior. Marked changes in levels of aspartate occurred during time periods when little or no behavioral changes were observed.

Distinct states of feeding behavior were observed during 12 days of observation. These states were evaluated independent of adaptation time. State A represents the apparently normal feeding behavior seen in toads prior to hyperosmotic adaptation. In state B, feeding behavior is extremely low or absent. State C represents an intermediate state in which there is attempted feeding behavior but little catching of prey. We have designated apparently normal feeding behavior in hyperosmotically-adapted toads as state D. Plasma osmolality of toads in states B, C and D were essentially identical. A comparison of the PNTAA levels in the cerebral hemispheres of toads of state A vs. state B and state B vs. state D indicated a correlation between feeding behavior and levels of aspartate, glutamate and GABA. If both methods of analysis (i.e., pattern analysis and content analysis) are applied, only the PNTAA glutamate and GABA show a consistent correlation with feeding behavior in the cerebral hemisphere. Results of similar analyses of PNTAA in the optic lobes and the olfactory bulbs of these toads will be presented. (Supported by the Medical Research Service of the Veterans Administration.)

59.1 INFEROTEMPORAL NEURON DISCHARGE IN VISUAL MEMORY. Joaquin M. Fuster and John P. Jervey*. Department of Psychiatry and Brain Research Institute, School of Medicine, University of California, Los Angeles, Calif. 90024.

This research is an attempt to elucidate the involvement of the neurons of the inferotemporal cortex (area TE, Bonin and Bailey, 1947) in higher visual functions. To that end, single-unit activity was explored with microelectrodes in the inferotemporal cortex of nine monkeys performing a task (delayed matching-to-sample) that required the perception and temporary retention of colors or patterns presented in a translucent plastic disk. The activity of some 500 task-related units was recorded extracellularly and statistically analyzed.

A large majority of the inferotemporal units investigated showed an excitatory reaction on appearance of the stimulus. About thirty percent showed different reactions depending on which color appeared in the disk. Many units exhibited activated firing in the period (16 - 20 sec.) during which the animal had to retain the stimulus to meet task demands. Differential (i.e., color-dependent) firing during the retention period was seen in about one-tenth of the cells. Cells activated during that period, whether differentially or not, were found particularly common in the cortex of the lower bank of the superior temporal sulcus. By contrast, inhibition and lack of differentiation in the retention period were the rule among units in a region outside of inferotemporal cortex (i.e., upper bank of that sulcus). The use of color/pattern combinations revealed that some inferotemporal units responded differently to a color depending on whether or not that color had to be attended to in order to meet the requirements of the task. Other units, however, appeared attuned to physical properties of the stimulus regardless of context.

The results of this study indicate that inferotemporal neurons participate in encoding and temporary retention of visual information. The latter function seems to be primarily represented in the cortex of the ventral bank of the superior temporal sulcus.

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59.2 VISUOTOPIC ORGANIZATION ON THE PRELUNATE GYRUS IN THE RHESUS MONKEY. W. M. Maguire* & J. S. Baizer. Division of Neurobiology, Physiology Dept., SUNY/AB Med. Sch., Buffalo New York 14226.

We have studied the visuotopic organization of the dorsal two thirds of the prelunate gyrus and surrounding cortex, a region comprising the most dorsal and medial parts of V4, as anatomically defined by Zeki. Cells and cell clusters were studied in the awake monkey trained to maintain fixation on a small spot of light.

We have found two topographic representations of the lower contralateral visual quadrant, including the central 30^{o} of the visual field. Together, they occupy the middle third of the gyrus and extend into the anterior bank of lunate sulcus and the posterior bank of superior temporal sulcus. They are contiguous, joined by a representation of the vertical meridian and the periphery. Their peripheral representations lie near the lip of the posterior bank of the superior temporal sulcus, a few millimeters above where the Sylvian fissure joins it.

Receptive fields cluster around the vertical meridian and become increasingly central as one moves diagonally across the gyrus in a generally ventral direction moving into the anterior bank of lunate sulcus. The more ventral area increases considerably in size as one moves into areas of central representation. The area ventrally covers the gyrus completely, occupying parts of superior temporal sulcus and anterior lunate as well. Its horizontal meridian is down in superior temporal sulcus. The foveal boundary is as yet undetermined.

The more dorsal area contains a representation of part of the upper contralateral field as well as the lower. The horizontal meridian runs in a generally posterior direction across the gyrus, beginning a few millimeters above the level of the Sylvian fissure and extending into the anterior bank of the lunate sulcus. A complete representation of the central 10^{o} of the upper visual field lies dorsal to the meridian.

Dorsal to these two areas, cortex remains visually responsive, though more dorsally it becomes increasingly difficult to drive. We are as yet unable to characterize the apparently haphazard organization here.

The topography of the gyrus is made difficult to see by large receptive fields and considerable field scatter. We have found it can be clearly revealed by careful plotting of multiunit receptive field centers and the use of many closely spaced tracks in a single animal.

59.3 PATTERNS OF 2-DEOXYGLUCOSE LABELING IN EXTRASTRIATE VISUAL CORTEX OF UNSTIMULATED AND UNIDIRECTIONALLY STIMULATED MACAQUE MONKEYS. A.Burkhalter*, D.C.Van Essen and J.H.R. Maunsell. Division of Biology, California Institute of Technology, Pasadena, CA 91125.

Physiological recordings in the posterior bank of the superior temporal sulcus (STS) of the macaque monkey have shown a high incidence of direction selective neurons in the middle temporal area (MT) and an area medial to it (Van Essen et.al.,J.Comp. Neurol.:198,1981).

We have used the 2-deoxyglucose (2DG) labeling technique to examine the spatial organization of cells in the STS having similar direction preferences. Animals were anaestethized with N_2O and paralyzed prior to 2DG injection. The pattern of labeling in the right hemisphere of an animal exposed to left-to-right stimulation in the left hemifield was compared to that in the unstimulated hemisphere and in both hemispheres of a control animal given no visual stimulation.

In both hemispheres of the control animal the labeling pattern was remarkably non-uniform throughout extrastriate cortex. Labeling was usually densest in layer IV, but isolated patches in other layers as well as radially oriented columns of high density were common. The distribution of columnar or patchy labeling was not entirely random, in that the labeling was consistently more dense along the V1/V2 border and along the margin of MT. Labeling within MT showed occasional patches and columns. There was no obvious asymmetry in the labeling of the two sides, but the detailed patterns were not identical.

In the experimental animal there was a significant asymmetry in the labeling within the STS. This was especially pronounced in the dorsal part of MT, where the visual periphery is represented and where the stimulation was probably most effective. In this region there were distinct columns on the stimulated side and more uniform labeling, albeit with some patchiness on the unstimulated side. Columns on the stimulated side were 0.2 - 0.4mm wide, spaced about 1mm apart and aligned with columns on neighboring sections over a dorso-ventral extent of about 1mm. In the STS lateral to MT, where direction selectivity is not a prominent feature, distinct columns of about the same size and spacing were observed on the stimulated side, while the patches on the unstimulated side as well as in the control animal were less sharply defined.

These results suggest that stimulation altered the pattern of 2DG uptake in the STS and that the representation of stimulus direction in MT is at least to some degree organized in columnar fashion.

59.4 STRUCTURE AND DISTRIBUTION OF FUNCTIONAL COLUMNS IN MONKEY VISUAL CORTEX. M.L.J. Crawford, J. Miears* and L. Black*. U. of Texas, Houston, Texas 77025.

By scanning microdensitometry, we have mapped the distributions of 2-Deoxyglucose patterns induced in the visual cortex of monkeys stimulated by luminance-matched grating patterns. Serial assembly of density patterns illustrates a mosaic of columns throughout striate cortex which differs in spacing from foveal to more peripheral visual field representations.

Scanning the striate columns across the cortical layers shows a density profile difference between columns induced by black and white and color stimulation. Binocular black and white stimulus induced columns have two density peaks, one in layer 6 and another in layer 4, with relatively less activity in superficial layers. Luminance matched red and green stimulation produces high densities in lower layer 4 and layer 5 with a high density in what appears to be layer 3. The two patterns appear similar in structure, but the color pattern is shifted in phase toward more superficial layers.

Pre-striate patterns are mapped and shown to be stimulus dependent but do not seem to change in V2 between color and luminance stimulation. Distributed throughout the posterior bank of lunate and extending downward to occupy both banks of the inferior occipital sulcus, these high density patterns appear as spots or triangles with the base deep in layer 5 and 6 and virtually no pattern in the superficial layers. Serial profiles show these columns to wax and wane in density as they course in approximately parallel rows.

59.5 DYNAMICS OF FIRING PATTERNS IN THE VISUAL CORTEX. J. Patera*, Center for Applied Mathematics, University of Montreal, Montreal, Quebec, Canada and Gordon L. Shaw, Physics Department, University of California, Irvine, CA 92717.

Mountcastle has presented a columnar organizing principle for the functioning of the neocortex in which there are basic irreducible processing units: minicolumns of neurons. The minicolumns are coupled together into columns or networks presumably resulting in sophisticated spatial-temporal firing capability, that is, sophisticated processing. Consider the primary visual cortex where Hubel-Wiesel analyses established the orientation specificity (as well as other attributes such as left-right dominance) to light bar stimuli of the minicolumns. It was suggested (G. Shaw, P. Rinaldi, K. Roney, to be published) that sequences of rotating light bar stimuli be used along with one or several closely spaced recording microelectrodes to study the possible dynamics or flow of firing activity among the minicolumns in the column (the Hubel-Wiesel hypercolumn). An investigation of these effects requires variation of a number of parameters associated with the stimulus. We present a framework for developing and describing in a simple pictorial manner the various relevant sequences of time dependent stimuli. The measured firing responses can then be placed on the same plot and the "effective connectivity" between neurons in different minicolumns can be deduced. These plots are also used to design and interpret psychophysics experiments analogous to the electrophysiology one noted above.

59.6 NUMBER OF NEURONS IN LAYER IV OF A CORTICAL "MODULE." Bennett Solnick*, Thomas L. Davis, and Peter Sterling. Dept. of Anatomy, Univ. of Penna. Sch. of Med., Phila., Penna. 19104.

A cortical column might be formed, according to Mountcastle, by replication of a smaller, precisely-wired neural circuit which he termed a "module." In cat area 17 the smallest modular unit may be that which analyzes with one eye (half of the ocular dominance hypercolumn) a small region of visual field for stimulus orientation over 10 degrees (one-eighteenth of the orientation hypercolumn; Hubel and Wiesel, '74). The tangential dimensions of such a module are 500 X 48 μm and the volume occupied in this module by layer IVab (250 μm thick) is $6 \times 10^6 \mu m^3$. We have estimated the total number of neurons in this volume and also the upper limit of the number of cells belonging to several specific cell types.

Three series of one-micron thick autoradiograms were prepared from tissue in which exogenous ^{3}H-GABA had been accumulated by certain neuronal types. The total number of neurons was estimated by counting neuronal nucleoli and dividing by the average number of sections in which a nucleolus appeared. We estimated the number of type II stellates (large, dense somatic distribution of round and flat vesicle endings) by counting all large neurons (17-22 μm) that showed, in an electron micrograph of an adjacent thin section, a high density of round and flat vesicle endings. The number of neurons accumulating exogenous GABA at 3-10 times background were also counted; these are known to comprise several subgroups (Hamos, Davis, and Sterling, this Volume).

For that part of a module occupied by layer IVab, the total number of neurons was about 350. Of these, no more than 12 (3.5%) could be class II. The actual number is probably smaller since there were indications within this group of heterogeneity. About 28 cells (8%) accumulated GABA. Since there are at least four subgroups of these cells, the maximum number belonging to a single type is probably about 1-2%.

That the receptive fields of individual neurons scatter means that the patch of visual field "seen" by the module is larger than the individual receptive fields. If every point in this patch is to be seen by at least one member of each cell type, then several members of each type must be present in the module. The present findings suggest, therefore, that the number of each cell type present within a module may be close to the minimum needed to give complete coverage. At this level of organization there may be very little "redundancy". (Supported by NEI grant EY00828 and NINCDS NS05881).

59.7 SEVERAL GROUPS OF NEURONS IN LAYER IVAB OF CAT AREA 17 ACCUMULATE ^{3}H-GAMMA-AMINOBUTYRIC ACID (GABA). James E. Hamos, Thomas L. Davis and Peter Sterling. Dept. Anat., Univ. Pa., Phila., Pa. 19104.

In earlier experiments, we found that approximately 10% of the neurons in layers II-VI in cortical area 17 accumulate ^{3}H-GABA. The neurons in layer IV were stellate and sparsely contacted by synaptic terminals. In this study, we have examined GABA-accumulating neurons in layer IVab in greater detail and found evidence of considerable morphological heterogeneity.

We injected into the visual cortex .02-1μl of ^{3}H-GABA (2.5×10^{-4}M) or a mixture of ^{3}H-GABA (2.5×10^{-3}M) and 'cold' β-alanine (1.5×10^{-5}M) and perfused with aldehydes one to two hours later. Blocks near the injection sites in area 17 were processed for electron microscopy and series of consecutive thin sections were prepared as electron microscope autoradiograms. By partially reconstructing the cell bodies of GABA-accumulating neurons, we were able to group them according to differences in level of GABA accumulation, soma shape and size, distribution of terminals on the soma, and ratio of flat vesicle to round vesicle terminals.

Certain neurons, as reported previously, were heavily labeled (>10X background) and had a sparse distribution of somatic synaptic terminals (<20 terminals/100μm^2). This group could be further subdivided into medium-sized spherical cells (11-15μm in diameter) and large fusiform cells (20-25μm X 7-12μm). Another group of neurons was distinctly but more moderately labeled (2.5-6X background) and had a dense distribution of somatic synaptic terminals (>20 terminals/100μm^2). These neurons could be subdivided into small spherical cells (10-12μm) and larger spherical ones (15-18μm).

GABA is important in generating certain properties of the receptive fields of simple and complex cells (Sillito, 1975). Should the GABA-accumulating neurons prove to be GABAergic, it will be of interest to determine which of these morphologically different cell groups might be responsible for the various physiological effects. (Supported by NEI grants EY00828 and EY05397).

59.8 CHANDELIER CELLS IN RAT VISUAL CORTEX. Alan Peters, Dept. of Anatomy, Boston Univ. Sch. of Med., Boston, MA 02118.

Golgi impregnated chandelier cells in rat visual cortex have been examined by both light and electron microscopy, using the combined Golgi/EM technique of gold toning. All of the impregnated chandelier cells encountered have been in layer II/III. Many are present throughout area 17, but their numbers increase at the area 17/18a border, and to a somewhat lesser extent at the area 17/18 border. Most of the chandelier cells are bitufted neurons with groups of dendrites extending from the upper and lower poles of the cell body, although some have a more multipolar configuration. The cell bodies are characterized by a pale nucleus surrounded by perikaryal cytoplasm rich in cisternae of rough endoplasmic reticulum and ribosomes. Both the cell body and the sparsely spinous dendrites receive axon terminals forming asymmetric and symmetric synapses. The most characteristic feature of these neurons is the axon, which arises from either the lower pole of the cell body, or from the base of one of the dendrites in the lower tuft. It forms branches which spread laterally and terminate in vertical strings of boutons. The boutons in each string form symmetric synapses with the axon initial segments of layer II/III pyramidal cells, the uppermost bouton in each string being 8-14 μm distant from the pyramidal cell axon hillock. The boutons of the chandelier cell axon are irregular in shape and packed with pleomorphic synaptic vesicles, so that they are readily identified in thin sections. Some layer II/III pyramidal cells seem to receive boutons from more than one chandelier cell, and still others appear to receive no chandelier cell synapses. Evidence is presented to show that the axonal boutons of chandelier cells give a positive reaction with antibody to glutamic acid decarboxylase (GAD), the enzyme which synthesizes GABA. Hence chandelier cells are GABA-ergic neurons and are probably inhibitory in function. Other GAD-positive axon terminals synapse with the more proximal portions of the pyramidal cell axon initial segments, as well as with their axon hillocks, cell bodies and dendrites. These latter terminals are probably derived from the smooth or sparsely spinous multipolar stellate cells, so that at least two different types of non-pyramidal cells inhibit the layer II/III pyramidal cell of rat visual cortex.

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59.9 COMPARISON OF THE RESPONSE PROPERTIES OF THREE TYPES OF MONOSYNAPTIC S CELL IN CAT STRIATE CORTEX. Michael J. Mustari, Jean Bullier* and Geoffrey H. Henry*. Dept. of Physiology, Australian National University, Canberra, A.C.T.

The receptive field properties of three types of S cell receiving a monosynaptic input from the LGN were quantitatively studied in the striate cortex of the anaesthetised and paralysed cat. The nature of the afferent input to these units was identified from their response latency to electrical stimulation delivered at different sites in the optic tract and optic radiations. From these latency measurements we determined whether the cell under study received a direct input from the LGN and whether this input originated from brisk transient (BT) or brisk sustained (BS) cells (Bullier, J. and Henry, G.H., J. Neurophysiol. 42:1251, 1979).

Two types of monosynaptic S cell were located in lamina 4, one type received a fast conducting input from the fast stream (BT) while the other type received its input from the slow (BS) stream. The third group comprised monosynaptic S cells located in lamina 6. When response properties of the 3 types of S cell were compared no significant differences were observed in direction selectivity, ocular dominance, binocular interaction, the strength of side band inhibition, spontaneous activity, or in the persistence of the response to flashing stimuli.

Class differences were found in the size of the receptive fields. S cells in lamina 4a, belonging to the fast stream in general, had larger receptive fields than their counterparts in lamina 4b driven by the slow stream. If 1° is taken as a cut off value, 80% of S cells in the fast stream were found to have receptive field measurements (in both the dimensions parallel and perpendicular to the optimal orientation) greater than the cut off while in 80% of S cells in the slow stream each of these two dimensions was less than the cut off value of 1°. Both of these S cell types exhibited strong end zone inhibition. By contrast the monosynaptic S cells of lamina 6 had weak or no end zone inhibition and had receptive field lengths (parallel to optimal orientation) in the range of 2-6°. In the dimension perpendicular to the line of optimal orientation there was no observed distinction between lamina 4 and lamina 6 S cells. In their velocity preference most (80%) fast input S cells of lamina 4 responded to stimuli moving faster than 10°/sec while slow input S cells ceased to respond when stimulus velocity exceeded this level.

The presence of quantitative differences in receptive fields that are essentially similar to one another suggest that fast and slow input S cells of lamina 4 perform complementary functional roles in the striate cortex.

59.10 CORRELATION BETWEEN VELOCITY CHARACTERISTICS AND RESPONSIVENESS TO STATIONARY STIMULI IN VISUAL CORTICAL NEURONS OF THE CAT. J. Duysens, G.A. Orban and O. Verbeke*. Lab. Neuro- en Psychofysiologie, KULeuven, Campus Gasthuisberg, B-3000 Leuven, Belgium.

In the visual cortex one finds cells which respond exclusively to slow stimulus movement (velocity low pass type, VLP) and others which prefer very fast movement (velocity high pass type, VHP; Orban et al., J. Neurophysiol., in press). To understand the mechanisms underlying the differences between these two velocity types the responses to stationary presented stimuli were compared with the responses to moving stimuli in 66 cells from areas 17, 18 and 19 in paralyzed and N_2O anesthetized cats. A narrow bar of light was projected either over different parts of the receptive field for a fixed period of 1 second or else at a fixed location but with different durations ranging from 20 to 5000 msec.

VLP cells were found to require much longer minimal durations of stationary stimulation (> 80 msec) than VHP cells which fired even for stimuli as short as 20 msec provided high contrast and optimal slit width were used. The minimal duration of stimulation could be used to accurately predict the upper cut-off velocity of VLP cells indicating that the inability of these cells to respond at high velocities is mainly linked to their high demand for temporal summation. VLP cells were also found to respond tonically and with long latency (median = 254 msec) to flashed bars of light whereas VHP cells discharge phasically and with short latency (median = 58 msec). Some cells exhibited a velocity response profile intermediate between the VHP and VLP type. These cells, which responded well to slow or fast movement but little to medium velocities, showed both phasic and tonic response components when presented with stationary stimuli. No strict correlation was found between the different velocity types and the presence or absence of spatial overlap of ON and OFF zones.

It is concluded that VLP and VHP cells constitute distinguishable populations not only on the basis of their velocity characteristics but also with respect to their response properties in the presence of stationary stimuli.

59.11 RESPONSE PROPERTIES OF CELLS IN THE VISUAL CORTEX OF THE RAT. R.A. Burne, J.G. Parnavelas, and C.-S. Lin, Dept. of Cell Biology, The Univ. Tx. Health Sci. Ctr., Dallas, Tx. 75235

This study was undertaken with the general aim of correlating structural and functional properties of single neurons in the visual cortex, area 17, of Long Evans pigmented rats. Extracellular spike responses to visual stimuli presented manually or by a computer-controlled system were recorded in animals anesthetized with 1% halothane. Post-stimulus time histograms and raster analyses were used to quantify response characteristics following presentation of visual stimuli. The morphological structure of functionally identified cells was determined with intracellular injection of horseradish peroxidase (HRP). Beveled glass micropipettes (20-70 Megohms) containing 4-8% HRP in 0.2 M KCl with 0.1 M Tris buffer at pH 7.6 were used in this study.

Visually responsive cells comprised 90% (296/327) of the cells recorded in area 17. Five percent of these cells responded only to stationary stimuli with either on-center (9/15; 60%), off-center (2/15; 14%) or on-off (4/15; 26%) properties. The remaining neurons (95%) responded to both stationary and moving stimuli or only to movement. They were classified as complex, simple, hypercomplex or non-oriented. Complex cells comprised the majority (122/281; 44%) of the movement sensitive cells and were distinguished from simple cells (77/281; 27%) by 1) the presence of spatial overlap of excitatory peaks evoked by a stimulus moving through the field in opposite directions, 2) the absence of inhibitory sidebands and 3) the lack of spatial summation in the receptive field. Hypercomplex cells (35/281; 13%) differed from other cell types by demonstrating a reduction (> 60%) in response frequency as the preferred stimulus was elongated beyond the excitatory region. Non-oriented cells (45/281; 16%) responded to moving stimuli over a wide range of orientations.

Of the cells successfully filled with HRP, complex cells (15) were pyramidal in morphology and located in layers II through VI. Simple cells (4) were both pyramidal and non-pyramidal in appearance and were located in layers II & III and IV. Finally, hypercomplex cells (3) were pyramidal in appearance and their perikarya were situated in layers II & III and V.

Our results suggest that neurons of the rat visual cortex have well defined receptive fields and have properties similar to those reported for animals with more developed visual systems.

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59.12 VESTIBULAR SYSTEM MODIFICATION OF SOME NEURONAL CHARACTERISTICS IN THE VISUAL CORTEX OF THE CAT. R. H. Lahue, Jr.*, S. Reinis, D. S. Weiss*, K. E. Money and J. P. Landolt, (SPON: R. G. Marteniuk) Univ. of Waterloo, Waterloo, & DCIEM, Toronto, Canada

The intertrial firing rate and orientation specificity of cells in the visual cortex of the cat as well as the size, shape and retinal position of their receptive fields have been shown to be affected by head and body tilt. To further analyze this phenomenon, some of these characteristics have been investigated following the administration of two nystaganogenic agents, deuterium oxide and mannitol, and following galvanic stimulation of the vestibular system. While these manipulations did not affect receptive field position, they did lead to alterations in receptive field size and shape and intertrial spontaneous firing rate. Changes in area were often biphasic with a brief initial decrease followed by an extensive and extended increase. Changes in receptive field area and spontaneous firing rate were not always parallel. Neither receptive field characteristics nor spontaneous firing rate were affected in labyrinthectomized animals subjected to the same manipulations. Furthermore, the spontaneous firing rate of non-visual cortical cells was unaffected. These experiments demonstrate that the vestibular system participates in the control of activity of visual cortical cells and that functional modification of cell characteristics as a result of head tilt is not artifactual.

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59.13 EFFECT OF LABYRINTHECTOMY ON VISUAL CORTICAL CELL CHARACTERISTICS IN THE CAT. S. Reinis, R. H. Lahue Jr.*, D. S. Weiss*, J. P. Landolt and K. E. Money. U. of Waterloo, Waterloo, and DCIEM, Toronto, Ont., Canada.

Receptive field size, shape and retinal position, as well as optimal stimulus parameters and intertrial firing rates of complex cells in cortical area 18 were examined in terms of their responses to head tilting of 10^{o}, 20^{o} and 30^{o} in both intact and labyrinthectomized cats. In intact animals, receptive field size changed in all cells in a more or less regular fashion as a function of head tilt. Receptive field position and intertrial firing rate also varied though less regularly. In labyrinthectomized animals changes in receptive field area were usually within the probable limits of experimental variability. However, the variability in receptive field position and intertrial spontaneous firing rates in labyrinthectomized animals equalled that observed in intact animals. These results indicate that several characteristics of visual cortical cells are altered by head tilt, but vestibular input is not the only factor controlling these changes.

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59.14 EFFECTS OF CONVERGENT SQUINT IN CATS ON TEMPORAL PROPERTIES OF STRIATE NEURONS, Michael S. Shansky,* Yuzo M. Chino* and Wayne L. Jankowski,* Fredrich A. Banser,* Illinois College of Optometry, Chicago, IL.

Kittens were reared with a surgically-induced convergent squint in one eye (via section of the lateral rectus during the third post-natal week), the temporal response properties of cortical cells were measured and comparisons made between the squinting eye, non-squinting eye and normally reared cats. The best responses to varying drift rates of sinusoidal gratings of an optiimum spatial frequency were shifted to lower temporal frequencies in the strabismic cats for both the operated and non-operated eyes compared to normal cats. Likewise the temporal high frequency cutoffs were significantly lower among all the cell groups in the squinting animals. In addition, a dramatic increase in both latency of response to chiasm stimulation and visual stimulation was found for the squinting and non-squinting eyes in the experimental animals, such that the latency values were, in many cases, more than twice those found in normal cats. Spontaneous activity was also significantly higher in the strabismic cats, but no differences were found in the peak responses to a moving slit of light. These results suggest that animals reared with abnormal interocular alignments will suffer major neurophysiological deficits with regard to processing temporal information.

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59.15 A CONVERGENT SQUINT IN KITTENS ALTERS SPATIAL RESPONSE PROPERTIES OF STRIATE CORTEX UNITS, Yuzo M. Chino,* Michael S. Shansky,* Frederick A. Banser,* and Wayne Jankowski,* Illinois College of Optometry, Chicago, IL.

Spatial properties of striate neurons were investigated in cats reared with a unilateral, surgically-induced convergent squint. Extracellular recordings were made when the animals were one year old. Contrast sensitivity functions in the operated cats, extrapolated from responses to drifting sinusoidal gratings, revealed shifts to lower spatial frequencies of both the peaks and high-frequency cut-offs.

Receptive field dimensions were significantly larger in the squinting animals, the orientation tuning (half-width at half-height) was far less sharp in normally-reared cats.

Some properties (e.g. direction selectivity) appeared to be unaltered in the strabismic animals, but overall we conclude that there is a significant modification of receptive field properties in the cortices of cats sustaining convergent squint during development.

(Supported by Grants EY-01444 and EY-03588)

59.16 TEMPORAL INTEGRATION IN THE VISUAL SYSTEMS OF THE CAT AND HUMAN. D. Brenner, E. Kaplan* and R. Shapley* Rockefeller University, New York, NY 10021.

We investigated the temporal integration of visual signals in the nervous system by measuring the phase of the neural response as a function of temporal frequency. Stimuli were sine wave gratings undergoing contrast reversal with a sinusoidal time course at temporal frequencies from 0.5Hz to 80Hz. The spatial frequency range was 0 to 3c/deg and 0.05 to 0.4 peak contrast. Average luminance was in the range 20-60 cd/m^2. Neural responses were averaged and Fourier analyzed to yield response amplitude and phase at the modulation frequency and its harmonics. For 47 cells of the cat lateral geniculate nucleus, and for 32 geniculate cell/s-potential pairs, the phase was proportional to temporal frequency from about 3Hz to about 30Hz. The slope of the line was approximately 40 msec. for the geniculate cells and their retinal inputs represented by s-potentials. Spatial frequency and contrast had rather little effect on the slope of the phase line in the geniculate or retina. The slopes were similar for X and Y, and "on" and "off" cells.

The linear phase vs. temporal frequency dependence is consistent with a constant time delay, or latency, but it is also consistent with a more plausible model, namely a cascade of neural low-pass filters. The slope of 40 msec. would be the sum of the time constants of the filters in the cascade.

Visually evoked cortical responses (to the same kind of stimuli) in cat and in man were subjected to the same analysis. The cat evoked potentials were measured with electrodes placed within area 17 of the cat's cortex. The human evoked responses had been measured previously by neuromagnetic recording from the occipital cortex. Both sets of evoked response data yielded similar linear phase vs. temporal frequency graphs, with slopes of approximately 80-120 msec. In terms of the filter cascade model, there is an additional stage of temporal filtering between the geniculate input and the units which generate the cortical evoked response. This stage of cortical temporal filtering has an approximate total integration time of 60 msec. The similarity between results in cat and in man suggests a similarity in cortical mechanisms of temporal integration.

This work was supported by NEI grants EY1472 and EY188.

59.17 CRANIAL, SUBDURAL AND INTRACORTICAL RESPONSES TO VISUAL STIMULI IN THE AWAKE RHESUS MONKEY. G. Dagnelie*, E.H. van der Marel* and H. Spekreijse* (SPON: M.H. von Meijenfeldt). The Netherlands Ophthalmic Research Institute, 1005 EK Amsterdam, Netherlands.

The search for the origin of the visually evoked potential (VEP) can be advanced by the use of invasive recording techniques. Since the rhesus monkey's visual cortex presents a structure analogous to that of man we have compared VEPs recorded at the scalp of monkey to those recorded simultaneously with cranial, subdural and intracortical electrodes.

Four rhesus monkeys were trained to fixate at a TV screen on which checkerboard and bar patterns as well as homogeneous fields could be presented. After establishing the VEP distribution with scalp electrodes a chronic preparation was obtained by placing five stainless steel screws in the skull bone and three electrode bundles (each containing seven recording sites at 5 mm intervals) in the cerebrospinal fluid over the temporo-occipital cortex. When the relationship between scalp and subdural VEP had been determined two electrode bundles containing seven recording sites at 0.3 and 0.5 mm intervals were inserted perpendicularly 2.5 cm from the midline into area 17. For those components of the VEP that originate close to these bundles it was expected that a change of polarity would be observed from superficial to deep cortical layers. A connector on the frontal part of the skull permitted a free choice of derivations during the experiment.

The following conclusions were reached:

- VEPs from bone electrodes bear a strong resemblance to VEPs recorded with scalp electrodes
- VEPs from subdural electrodes show a stronger dependence on electrode location than bone or scalp derivations
- The subdural VEP to luminance stimuli is less localized and represented more occipitally than those to pattern stimuli
- In subdural VEPs the different cortical origin of the response components can be established more easily than in scalp or bone recordings
- In the intracortically recorded VEP a change of polarity of response components is only observed under a limited number of stimulus conditions

59.18 Neural Circuitry of the Neocortex examined in the in vitro brain slice preparation. N. Chiaia, C. Shaw and T. J. Teyler. Neurobiology Program, N.E. Ohio College of Medicine, Rootstown OH 44272 and Psychology Dept., Dalhousie Univ., Halifax, Nova Scotia B3H 4J1.

The in vitro brain slice technique has been applied to the study of the neocortex. Cortical blocks were removed from adult rats deeply anesthesized with halothane, sectioned coronally at 400-700 u, and placed in a brain slice chamber. Cortical slices typically showed normal histology and spontaneous and evoked neural activity for 8 hours or longer. Single unit and evoked potential recordings were made from different layers of the cortex using micropipettes. Evoked potentials to afferent simulation of differing intensity and frequency and from different cortical layers were analyzed.

Evoked potentials from all but the most superficial layers of the cortex showed a characteristic 6 component response to simulation of underlying white matter. This evoked potential closely resembled cortical responses recorded in vivo by other investigators following afferent stimulation. The response amplitude of all components increased as stimulus intensity was raised. Radial movement of the recording electrode showed the components 1, 2, and 3 had their largest amplitude in the deepest cortical layers. Component 4 reached its greatest amplitude and shortest latency in layer 4, and components 5 and 6 reached their greatest amplitudes in the more superficial layers. The frequency following for various components was measured showing greater decline in amplitude for components 4 through 6 than for 1 through 3.

These results, together with those from in vivo investigations suggest that the first 3 components represent afferent fiber input. Component 4 represents the initial postsynaptic response which is localized in layer 4. Components 5 and 6 represent later additional cortical activity. Further support for the intercortical orgin of component 4 was provided by lateral intercortical stimulation within layer 4 giving an evoked potential composed mostly of component 4. With lateral movement of the recording electrode in layer 4 the evoked potential disappeared in under 1mm, suggesting a fairly restricted afferent input to the recording site. These results suggest that the cortical brain slice may be an appropriate model system in which to study cortical neural circuitry.

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59.19 QUALITY OF COLOR VISION OF DECORTICATE GROUND SQUIRRELS. E. Kicliter. Lab. of Neurobiology and Dept. of Anatomy, Univ. of Puerto Rico Sch. of Med., San Juan, P.R. 00936.

Ground squirrels maintain color vision after removal of their visual cortex, but the quality of the persisting color vision has not been studied. Two adult ground squirrels (Spermophilus tridecemlineatus) were trained to perform both brightness and color discrimination tasks in a darkened two-choice visual discrimination apparatus. The color task was based on pseudoisochromasy (Kicliter and Loop, Vision Res. 16: 951-956, 1976). After initial acquisition of these discriminations, the size of the stimuli was varied and the minimal target size for color and brightness discrimination was determined. I then determined the spectral sensitivity of the animals using an increment threshold method at a background luminance of 6.1 cd/m^2. The posterior neocortex, including the cortical target of the dorsal lateral geniculate, was then removed in two stages and the three functions were again determined for each animal. The results showed no difference in target size required for color discrimination. Some performance decrement for brightness discrimination occurred with larger target sizes. The postoperative spectral sensitivity curves, like the preoperative curves, were bimodal. Postoperatively there was some sensitivity loss extending across the spectrum. That these changes were minor indicates that neither the discrimination of the color of small targets nor photopic spectral sensitivity requires the integrity of the geniculocortical system. (Supported by NIH grant NS-07464).

59.20 EFFECTS OF TELENCEPHALIC VISUAL SYSTEM LESIONS ON REVERSAL AND DIMENSIONAL SHIFT IN TURTLES (CHRYSEMYS PICTA). J. Cranney and A. S. Powers, Dept. of Psychology, Bryn Mawr College, Bryn Mawr, PA 19010.

There are two regions of the telencephalon of reptiles that receive visual projections from the thalamus: the core nucleus of the dorsal ventricular ridge (CN) and the dorsal cortex. The present research was undertaken to examine whether lesions of these structures produce different effects on visual tasks. Previous research had led us to expect that turtles with CN lesions would have sensory deficits while turtles with cortical lesions would have deficits that were not sensory, but rather associational.

We studied the performance of 16 turtles (Chrysemys picta) that were given lesions of the CN or dorsal cortex or sham lesions. In a standard two-key situation, all turtles were given a series of ten visual discrimination problems in sequence. On each problem, two dimensions were present, pattern (horizontal vs. vertical stripes) and color (red vs. green), one relevant and one irrelevant. The turtles were run on each problem until they reached a criterion of 85% correct initial choices. The correct choice on each problem was as follows:

(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)
H+	R+	V+	G+	V+	H+	G+	R+	H+	R+

Two of these problems, (6) and (8), are reversals of the previously learned discriminations. The rest all involve shifts of dimension, from pattern to color or vice versa.

For the most part, our hypotheses were confirmed. Animals with CN lesions had a deficit on problems (1) and (3), no deficit on problem (2), and no obvious deficit on any of the other problems. We interpret these findings to mean that CN lesions produce a sensory deficit that recovers with practice. Recovery may be due to the turtle's learning to compensate for the deficit or to the gradual takeover of function by other structures or to both these factors. The deficit appeared to be specific, in that it was not found on color problems. Cortically-lesioned animals had no deficit on problem (1), although they showed a deficit on problem (3), a dimensional shift to pattern, which was a reversal of the first pattern problem, and on problem (6), which was a pattern reversal. Thus it appears that lesions of the cortex damage some associative mechanism that makes it possible for a turtle to change its responses from one situation to another. In spite of the visual projection to the dorsal cortex, lesion of this area does not seem to produce a sensory loss on problems of moderate difficulty.

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60.1 SYNAPTIC SPECIFICITY OF REGENERATING RETINAL TERMINALS IN THE GOLDFISH OPTIC TECTUM. M.J. Airhart* & R.M. Kriebel* (SPON: M. Moffroid). Dept. of Anatomy & Neurobiology, The University of Vermont, Col. Med., Burlington, VT 05405.

Regenerating axons of the goldfish retinotectal pathway have been used extensively as a model system for studies on fiber specificity. These studies have measured axonal specificity on the basis of the retinotopic distribution of regenerating fibers using electrophysiological recording techniques and light microscopic autoradiography. Neither of these two assays allows determination if specificity exists at the synaptic level. The quantitative aspects of synapse formation and the synaptology of regenerating retinal terminals has never been used as a measure of specificity. Therefore, the purpose of this study was to answer the following question: to what extent do regenerating retinal terminals reestablish their original morphology, synapse density, and synaptology? An electron microscopic study of the normal retinotectal pathway and its synaptic neuropil, the stratum opticum(SO) and stratum fibrosum et gresium superficiale(SFGS), was done to provide baseline information for studies involving retinal synaptic specificity. A population of retinal terminals was identified in the SO and SFGS using a combined short term and long term electron microscopic degeneration study. The retinal terminal was found to exhibit a combination of morphological features that allowed direct identification of control and regenerated retinal synapses. Retinal synapse density, spherical vesicle associated synapse density (S) and total synapse density were determined from a standardized sample area ($621\mu^2$). The results showed little variability in synaptic parameters between control animals. Retinal terminal synaptology was examined both qualitatively and quantitatively. Retinal terminals did not synapse on cell soma or proximal dendrites, but rather on relatively small postsynaptic profiles. The size of the postsynaptic profiles fell within two area distributions: 87% size range $0.01\text{-}0.4\mu^2$ and 13% size range $0.41\text{-}1.10\mu^2$. This control data was used to evaluate the three experimental parameters used in defining synaptic specificity. Qualitative and quantitative changes in the retinal synapse population as well as the synapse densities of two related parameters, total synapse and S synapse densities, were examined in the SFGS during optic nerve regeneration. As early as 16 days postcrush, regenerating retinal terminals were observed in the SFGS. By postcrush day 63, retinal synapse density, S synapse density, and total synapse density were all equal or within 87% of control values. Further, the data strongly suggested that retinal synapses form a normal synaptology. (Supported by PHS 5429-16-19).

60.2 EFFECT OF DELAYED AFFERENT ARRIVAL ON SYNAPSE LOCALIZATION ON THE AMPHIBIAN MAUTHNER CELL. S.M. Leber and P.G. Model. Dept. Neuroscience, Albert Einstein College of Medicine, Bronx, N.Y. 10461.

The spatial patterning of inputs upon a neuron can be very precise. We have disrupted normal afferent-target cell temporal relationships in the development of an identified vertebrate central neuron to see whether temporal coordination is required for the correct localization of afferent terminals. The Mauthner cell (M-cell) of the axolotl (Ambystoma mexicanum) receives synapses from the ipsilateral vestibular nerve (nVIII) only on the ventral surface and branches of its lateral dendrite. The nVIII terminals (club endings) are distinctive at the EM level.

The ear and vestibular ganglion are of ectodermal placodal origin. The earliest axons from the ganglion enter the medulla at Harrison stages (s) 34+/35-, at about the same time that the M-cell begins to differentiate. To delay axonal ingrowth, prospective ear/ganglion placodes from younger (s23) embryos were unilaterally grafted in place of ears and ganglia from older (s33/34) animals. LM examination of embryos over successive days following surgery showed that a significant delay in the ingrowth had occurred. During this delay, the M-cell's lateral dendrite continued to grow and the experimental axons thus reached a larger and more differentiated target than did the control axons.

LM examination of 21 mm feeding larvae revealed that the grafts had developed into anatomically normal ears and nVIII ganglia. The vestibular nerves entered the medullae at the correct location. The M-cells on the operated side were normal, but the branches of the proximal lateral dendrite exhibited increased variability. EM examination of these cells showed that club synapses had indeed formed despite the late arrival of the axons, and comparison with control M-cells revealed a similar distribution of the club endings on the ventral surface and branches of the proximal lateral dendrite. Fewer club synapses, however, were seen on the operated side.

Thus disruption of the coordination of afferent and target cell differentiation does not prevent correct synapse localization. The data conflict with models which attempt to account for such spatial patterning solely on the basis of spatio-temporal relationships during development.

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60.3 ESTABLISHMENT OF CORTICAL TOPOGRAPHY IN HAMSTER PYRAMIDAL TRACT NEURONS DURING DEVELOPMENT. K. Kalil and T. Reh. Department of Anatomy and Neurosciences Training Program, University of Wisconsin, Madison, WI 53706.

Pyramidal tract neurons in the adult hamster are topographically organized in the sensorimotor cortex. Injections of HRP into the dorsal column and trigeminal nuclei, and the cervical, thoracic, and lumbar spinal cord show that retrogradely labeled neurons occupy a continuous band in the deep part of layer V. The origin of the pyramidal tract extends in the A-P direction from the level of the posterior commissure to the very frontal pole and mediolaterally from the midline to the rhinal sulcus to encompass MI, SI, and SII. Within this region the lumbar area is confined to a narrow posteromedial strip of cortex. The representation of the cervical cord lies primarily rostral and lateral to the lumbar area, but some overlap occurs between caudal cervical and rostral lumbar cortex. The trigeminal region lies primarily lateral to cortical areas projecting to the spinal cord.

Experiments were undertaken to determine whether this topography is already present early in development or whether it emerges from an initially overlapping projection. Infant hamsters from 5-21 days of age were injected with lectin conjugated HRP (Sigma) into localized regions of the spinal cord and medulla, sacrificed 24-48 hours later, and the brains processed according to the TMB method of Mesulam. The use of lectin conjugated HRP resulted in very sensitive cortical labeling.

We found that cortical neurons occupying a continuous band in layer V had already established their adult topography as early in development as the pyramidal tract axons had grown out to their targets and could be retrogradely labeled. In contrast to a previous report in the rat (Bates and Killackey, 1980, Neurosci.) we found no change in the extent of the cortical distribution of pyramidal tract neurons during development. Since cortical representations did not overlap significantly, it is unlikely that topography was established by the elimination of axon collaterals. Further, cortical cell migration is ending and neuronal differentiation still proceeding at the earliest ages examined in the present experiments. Thus the establishement of cortical topography is an early developmental event which occurs prior to or during target innervation, and may therefore be independent of synaptogenesis.

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60.4 MOTOR INNERVATION OF THE CHICK HINDLIMB AFTER DORSAL-VENTRAL LIMB ROTATIONS. B. Ferguson* (SPON: C. F. Stevens) Biology Department, Yale University, New Haven, CT. 06511.

Specific motoneuron (MN) nuclei in characteristic positions in the rostro-caudal and medio-lateral (ML) planes of the spinal cord innervate particular muscles selectively in the developing chick hindlimb (Landmesser, L. (1978) J. Physiol. 284:371;391). The position of MNs in the spinal cord is related to the embryonic origin but not to the function of the muscle (Landmesser, L. (1978) and Ferguson, B. (1978) Soc. for Neurosci. Abstr. 4:111). All MNs innervating muscles derived from the dorsal (D) muscle mass (MM) are situated laterally, ventral (V) MM situated medially in the spinal cord. In view of the strict correlation between MN position and muscle origin, DV limb bud rotations were done at stages 16-17 (Hamburger and Hamilton), prior to MN outgrowth. Horseradish peroxidase (HRP) backfills of 10 stage 34-37 embryos showed that MN innervation remained specific for both muscles of the thigh and shank regardless of altered limb orientation (Ferguson, B. 1978). Since this result could be due to selective MN outgrowth or random MN outgrowth with death of incorrect connections, motor nuclei innervating D or V shank muscles were localized prior to MN cell death in 9 st. 29-30 embryos. In every case the position of stained MNs in the cord was normal. Thus MN axons can detect and respond to a DV limb rotation to retain specific synaptic connections. Motor innervation of the limb thus cannot occur by a passive mechanism.

Normally, axons destined for D or V shank collect within each spinal nerve before spinal nerves converge. Reconstructions of st. 29 embryos with DV rotated limbs show that HRP stained MNs collect more distally, after the spinal nerves converge to form the sciatic plexus. As in controls, axons destined for V shank collect medially, D shank laterally. Unlike controls, just after or as axons collect, they shift position in the ML plane, to maintain normal orientation in the rotated limb. This delay in grouping of axons in DV rotated limbs could result from conflicting positional cues as axons pass from unaltered to DV rotated tissue. The shift in axon position within the nerve trunk indicates that growing axons actively orient by reference to their environment. The nerve patterns in these embryos suggest strong mechanical effects on nerve branching and spinal nerve convergence. MNs innervating a muscle were always appropriate, but not all cord segments normally innervating a muscle did so in embryos with DV rotated limbs due to some spinal roots not entering normally into the plexus; instead, branching to the tail, body wall, or ending in a neuroma. The nerve branching pattern was abnormal and differed in each embryo due to slight shifts of the limb in anterior-posterior and/or proximal-distal axes.

These experiments show that MNs respond actively to positional cues during outgrowth and can compensate for a DV limb rotation by altering both their position in a nerve trunk and path through the limb. Also, mechanical factors affect the paths of MNs in the limb. (Supported by NIH Grant NS10566 to L. Landmesser.

60.5 ONTOGENETIC CHANGES IN THE TARGETS OF LAYER Va BARREL FIELD NEURONS. H. P. Killackey and G. O. Ivy. Dept. of Psychobiology, Univ. of Calif., Irvine, CA 92717.

We have previously demonstrated (Ivy and Killackey, J.C.N. 195, 1981) that during the first postnatal week, the callosal projection neurons of rat parietal cortex are distributed in continuous bands. In particular, cells in lamina Va of the barrel field area can be densely labeled by contralateral injections of horseradish peroxidase (HRP). However, during the second postnatal week, these cells gradually cease to be labeled after contralateral HRP injections, even though neurons in adjacent "callosal" regions are heavily labeled from the same injections. In the present study, we decided to investigate the fate of these layer Va barrel field neurons which formerly projected across the corpus callosum.

First, in order to determine if these cells die, we used the fluorescent dyes fast blue and true blue as neuronal fate markers. We injected either fast or true blue into one hemisphere of six postnatal day (PND) 6 rats and allowed them to survive until PND 20 to 25, well past the time at which most barrel field callosal neurons "disappear." We find that these former callosal projection neurons do not die, rather they remain labeled in the continuous neonatal pattern. Next, we sought to determine if these neurons project elsewhere. We accomplished this by temporal double labeling. We injected fast blue into the contralateral cortex of four rats at PND 7. Later, at PND 20 to 25, we injected nuclear yellow into the ipsilateral motor cortex and allowed a 4 to 16 hr survival time. This motor region is a known projection area of the barrel field (Akers and Killackey, J.C.N. 181, 1978) and we have confirmed in four rats with HRP that many of the neurons of origin of this projection are located in lamina Va. We find that many neurons in layer Va of the barrel field are double labeled. This suggests that many of the initial callosal projection neurons send axons to the ipsilateral motor cortex. Finally, we sought to determine if these dual projections are present simultaneously in the neonatal rat. In three PND 7 rats, we injected fast blue into the contralateral cortex and nuclear yellow into the ipsilateral motor cortex and allowed a survival time of 4 to 16 hr. Following this procedure, double labeled cells are present in layer Va of the barrel field.

We interpret our results as suggesting that the specific adult patterns of connectivity are achieved through mechanisms which include a selective elimination of collateral processes.

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60.6 CORTICOSPINAL AND CORTICOTECTAL NEURONS: MECHANISMS OF ONTOGENETIC CHANGES IN DISTRIBUTION. G. O. Ivy and H. P. Killackey. Dept. of Psychobiology, Univ. of Calif., Irvine, CA 92717.

During the first few postnatal days (PNDs) in the rat, both the corticospinal (CS) and corticotectal (CT) projection neurons have a continuous distribution in lamina Vb throughout most areas of the neocortex. However, after two weeks, each of these types of projection neuron achieves a more discrete distribution. This phenomenon parallels that which we have previously demonstrated for the callosal projection neurons (Ivy and Killackey, J.C.N. 195, 1981). We decided to investigate these distributional changes of CS and CT neurons in more detail utilizing the retrograde transport of fluorescent dyes.

First, we confirmed the adult pattern of CS and CT neurons in single animals using two different dyes (fast blue and nuclear yellow). We find that there are three populations of cells with different areal distributions: one projecting only to the spinal cord (CS), a second only to the tectum (CT) and a third to both places (CST). Next, we sought to determine the neonatal distribution of these projection neurons with double labeling techniques. In the neonate, we do not find areally distinct populations of projection neurons, rather we find all three types (CS, CT and CST) intermixed and widely distributed. Further, there are more double labeled (CST) neurons in the neonate than in the adult. Finally, we sought to determine if the adult distribution is achieved by a process of selective cell death or by a loss of collaterals. We accomplished this by temporal double labeling. We injected fast blue into the spinal cord of three PND 2 rats and later, at PND 15, we injected nuclear yellow into the tecta of these same rats and allowed them to survive for 8 to 24 hr. We find that many of the CS neurons which were retrogradely labeled with fast blue at PND 2 are still present at PND 15 to 25 and are distributed in a pattern similar to that of the neonate. Further, there are double labeled neurons in regions where they would not normally be found in the adult.

We interpret the present findings as indicating that the ontogenetic changes in the distributions of at least CS neurons are not due to selective cell death but, it seems, to a selective loss of collateral processes. The present results support and extend our findings on intracortical projection neurons (Killackey and Ivy, this vol.). Together, these findings suggest that selective process elimination may play an integral role in sculpting neocortical connectivity.

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60.7 DISTRIBUTION OF THALAMIC TERMINALS WITHIN NEOCORTICAL FIELDS OF THE REELER MOUSE. V.S. Caviness,Jr. and D.O. Frost E.K. Shriver Center, Waltham, MA 02154 and Yale University, New Haven, Conn 06510

The reeler mutation in mice causes inversion in the relative positions of the principal neocortical neuronal classes. The radial pattern of distribution of thalamocortical axons within the majority of neocortical fields of the mutant is studied by anterograde degeneration (Fink and Heimer) after large dorsal thalamic lesions. In the agranular fields at the lateral margin of the hemisphere terminals are diffusely distributed. In all other fields terminals are concentrated in either two or three tiers. An outer tier, overlaping the polymorphic cell zone, and a middle tier, overlaping the granule and the upper portion of the medium pyramidal cell zones, are present in all fields. Only these two tiers are found in fields where the medium pyramidal cells are distributed uniformly, (e.g.,fields 17,3,1,4 and 6). A third or inner tier is located at the lowest margin of the cortex and is present in fields where medium and small pyramidal cells are concentrated in a "lamina" at this level. (e.g.,fields 18a,41,40 and 3i).

The tiered distribution of thalamocortical terminals presumably reflects the distribution of receptive surfaces for these terminals. The basal dendrites of medium pyramidal cells, normally concentrated in neocortical layers III and IV,are, a principal synaptic target for thalamocortical axons.Observations from Golgi impregnations indicate that this dendritic class is dispersed widely in the lower half of the mutant neocortex in a fashion that does not match the tiered distribution of the axon terminals. Further, at all levels this dendritic class is abnormally intermixed with apical dendritic arbors of medium and small pyramidal cells, classes of dendrites normally not in receipt of large numbers of thalamic axon terminals. Thus terminals of the reeler thalamocortical projection may be concentrated in tiers at the cost of accepting an abnormal mix of dendritic classes as their synaptic partners.

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60.8 ANTEROGRADE AND TRANSNEURONAL TRANSPORT OF FLUORESCENT DYES IN THE RAT VISUAL SYSTEM. James N. Davis and Patterson McKinnon*. Neurology Research Laboratory, Duke Univ. Med. Ctr., Durham, NC 27710.

The retrograde transport of fluorescent dyes has proved useful in analyzing neuroanatomical projections. In order to evaluate anterograde transport, we injected 10 µl of 10% either Bisbenzimide (BSBZ), BSBZ and polyornithine, DAPI, Evans blue, or Propidium Iodide into the posterior chamber of the right eye of male 180-200 gram Sprague-Dawley rats. Three days later the brains and eyes were rapidly removed and frozen on dry ice. 16 µ frozen sections were dried on glass slides, cleared with xylene, mounted with Entellan and viewed with a Leitz Dialux microscope equipped for incident fluoroscopy.

Although all dyes could be detected in retinal ganglion cells, only BSBZ and DAPI could be detected in the brain. Of these two dyes BSBZ was present in the contralateral projection from the injected eye, while DAPI was only detected in the ipsilateral optic nerve, chiasm and contralateral optic tract in one animal. BSBZ was detected in cell nuclei in the ipsilateral optic nerve, optic chiasm, contralateral optic tract, contralateral dorsal and ventral optic tract nuclei, the contralateral lateral geniculate nucleus and the stratum griseum superficiale of the contralateral superior coliculus. Cells detected in the nerve, chiasm and tract were glial as judged by the light microscopic appearance and the known lack of neurons in these pathways. The large size and topography of the cells labeled in contralateral terminal areas suggested that these nuclei might be neuronal. Only one third of the animals injected with any dose (3 to 10 µl) of BSBZ demonstrated good labeling regardless of time of survival (12 hours to 7 days). Dose-dependent retinal necrosis and inflammation were present in all animals injected with polyornithine and BSBZ and in over half of the animals with BSBZ alone. Anterograde transport of BSBZ was not able to label the ipsilateral visual projections representing 5-10% of the total retinal projection. Furthermore no labeling was detected of the suprachrasmatic nucleus, another sparse projection. Thus anterograde transport under these conditions was not a sensitive technique for analyzing neuronal connectivity. However the presence of apparent transneuronal transport offers the intriguing potential of identifying target cells for a pathway using flourescent dyes.

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61.1 MOTOR INNERVATION OF THE CUTANEOUS TRUNCI MUSCLE. E. Theriault+ and J. Diamond. Dept. of Neuroscience, McMaster Univ., Hamilton, Ont. L8N 3Z5.

The cutaneous trunci muscle (CTM) of the rat is an extensive sheet of skeletal muscle that moves only the skin; it originates on the lateral aspect of the humerus (bilaterally) and inserts into the skin of the back and flanks. The muscle fibres run in a rostro-caudal direction and AChE staining shows end-plates in dorso-ventral rows at right angles to the longitudinal axis of the muscle. The reflex activation of CTM demonstrates local sign (see accompanying Abstract by Nixon et al.). While the sensory nerves that evoke this reflex are segmental, all the motoneurons of the CTM are in a circumscribed region of the spinal cord rostral to the sensory inputs that drive them. The motor axons emerge from the brachial plexus in 3-4 major nerves. Electrophysiological and histochemical (glycogen depletion) studies indicate that each of these nerves innervates a rostro-caudal (longitudinal) column of muscle; however the reflex activation of CTM involves dorso-ventral (transverse) segments of the muscle. This is an intriguing situation, and we are examining how the morphology of the CTM and the pattern of its innervation are designed to allow the segmental expression of the local sign character of its reflex activation. Retrograde horseradish peroxidase (HRP) labelling shows that the motoneuron pool is located in the ventral horns of C6, C7 and C8, and consists (on each side) of approximately 350 somata in a semi-lunar arrangement at the most anterior edge of the gray matter. However there is no obvious organization of the pool that corresponds with the major motor nerves. In preliminary experiments the injection of HRP directly into the muscle mass gave results suggesting that the motoneuron pool may have an intrinsic organization that corresponds to the "dermatomal" (dorso-ventral) pattern of the reflex response. In the salamander (Macintyre and Diamond, 1981, Proc. R. Soc. Lond. 211: 471) and in the rat (Jackson and Diamond, in preparation), the sprouting of intact sensory nerves is often confined to "domains" whose borders are similar to those of the sensory dermatomes. We are studying whether sprouting of remaining CTM motor axons (which is detectable by one week after partial denervation of CTM) is similarly constrained territorially, whether electrical activity in the intact motor nerve influences the rate of sprouting (as it does high-threshold sensory nerves (Nixon et al., Soc. for Neurosci. Abstr. #59.2, 1980)), whether ventral rhizotomies (of C6, 7 or 8) will reveal a possible segmental innervation of CTM, and if there is any selectivity of reinnervation of CTM by regenerating axons.

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+ M.S. Soc. (Canada) Research Student.

61.2 LOCAL SIGN IN A CUTANEOUS SKELETAL MUSCLE REFLEX IN THE RAT. B. Nixon, P. Jackson, E. Theriault+ and J. Diamond. Dept. of Neurosci., McMaster Univ., Hamilton, Ont. L8N 3Z5.

"Local sign" in a reflex describes the orienting of an evoked movement to the region where a sensory stimulus is applied. We are studying a mammalian reflex in which local sign can be described quantitatively, the activation in the rat of the cutaneous trunci muscle (CTM), a thin sheet of skeletal muscle that lies just below, and is inserted into, the back and flank skin. A forceps pinch of dorsal skin results in a localized contraction of both the underlying CTM, and that in the corresponding region of skin on the contralateral side; as the forceps pinch is moved rostrally or caudally so do the two foci of the reflex responses move. Low-threshold, (touch and pressure), stimulation is ineffective. The afferent input reaches the spinal cord through the segmental (T4-L3) dorsal cutaneous nerves (DCNs). All of the motor output to CTM however originates from a relatively localized region of the spinal cord at the C6-8 level, rostral to all the DCNs that drive the reflex (see accompanying Abstract by Theriault and Diamond). When we electrically excite a DCN and record from that DCN and the CTM motor nerves we find that two groups of afferent fibres (in the Group III (Aδ) and C categories) can evoke the reflex. In a 250 g rat excitation of a single DCN at threshold for activation of Group III fibres results in a brief EMG with a rostro-caudal spread of 3-4 cm; the location where the EMG has the shortest latency (12-15 msec) is always 1-1.5 cm rostral to the centre of the receptive field of the stimulated nerve. Supra-threshold stimulation causes a more generalized CTM response over several cm; with a large enough stimulus a second brief EMG of longer latency (40-50 msec) is elicited, clearly associated with the appearance of a C fibre potential in the excited DCN. With a just-threshold stimulus this second reflex response is localized in much the same way as the early one. The local sign of the reflex can thus be measured physiologically, and in terms of shortest latency of the responses at "threshold" excitation of two groups of sensory afferents, and is clearly related to a skin region that includes the "sensory dermatome" of the segmental afferent input. We are currently investigating the effects on the development of the local sign character of this polysynaptic reflex of (1) neonatal capsaicin treatment (to reduce or eliminate particularly the C fibres), and (2) changes in the locations and sizes of sensory or motor fields in neonates produced by e.g. nerve sprouting after partial denervation, and by selected nerve redirections and regeneration.

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+ M.S. Soc. (Canada) Research Student.

61.3 MOTOR UNITS DIVERSIFY IN SIZE AS SYNAPSE ELIMINATION PROCEEDS IN THE NEONATAL RABBIT SOLEUS MUSCLE. Herman Gordon and David C. Van Essen, Division of Biology 216-76, California Institute of Technology, Pasadena, CA 91125.

During the first two weeks of post-natal development, motor units of the rabbit soleus muscle shrink 4- to 5-fold in average size as redundant synapses are eliminated. We have examined the distribution of motor unit sizes throughout this period in order to address several models of how synapse elimination might occur. Motor unit sizes were estimated by stimulating single motor axons in ventral root filaments and measuring the resultant muscle tensions in an in vitro preparation. In muscles aged 0-5 days postnatal the width of the distribution of motor unit sizes (twice the standard deviation) was .96 times the mean, whereas the width of a similar distribution for muscles aged 12-18 days postnatal was 1.86 times the mean. A few of the older motor units were even comparable in absolute size to the largest units from younger muscles, suggesting that they had escaped extensive loss of synapses. Also, we observed a significant number of extremely small motor units <1/10 of the mean in size.

These results indicate that (1) a uniform down-scaling of motor unit sizes does not occur and (2) small motor units are not at a competitive advantage over large units during synapse elimination. With regard to the latter conclusion it will be important in the future to determine whether the distribution of motor unit sizes in the adult rabbit soleus muscle is as uniform as that reported for the rat, as this could imply a secondary stage of motor unit reorganization.

Interestingly, models in which synapses are lost at random with respect to size of motor units are consistent with an increase in variability. On the other hand, the diversification could also reflect differing abilities of the motor neurons, independent of their sizes, to compete for targets. One possibility involves a recent claim by Miyata and Yoshioka (J. Physiol., 309:631-646, 1980) that only motor neurons from the more rostral of the two spinal roots innervating the rat soleus muscle undergo synapse elimination. Such a situation would indeed result in a diversification of motor unit sizes. However, when we looked in the rabbit soleus we found marked synapse elimination and diversification of motor unit sizes in innervation from both spinal roots, although a slight bias may exist for elimination of synapses from rostral motor neurons.

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61.4

Withdrawn

61.5 DEVELOPMENT OF THE INTERLIMB LOCOMOTOR PROGRAM IN BULLFROG TADPOLES. Donald J. Stehouwer and Paul B. Farel. Dept. Physiol., Sch. Med., Univ. N. Carolina, Chapel Hill, N.C. 27514

Throughout most of larval development, tadpoles use only axial muscles to swim, holding their hindlimbs in an extended position against the tail. By Stage XVII, the hindlimbs participate in swimming either by alternate or by simultaneous thrusts. The present study uses the isolated CNS to examine the development of the neuronal activity underlying this behavioral change and compares this development to the endogenous patterned bursting of primary motoneurons present throughout larval life and known to underlie swimming (Stehouwer and Farel, Brain Res., 1980; 1981).

Primary motoneurons, which are born embryonically, persist only through larval stages, innervate axial musculature and are physiologically and anatomically well-developed, even in young larvae. In contrast, motoneurons innervating the hindlimbs form the lumbar lateral motor column (LMC) and are not born until larval stages. Primary and LMC motoneurons of the larva send their axons to the periphery in different fascicles of the ventral root. These fascicles can be dissected apart and studied separately in electrophysiological experiments.

Prior to Stage XI, axons of LMC motoneurons show a tonic burst discharge coinciding with episodes of patterned burst activity in axons of primary motoneurons. The tonic burst in LMC axons appears to underlie the hindlimb extension maintained during swimming at these stages of development. However, by Stage XIV, patterned burst activity develops in LMC motoneurons and bears a 1:1 frequency relationship with activity in primary motoneurons. In contrast to primary motoneurons, which always burst alternately on the two sides, ventral root axons of LMC motoneurons show both alternating burst activity (the bursts being phase-shifted 180° with respect to bursts in ipsilateral primary motoneurons) and bilaterally synchronous bursts. These two modes of activity can be seen in the same animal and probably underlie the two different behavioral uses of the hindlimbs during swimming.

Because the activity of motoneurons innervating flexors and extensors is combined, the bursts of activity recorded from LMC motoneuron axons in the ventral roots are less discrete than bursts recorded from primary motoneuron axons. More discrete bursting can be recorded from peripheral nerve branches. Bursts recorded from different branches showed varying phase relationships.

These results relate the development of hindlimb use during swimming to changes in motor program activity recorded in vitro and provide a preparation for investigating the cellular events underlying development of motor rhythmicity.

61.6 CHARACTERISTICS OF A CORTICOSPINAL TRACT WITH AN ABBERANT TRAJECTORY. B.S. Bregman and M.E. Goldberger. Department of Anatomy, The Medical College of Pennsylvania, Philadelphia, PA. 19129.

Tactile placing (TP) is spared following neonatal spinal hemisection (L1, dorsal columns spared) but is abolished by adult lesion (Bregman and Goldberger, 1980). The anatomical basis of spared TP is the presence in neonatal operates (NO) only, of corticospinal input from the hemisphere contralateral to the lesion. This was determined by HRP injections into the spinal enlargement in adult animals caudal to the neonatal or adult hemisection, which revealed labeled cells in the contralateral sensorimotor cortex in NOs. (Bregman & Goldgerger, 1981). The spared CST cells are morphologically indistinguishable from normal, and are found in areas 4,3,1,2 and 5 in lamina V. In a representative NO, 746 cells were labeled. 83.4% were located in area 4, motor cortex, with substantially fewer in area 3, (8.4%); area 1-2, (3.7%); and area 5, (4.2%). Most cells are small to medium diameter but the range is 16-72μm. The distribution is unimodal with a mean diameter of 30.9 (± 1.7)μm. This distribution is different from that of published reports of cells of origin of the normal CST (Groos et al. 1978) where there was a larger mean cell diameter (41.9μm) and a bimodal distribution of cells. The unimodal distribution of spared CS neurons suggests that most of the large cells have: 1) died; 2) failed to reach the lumbar enlargement; or 3) failed to reach the adult size. Removal of NO's sensorimotor cortex in adulthood abolishes the spared TP and, when degeneration methods are used, reveals the aberrant CST pathway. In the cervical and thoracic segments the CST occupies a normal position in the dorsolateral funiculus. As the compact bundle of fibers approaches the lesion (L1) it disperses moving through the dorsal horn, medially. Most fibers then occupy the spared dorsal funiculus. Caudal to the lesion fibers remain in the dorsal funiculus, but as the lateral funiculus begins to reappear, degenerating fibers are found there too. They are not confined to the normal dorsolateral position but are dispersed in the lateral funiculus. Results indicate no regeneration of cut axons through the lesion site. Since rubro-, lateral vestibulo- and many reticulo-spinal neurons are unlabeled it appears that the plasticity of the late-developing corticospinal tract is not shared by other descending pathways which are more developed on the day of birth. The absence of brainstem-spinal tracts in the lateral funiculus may permit greater dispersal of the growing corticospinal axons. In spite of its aberrant course, the spared CST mediates sparing of some motor functions.

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61.7 PLASTICITY OF INFERIOR OLIVARY AFFERENTS FOLLOWING NEONATAL HEMICEREBELLECTOMY IN RATS. R.S. Swenson* and A.J. Castro. Depts. of Anatomy, National College of Chiropractic, Lombard, IL 60148 & Loyola Univ. Stritch School of Medicine, Maywood, IL 60153

Following neonatal hemicerebellectomy, the pontine gray and the inferior olivary complex (IOC) contralateral to the lesion show a considerable loss of cells. Ipsilateral cortical projections to the reduced pontine gray develop an anomalous crossed projection to the opposite side (Leong and Lund, Brain Res. 62: 218-221). The current study examined whether pathways normally terminating in the missing IOC will similarly remodel to the spared olive after neonatal hemicerebellectomy. Since previous work from this laboratory (Anat. Rec. 199:251A) has shown the rostral mesencephalon and caudal diencephalon to provide the heaviest input to the IOC of the rat, certain nuclei in this region, namely the subparafascicular nucleus and the nucleus of Darkschewitsch, were selected for the current study.

Tritiated leucine (50 uCi/ul) was stereotaxically injected into the subparafascicular and Darkschewitsch nuclei of 23 normal and 14 neonatally hemicerebellectomized rats under pentobarbital anesthesia (40mg/kg). Dense labeling was observed in the ipsilateral descending MLF in both groups. In the normal animal, these fibers distributed primarily ipsilaterally to all olivary subdivisions except the dorsal accessory olive, with a light contralateral distribution. In the neonatally hemicerebellectomized animals, many labeled fibers decussated to the spared side, 300-500u caudal to the rostral pole of the olive. This anomalous decussation terminated in the same olivary subdivisions that normally receive input from the subparafascicular and Darkschewitsch nuclei.

Although the abnormally increased crossed projection may result in a decrease in the normal inputs to the spared IOC, the observed remodeling indicates that the spared IOC receives a substantially more bilateral input from the subparafascicular and and Darkschewitsch nuclei.

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61.8 THE POSTNATAL DEVELOPMENT OF CORTICOPONTINE SYSTEMS ARISING FROM SENSORIMOTOR AND VISUAL CORTICES IN THE RAT. C.E. Adams*, G.A. Mihailoff and D.J. Woodward (SPON: R. Galosy). Depts. of Cell Biology and Physiology, The Univ. Tx. Hlth. Sci. Ctr., Dallas, TX 75235.

The impetus for this report evolved from a series of ongoing studies in this laboratory concerning the anatomy and physiology of cortico-ponto-cerebellar interactions. The postnatal development of the corticopontine system was studied in Long-Evans hooded rats beginning at postnatal day 1 (birth=day 0) and extending through postnatal day 20. Axonal trajectory and terminal field distribution of corticopontine fibers were visualized through routine autoradiographic procedures following injection of 0.10 μl ^{3}H-Leucine (65-75 μCi/μl; sp. act. 139 Ci/mmole) into sensorimotor (SM) or visual (VIS) cortices and allowing 24-48 hours survival. The developmental patterns in the sensorimotor and visual projections were quite similar and thus the following account represents the general features of this development except where specific variations are noted. Between day 1 and day 3, labeled axons were clearly present within the cerebral peduncle coursing longitudinally through the pontine nuclei ipsilateral to the injection site. Silver grain accumulation suggestive of sparse terminal labeling was also apparent over pontine gray neuropil during this period, being located rostrally after VIS injections and more caudally following SM injections. The period between day 3 and day 10 was characterized by an apparent increase in the density of terminal labeling. In the case of both SM and VIS projections the terminal labeling occupied the general regions of pontine gray where labeling has been observed in the adult although the pattern was clearly more diffuse than that seen in the adult. Between days 10 and 16 there appeared to be a distinct focusing or constriction of terminal projection zones for both SM and VIS systems. The five longitudinally oriented columnar zones which have been shown to receive somatotopically organized SM input in the adult were clearly delineated by postnatal day 16. In addition, the relatively sparse contralateral SM projections observed in the adult were in place by day 13. Thus, by postnatal 16, both SM and VIS corticopontine projection systems had attained their adult configuration. These findings correlate well with a previous Golgi study of postnatal neuronal maturation in the pontine nuclei which demonstrated an emergence and proliferation of dendritic spines between days 10 and 12. Ultrastructural studies in adult rats have shown that corticopontine axons synapse primarily on spines and distal portions of the dendritic tree. Supported by NS12644 (NIH), BNS8004853 (NSF), BNS77-01174 (NSF) and the Biolgoical Humanics Foundation.

61.9 THE DEVELOPMENT OF SEROTONINERGIC PATHWAYS WITHIN THE SPINAL CORD. STUDIES USING THE NORTH AMERICAN OPOSSUM AS AN EXPERIMENTAL MODEL. T. Cabana*, F. DiTirro, R. Ho and G.F. Martin, (SPON: E. Keith Michal). Dept. of Anatomy, School of Medicine, The Ohio State University, Columbus, Ohio 43210.

Brainstem neurites, some of which transport monoamines, were reported to grow into the opossum's spinal cord very early in development (Martin et al. 1978). In the brainstem, 5HT neurons were identified by the Falk-Hillarp technique within raphe and adjacent areas of the reticular formation as early as 5 days after birth (17 days after conception) (Humbertson and Martin, 1978). In opossums of the same age, injections of horseradish peroxidase into the thoracic cord produced labeling of neurons in comparable areas of the brainstem: the presumptive nuclei pallidus, obscurus and magnus raphae and the adjacent reticular formation.

A series of pouch young opossums was processed by the peroxidase-antiperoxidase technique of Sternberger (1970) for the presence of serotonin (5HT). 5HT-containing varicosities were present within the spinal cord as early as the sixth (6) postnatal day. They were located within dorsolateral areas of the marginal zone where they were most numerous, as well as within ventral, lateral and dorsal regions. Furthermore, there was evidence for growth of 5HT axons into the intermediate (mantle) zone. At cervical and rostral thoracic levels they occur within lateral regions of the dorsal horn, around the central canal and within the ventral horn; at more caudal levels they were restricted mainly to lateral areas of the dorsal horn. Of particular interest is the existence of 5HT-containing cell bodies ventral to the central canal. By postnatal day 13 the distribution of 5HT neurites is comparable at all levels of the cord. They continue to grow into the intermediate zone and by day 22 are numerous within the lateral parts of the dorsal horn (presumptive laminae IV-VII), the intermediolateral cell column, the presumptive lamina X and the ventral horn where they approximate presumed motoneurons by at least day 31. 5HT elements were not found within laminae I and II until after postnatal day 51.

Thus, serotoninergic bulbospinal neurites innervate the presumptive laminae IV-VII of the dorsal horn, as well as autonomic and somatic motoneurons relatively early in development, but laminae I and II considerably later. In developing opossums, unlike the adult, 5HT cell bodies were identified ventral to the central canal. Supported by BNS-80-08675. We thank Dr. Robert Elde for the 5HT antibodies.

61.10 EFFECTS OF NEURAL BLOCKADE ON BEHAVIORAL DEVELOPMENT IN XENOPUS EMBRYOS. Lanny J. Haverkamp and Ronald W. Oppenheim. Neurobiol. Progr., Univ. N. Carolina, Chapel Hill, NC 27514 and Neuroembryol. Lab., Dorthea Dix Hosp., Raleigh, NC 27611.

The dictum that neural function during embryogenesis is without effect on later behavior, and by implication without effect on the development of early neural networks, is largely based upon the experiments of Harrison, Matthews and Detwiler, and Carmichael done in the first third of the century. Since the conclusions drawn from these works are not entirely warranted by the reported data and since a repetition of the studies (Fromme,1941) reached an opposite conclusion, we have conducted a quantitative and more thorough study using the same basic procedures.

Embryos were immersed in solutions of either chloretone ($3x10^{-4}$by wt.) or xylocaine ($1.5x10^{-4}$) prior to the onset of embryonic motility (st.17). These animals were removed from the drug solution at st.35 (about 24 hr. later) by which time normal animals are hatching and will readily swim. Stage-matched normal animals from the same spawning were compared to experimental as well as to control animals immobilized by the drugs for 1/2 hr. at st.35. Spontaneous activity and responses to tactile stimulation were recorded on videotape 1 and 2 hr. after removal from the drug solutions and at st.40 and 44 (about 24 and 48 hr. after removal). In a third experiment, animals injected with 2ug -BTX at st.23 (resulting in 20-28 hr. immobility) were compared to vehicle-injected control animals at st.41 and 44. Spontaneous behavior and response to repeated substrate vibration were recorded for all groups at 10 days of age (st.48). The videotape records were replayed at reduced speed and the animals' movements traced onto a computer graphics tablet. Various parameters of activity were then derived through computer analysis.

The spontaneous and induced activity of the experimental animals was clearly subnormal for the first 2 hr. after removal from the drug, a result attributable to residual drug effect. At st.40 (about 24 hr. after release from the drug) levels of activity and responsiveness of experimental animals were comparable to those of normal embryos. At st.44 and 48, experimental and normal animals were qualitatively and quantitatively indistinguishable.

These experiments confirm the early studies and reconcile them with the disparate findings of Fromme. They also indicate that the long-term effects of functional deprivation in early postnatal systems are without parallel during the earliest stages of neural development. Further experiments involving longer-term immobilization and more precise characterization of behavior during the first 24 hr. after drug release are in progress. Neuroanatomical effects of this functional blockade are also under investigation.

61.11 HABITUATION OF MOTOR ACTIVITY: GENETIC AND DEVELOPMENTAL ASPECTS. Donna G. Atwater*, Janice E. Gellis*, Walter C. Low, Michael M. Myers*, David Whitehorn, and Edith D. Hendley. (SPON: R. Rotundo) Dept. of Physiology and Biophysics, Univ. of Vermont, Burlington, Vermont 05405.

The spontaneously hypertensive rat (SHR) has been shown to exhibit higher levels of motor activity in comparison to their parent strain, the Wistar Kyoto (WKY). In addition to genetic differences, evidence suggests that the level of motor activity in the SHR decreases with age. We now report that genetic and developmental factors also influence the habituation of motor activity in the SHR, WKY, and their progeny, the F2 generation.

The F2 generation was bred using SHR males and WKY females. Seven pairs of SHR/WKY rats were randomly mated to produce an F1 strain. Mean systolic blood pressure (BP) of the SHR males was 190±3 mmHg, BP for the WKY females was 110±1 mmHg, BP for the F1 males was 139±2 mmHq, and the BP for the F1 females was 130±1 mmHg. F1 animals were then randomly bred to produce the F2 generation, with BPs of 151±5 mmHg.

Measurements of motor activity were made with an automated activity cage (30cm X 30cm X 25cm) consisting of four light sources and photodetectors, coupled with a digital counter to monitor the movement of the animals. Each animal was placed in the activity cage for four 15 minute trials during a single day, with the time between each trial ranging from 60 to 75 minutes. The activity of each trial was expressed as a percentage of the total activity of the first trial.

Adult male SHRs (32 weeks) were found not to habituate with repeated trials, whereas adult male WKYs (32 weeks) exhibited a continual decrease in total activity over four trials. Young male SHR, WKY and F2 rats (12 weeks) also exhibited a decrease in total activity over four trials. However, rate of habituation for the F2 rats was significantly greater than that for the SHRs ($p<0.001$, 2 way ANOVA): and the rate of habituation for the WKYs was significantly greater than that of the F2 rats ($p<0.001$, 2 way ANOVA). No difference was observed in the rate of habituation between the adult WKY males (32 weeks) and the young WKY males (12 weeks).

These results suggest that there is a genetic influence in the rate of habituation of motor activity in the comparison of adult SHR and adult WKY males, and in the comparison of young F2, SHR and WKY rats. Furthermore, the inability of adult SHRs to habituate compared with young SHRs is suggestive of developmental alterations in responsiveness of SHRs to their environment.

This work was supported by PHS grant 5429-19-18, HL 24110, and grants from the American Heart Association and its Vermont affiliate.

61.12 MORPHOLOGY AND PHYSIOLOGY OF THE CLAW CLOSER NEURONS IN SNAPPING SHRIMP. John A. Wilson and DeForest Mellon Jr. Department of Biology, University of Virginia, Charlottesville, VA 22901.

Adult Alpheid shrimp possess the unique ability to transform a pincer claw into a snapper claw following the loss of the snapper or section of the nerve trunks to the claw. Changes in both the diameters of the cell bodies and several properties of the muscle have been well documented to occur during transformation (Mellon, in prep. for review); however, the electrical and morphological properties of the neurites and axons have not been studied. We have therefore examined the anatomical and biophysical properties of snapper and pincer closer neurons.

Double barreled microelectrodes were used to impale the axons of the neurons to the claw closer muscle 100-500 uM distal to where the nerve leaves the ganglion. Input resistance was determined by recording the change in voltage in one barrel of the electrode in response to a measured current pulse passed from the other barrel. Our comparison of input resistances for the four snapper closer neurons shows them to have equal input resistances to those of their contralateral homologues, the pincer closer neurons. This finding allowed us to predict that the axonal and dendritic morphology should also be similar for both the snapper and pincer neurons. We therefore intracellularly stained the eight neurons with cobaltous ions, Timm's intensified the ganglia, and examined the resultant preparations. The diameter of the neurites and length of the major branches for pairs of contralaterally homologous neurons are the same.

Furthermore, we have measured the length constant and found that our recording site should be less than 1λ away from the terminations of all the branches even though they change diameter. We therefore found no biophysical or anatomical differences between the axons and neurites of the snapper and pincer closer neurons which can explain their different roles in behavior.

We interpret our findings to support Ritzman's (J comp. physiol. 1974) contention that the neural circuitry for the snapping behavior is present in both the snapper and pincer. As we, like Ritzman, have observed pincers to "snap", the only necessary neural change may be a supplementation of the sensory input. Claw transformation may be therefore primarily a process of growth and specialization of the claw and the closer muscle with the only major change in the closer neurons being the increase in the diameters of their cell bodies. Supported by USPHS Research Grant NS 15006 (to DeF. Mellon).

61.13 MATCHING OF MOTOR AXON AND MUSCLE FIBER PROPERTIES DURING AN ALTERATION IN CRAYFISH MUSCLE FIBER GROWTH. Gregory Lnenicka* and DeForest Mellon, Jr. Dept. of Biology, Univ. of Virginia, Charlottesville, VA, 22901

During growth of the most lateral muscle fibers of the crayfish superficial flexors, the input resistance decreases as predicted by cable theory. Meanwhile, the amplitude of the EJP produced by axon 6 in the most lateral muscle fibers remains constant. Factors contributing to the stability of the EJP amplitude during growth are an increase in the quantal content of release at synaptic sites, and an increase in the current produced by a quantum of transmitter (Lnenicka and Mellon Neurosci. Abstr. 6 497) We wish to determine if these factors can be regulated by the rate of muscle fiber growth.

By surgically reducing muscle fiber tension, we have produced a decrease in muscle fiber growth. This procedure was performed unilaterally on single abdominal segments in small animals (carapace length 1.4 cm.). After two months of growth (carapace length 3.0 cm.), the most lateral muscle fibers on the operated side had increased their radius by 2.3x while on the control side the radius increased by 4.4x. If the muscle fiber electrical constants are not different on the two sides, cable theory predicts the input resistance to be 2.7x greater on the operated side. However, this predicted difference in the input resistance was not reflected in the EJP amplitude. Preliminary results show no significant difference in EJP amplitude between the two sides.

Experiments are in progress to determine how the normal EJP amplitude is maintained during abnormally slow muscle fiber growth. Possible explanations currently being explored include the following: a change in the muscle fiber electrical constants, resulting in an abnormally low input resistance for the experimental muscle fibers; the quantal content of release and/or the current produced by a quantum of transmitter increases at a less than normal rate. (Supported by a grant from the Muscular Dystrophy Association.)

62.1 β-ADRENERGIC RECEPTORS IN RAT BRAIN MICROVESSELS: CHANGES DURING AGING. M. Trabucchi, H. Kobayashi+, P.F. Spano+ Depts. Pharmacol. and Pharmacognosy, Univ. of Padua and Milan , Italy.

Recent observations suggest a possible neurogenic control of cerebral microcirculation mediated by a number of receptors for neurotransmitters located on brain microvessels. This system may be relevant in the control of vascular function during aging. We characterized the β-adrenergic receptors located on brain microvessels using (^{125}I)-iodohydroxybenzylpindolol (IHYP) as a radioligand. The brain microvessels have been prepared using albumine flotation and glass beads filtration technique from gray matter of rat cortices. The preparation, predominantly composed of capillaries and free from neural and glial elements, was subjected to radioreceptor assay. The binding is linear with protein concentration up to at least 80 μg per tube. It was saturated at 200 pM of IHYP concentration. The K_D value calculated by Scatchard analysis was 69 ± 8 pM. The maximum bound was 108 ± 6 fmol/mg protein. The binding reached the equilibrium within 30 min and was dissociated by addition of (-)-propranolol. The inhibitory effects of isomers of propranolol and isoproterenol on this binding showed that (-)-isomers were two orders of magnitude more potent than the (+)-isomers.

The binding was inhibited by isoproterenol, epinephrine and norepinephrine with Ki values of 2×10^{-7}M, 2.5×10^{-6}M and 1.2×10^{-5}M, respectively. A modified Scatchard analysis of the inhibitory effects of practolol, metoprolol and zinterol on IHYP binding shows that the proportion of β_2-receptors was about 80% of total β-adrenergic receptor population. The scatchard analysis of the IHYP binding to microvessels prepared from cortices of aged (24 months) and mature (3 months) rats shows that the density of receptor sites is reduced by aging (B_{max}: 84 ± 4 fmol/mg prot and 106 ± 5 fmol/mg prot respectively) without changes in the affinity constant (K_D: 75 ± 9 pM and 71 ± 7 pM, respectively). This result suggests that the changes in brain circulation observed during aging may be at least partially due to a reduction of β-adrenergic control of cerebral microvessels. Moreover these functional alterations are in the same line with the changes observed during aging at neuronal level.

62.2 AGED DEGENERATING PATHWAYS ARE REFRACTORY TO AXONAL PENETRATION. Michel Paré* and R. Levine. Dept. of Biology, McGill University, Montréal, Québec, Canada H3A 1B1

We have examined the effect of aging of degenerating pathways in the brain of the goldfish on their suitability as conduits for regenerating axons. Our experiments were performed as follows. We removed the right eye of 25 goldfish. These animals were then allowed to recover from surgery for times varying from 2 to 12 weeks, after which the right tectal lobe was removed. In some animals the eye and the tectal lobe were removed at the same time. In all cases animals were allowed to survive for varying lengths of time following tectal lobe removal and then sacrificed 24 hrs. after intraocular injection of 30 μCi ^{3}H-proline. The brains of these animals were then processed for autoradiography to allow us to visualize the trajectories of optic fibers which were growing through the brain following the removal of their target structure (the tectum).

We found that if the eye and the tectal lobe were removed at the same time, optic fibers from the remaining eye grew to the remaining tectal lobe through, among other pathways (Lo and Levine, 1981, Br. Res. 210: 61), the transverse commissure and the denervated optic tract of the opposite side. The penetration of the degenerating optic tract by large numbers of fascicles was seen in animals which had survived as long as 4 weeks after enucleation before undergoing tectal lobe removal. On the other hand, in animals which survived for 8 or 12 weeks after enucleation, the majority of the regenerating optic fibers appeared to grow preferentially through the transverse commissure. While these animals did show some invasion of the optic tract it was always far more sparse than the earliest post enucleation animals and often did not fill the entire tract cross section.

On the basis of these observations we conclude that the character of degenerating pathways may change over time in such a fashion as to make those pathways relatively refractory to invasion by regenerating axons.

62.3 THE EFFECT OF AGE ON BRAIN CAPILLARIES. P. Hicks, C. Rolsten*, D. Brizzee* and T. Samorajski. Texas Research Institute of Mental Sciences, Houston, Texas 77030.

The effect of age on brain capillaries has been described in human, primate and rat cortex (Hunziker et al, J. Gerontol., 1979; Burns et al, J. Gerontol., 1979; Knox and Oliviera, Acta Neurpathol., 1980). However, there are no reports of the effect of age on capillaries from non-cortical brain regions. Therefore, we have measured several capillary components from the rat frontal cortex (FC) and hippocampal CA 1 region (HC) to determine if regional differences occur in capillary morphology with aging.

Male Fischer 344 rats 3-, 9- and 24-months old were prepared for electron microscopy following intracardiac perfusion. Capillary measurements were made with a planimeter from photomicrographs enlarged to 30,000X. Corrections were made for differences in photographic enlargement.

There is an age-related increase (30%) in the cross-sectional areas of the basement membrane of both the FC and HC. There are no age-related differences in the cross-sectional areas of the capillary endothelial cells in either brain region. However, the cross-sectional area of the capillary lumen increases (39%) with age in the FC and decreases (11%) with age in the HC. The pericyte cross-sectional area is greater in the HC than in the FC.

These results indicate that age-related changes occur in both the FC and the HC capillaries, but the changes may differ between brain regions.

Age	N		Basement Membrane	Endothelial Cell	Lumen	Pericyte
3	4	FC	0.69	1.96	11.5	0.50
		HC	0.71	2.00	14.3	0.84
9	3	FC	0.72	2.03	13.6*	0.53
		HC	0.72	1.91	14.5	0.56*
24	5	FC	0.90**	2.08	16.0**	0.53
		HC	0.90**	2.13	12.9*	0.76

Capillary cross-sectional areas are given in μ^2. N= the total number of rats used at each age. A minimum of 10 capillary profiles were measured from each rat. *p<.05 and **p<.01 when compared to the 3- or 9-month old rats.

62.4 MORPHOMETRY OF AGING NEURONAL NUCLEI. MB. T. Buschmann, J. S. Geoffroy* and A. LaVelle. Dept. Anat. & Gen. Nursing, Coll. Med. & Coll. Nursing, Univ. Illinois Med. Ctr., Chicago, IL 60612.

Changes in the diameters, areas and perimeters of nuclei and changes in the nuclear envelope (NE) form factor which indicates the spherical or nonspherical character of the nucleus, and in nuclear envelope invagination (NEI) length and number of neocortical neurons were studied in selected ages from the lifespan of the golden hamster. The ages ranged from newborn to 700 days.

The animals were anesthetized, perfusion fixed and epoxy embeded via standard EM procedures. Thick sections (1 μm) were stained with toluidine blue. The slides were coded by an uninvolved individual so that the data were collected blindly. Camera lucida drawings were made of all layer V pyramidal nuclei in one section from each slide that exhibited apical dendrites and ample cytoplasm. The measurements of the area and the perimeter of each profile were obtained by computer-assisted image analysis (Bio-Quant, R&M Biometrics, Nashville, TN). From these measurements a form factor was calculated ($A4\pi/p^2$). Diameters and numbers of NEI were measured and counted and the boundary density (NE length per unit nuclear area) was calculated from the direct measurements of areas and perimeters (B/A = perimeter area) and were also determined by classical point counting stereology ($B_A = \frac{\pi}{2} \times \frac{Ii}{P_T \times 2d}$).

The data indicate that nuclear diameter, area, and perimeter increased with age and peaked at 15 days. After 15 days, the diameter and area decreased significantly although the perimeter remained constant due to the associated increase in nuclear envelope invaginations. Also because of these invaginations, the boundary density increased with age and peaked at 500 days. Correlative to this, the form factor calculation indicated that the spherical configuration of the nucleus at 5 days changed to a nonspherical form with age.

The increases in diameter, area, and perimeter are indicative of the rapid growth phase that occurs until 15 days. The subsequent constancy of nuclear perimeter and the decrease in nuclear diameter and area during maturation and aging, correlates with the associated increase in the nuclear boundary density which is due to the increase in the length and number of peripheral NEI which in turn is reflected in the form factor. Therefore, these results are verified by both the indirect stereology and the direct image analysis.

In conclusion, nuclear envelope invaginations, which begin to appear during the stages of rapid perikaryal growth, peak in number and dimensions at maturity and persist during subsequent aging. This is indicative of a relatively high level of continued synthetic activity.

62.5 SPIKE PROPAGATION IN DENDRITES OF YOUNG AND OLD HIPPOCAMPAL GRANULE CELLS. C.A. Barnes and B.L. McNaughton. Inst. of Neurophysiology, University of Oslo, Norway.

Both anatomical and neurophysiological experiments have shown a profound deafferentation of hippocampal granule cells in senescent rats. This loss (about 30%) of excitatory input to the granule cells could potentially be disruptive to information processing in this system unless it was compensated for in some way. Two forms of such compensatory change have, in fact been found: 1) a lowering of the discharge threshold and a decrease in the latency of the orthodromically elicited action potential in old granule cells; 2) stronger synaptic responses in the old animals (Barnes & McNaughton, J. Physiol. 309, 473-485, 1981).

One possible mechanism for the decrease in the threshold of the granule cells is that the area of electrical excitability could expand to include more of the proximal dendritic tree during senescence. Such an expansion would explain both the age differences in threshold and action potential latency. The experiment reported here was designed to test this hypothesis using current source density analysis to compare young and old rat fascia dentata.

Hippocampal slices taken from 13 young (8 mo) and 13 old (29 mo) rats were prepared using standard techniques. A fixed stimulating electrode was placed in the mossy fibres to antidromically activate the granule cells, and a movable recording pipette was placed 50 μm from the bottom of the cell body layer. Constant intensity stimuli were delivered and the corresponding field potential responses were recorded at 10 μm intervals along a line parallel to the dendritic axis. Current source densitites were computed by taking the second derivatives of potential versus spatial position.

There were no detectable differences between age groups in either the distance travelled by the peak inward current in the proximal dendritic tree, or in the current propagation velocity. The hypothesis of a shift in the action potential trigger zone predicted that the antidromic spike should have propagated further up the dendritic tree in old granule cells, on the assumption that the inward current was of a classical Hodgkin-Huxley type. It remains possible that there exists some slowly activated voltage-sensitive inward current which is responsible for spike initiation following synaptic (orthodromic) activation. This possibility is currently under investigation.

62.6 UPTAKE AND STIMULATED RELEASE OF [^{3}H]-DOPAMINE FROM STRIATAL SLICES OF YOUNG, MATURE AND SENESCENT RATS. John R. Whitaker*, Jeffrey M. Thompson and J.A. Joseph. Laboratories of Behavioral Sciences and Neurosciences, Gerontology Research Center, National Institute on Aging, Baltimore, MD 21224.

In previous experiments it was determined that aged rats, unilaterally lesioned in the substantia nigra, exhibit decreased rotational behavior following amphetamine administration (Joseph et al., J. Gerontology, 33, 643-649, 1978; Joseph et al., Neurobiol. Aging, 1, 119-125, 1980). These experiments and others suggested that these deficits may be the result of decreases in DA turnover, loss of DA receptors or reduced numbers of striatal neurons during senescence. The present experiments were carried out to determine whether age-related changes in presynaptic events occur in the striatum and might account for these rotational behavior deficits. Thus, we determined [^{3}H]-DA uptake and release following KCl or amphetamine administration in striatal slices from young (7 mos), mature (12 mos) and senescent (24 mos) Wistar rats.

Striatal slices were incubated in [^{3}H]-DA. Some slices were collected by vacuum filtration after increasing incubation times to determine [^{3}H]-DA uptake. Other slices were washed and placed in a multi-channel perfusion system and washed for 30 min. Timed perfusate samples were then collected before and during addition of KCl (80 mM) or amphetamine (10^{-6} - 10^{-2}M). The amount of [^{3}H]-DA released in each sample was determined.

No significant age-related differences in release were detected when the slices were stimulated by 80 mM KCl (Peak release as a percent of total radioactivty: 24.1%, 7 mo; 23.2%, 12 mo; 21.9%, 24 mo; $t(12) < 1$; $p > 0.05$). While there was a dose-dependent stimulation of release by amphetamine ($F(7,21) = 11.96$, $p < 0.001$; 3 (age) by 4 (dose) analysis of variance), no age-related differences in amphetamine stimulation were seen ($F(2,21) < 1$; $p > 0.05$). Uptake was maximum in all age groups by 15 min and no significant differences were seen as a function of age and time ($F(6,45) = 1.66$; $p > 0.05$).

These results indicate that the age-related motor-behavioral deficits measured by the rotational model are not produced by changes in striatal DA uptake and release, but may be more closely associated with other synaptic alterations, such as loss of striatal DA receptors which occur during senescence.

62.7 DECREASED SPROUTING AND DEGENERATION OF NERVE TERMINALS OF ACTIVE MUSCLES IN AGED RATS. J.L. Rosenheimer and D.O. Smith. Dept. of Physiology, University of Wisconsin, Madison, WI 53706.

The architecture of the end-plate region in the phrenic nerve-diaphragm muscle was examined in aged (28 mos) and control (10 mos) rats, upon cholinesterase staining of the end-plate and silver-gold impregnation of the axon and its terminal arborization. The average number of nerve terminals per end plate was 18 and 13 in the aged and the control animals, respectively; this difference is significant statistically. The number of motor axon collaterals, from which terminal branches emanate, projecting into the end plates, was not significantly different for the two age groups. Thus the increased number of terminal endings in the older animals is due to more frequent branching within the end-plate region. In 1.7% and 1.6% of the muscle fibers in the control and the aged rats, respectively, there was a second end plate; more than two end plates were never observed. There was no significant difference in the size of the end-plate region of the two age groups.

In 23% and 43% of the end plates of aged and control rats, respectively, there were signs of terminal degeneration. There were indications of sprouting in 10% and 41% of the end plates of the aged and the control animals, respectively. Among the aged rats, there were no cases in which both sprouting and degeneration were seen in the same end plate; both phenomena were observed in 11% of the young animals. Thus, degeneration and new growth appear to be independent processes which occur less frequently in aged rats.

Synaptic sites in animals of both age groups were examined in electron micrographs. The nerve terminals of the aged rats contained 1.38 times as many synaptic vesicles as those of the control rats; the average diameters of the synaptic vesicles in the control and the aged rats were 50 and 49 nm, respectively.

It is concluded that in nerve terminals of aged rats, there is a decreased rate of membrane degeneration and growth. In this preparation, the rate of degeneration declines more rapidly; therefore, the imbalance between these two processes results in greater numbers of nerve terminals and synaptic vesicles, although they may not all be functional.

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62.8 NEUROGENESIS IN THE DENTATE GYRUS OF THE 8 AND 11 MONTH OLD RAT. M.S. Kaplan* and D.H. Bell* (SPON: E. Reyes). Department of Anatomy, University of New Mexico School of Medicine, Albuquerque, NM 87131

Only a few investigators have attempted to study cell proliferation in the adult brain with radioautography after H^3-thymidine injection. The infrequent occurrence of labeled cells in the adult precludes any reasonable chance of observing labeled neurons with EM radioautography. To date we have identified apparent labeled neurons, with the light microscope, in the dentate gyrus granular layer of rats injected with H^3-thymidine at 8 and 11 months after birth. In the next few months these labeled granular neurons will be quantified then re-embedded and thin sectioned for subsequent electron microscopic identification.

Male rats, 8 and 11 months after birth, were given single intraventricular injections of H^3-thymidine (5uCi/injection), and after a 19 or 27 day survival the animals were perfused with a mixture of aldehydes. One micrometer plastic sections of dentate gyrus were cut, dipped in Kodak NTB-3 emulsion, and exposed at 4°C. In the resulting radioautographs, a cell was considered labeled if it had 4-5 grains over the nucleus, a value considerably above background. Although labeled cells in these mid-aged rats have not yet been re-embedded or examined in the electron micorscope (April 1981), they appear as typical labeled granular neurons previously described in the 3 month old rat (Science, 197: 1092-1094, 1977).

The population dynamics of granular neurons in animals aged 3, 8 and 11 months after birth are now being evaluated. Our preliminary data indicates that the labeling index of dentate gyrus granular neurons does not change much between these ages. If it is assumed that the S phase for the precursors of granular neurons is approximately 8 hours, then for every successive 8-hour period sample another equal percentage of labeled cells would be observed. Assuming steady additions of granule cells from 3 to 11 months these newly formed cells could result in considerable growth or turnover and implies a considerable plasticity in the hippocampus.

(Supported by University of New Mexico Research Allocations Committee Support Grant #R-5024.)

62.9

WITHDRAWN

62.10 MAPPING OF NEOSTRIATAL TRANSMITTER SYSTEMS AS A FUNCTION OF AGING. R. Strong*, T. Samorajski, and Z. Gottesfeld. (Spon: J. Schoolar). Texas Res. Inst. of Ment. Sci. and The Univ. of Tex. Med. Sch., Houston, Texas 77030

Functional imbalance of striatal cholinergic and dopaminergic neurotransmitter systems has been reported for age-related neurodegenerative disorders. Thus choline acetyltransferase (ChAT) and glutamic acid decarboxylase (GAD) have been found to be lowered in Huntington's disease, while dopamine (DA) is decreased in Parkinson's disease.

The functional association of dopaminergic and cholinergic neostriatal neuronal elements has been demonstrated pharmacologically, anatomically and electrophysiologically, in experimental animals. In addition, these neurotransmitter systems are reported to have a heterogeneous distribution within the striatum, suggesting differential functional organization for this region.

Since age related deficits of both pre- and post-synaptic dopaminergic and cholinergic neurochemical markers have been report ed for whole neostriatum of rats (Strong, et al., 1980, Finch, et al., 1980, Timiras, et al., 1980) it was of interest to compare the distribution of these neurotransmitter systems within the striatum and their alteration with age.

A total of 18 male Sprague-Dawley rats[1] age 6, 16, and 26 months were used in this study. Animals were decapitated, their brain was removed and frozen in dry ice, then cut coronally (300 μm thick) in a cryostat at -8°C. Tissue punches were removed from the central and lateral portions of 11 consecutive striatal sections and assayed for ChAT (Fonnum, 1976) and GAD (Fonnum, et al., 1970). For catecholamines, punches were removed adjacent to the central sites taken for ChAT and GAD and then assayed according to Coyle and Henry (1973). ChAT activity and DA levels manifested a heterogeneous pattern and, in general, were 50% higher rostrally than caudally and ChAT was higher laterally than medially. GAD and norepinephrine (NE) were more homogeneously distributed. Significant age-related changes in ChAT and DA were also manifested in a heterogeneous manner. There were extensive deficits (30-50%) of DA and ChAT in the rostral and caudal parts of the striatum but no significant changes in the other regions. There were no age-related changes in GAD while NE showed a lesser decrease compared to DA or ChAT.

The present data, especially the age related changes, is consistent with previous reports of, and further reiterates, the close synaptic association between the cholinergic and dopaminergic systems in the neostriatum.

[1]Supported by National Institute of Aging

62.11 INTRASTRIATAL INJECTIONS OF DOPAMINE-ACTIVE AGENTS AND AGE-RELATED ALTERATIONS IN BEHAVIOR IN THE RODENT. J. A. Joseph, J. R. Whitaker* and J. F. Cubells, Jr. Lab. of Behavioral Sciences, Gerontology Res. Ctr., National Inst. on Aging, NIH, Baltimore, MD 21224.

Age-related differences in sniffing, grooming and rotational behavior following intrastriatal injections of amphetamine (AMP) or dopamine (DA) were examined in young (N=7) and old (N=7) male and female (N=4 and 3 respectively) Wistar rats, unilaterally lesioned in the left substantia nigra with 6-hydroxydopamine. After recovery from surgery (1 week) all animals were injected (i.p.) with AMP (2 mg/kg) and rotations counted for 10 minutes. All animals had to exhibit at least 25 rotations or they were not used. One week after screening, several successive doses of AMP dissolved in .5 ul D_2O (pH adjusted to 5.5 to 6.0 using $NaHCO_3$ and 1 M HCl) were administered on alternate days (1 dose/day) through cannulae chronically implanted into the right (intact) striata. After this regimen DA, dissolved in .5 ul N_2 bubbled D_2O and pH adjusted to 5.5-6.0, was administered in a regimen similar to that for AMP. Vehicle injections were comprised of pH adjusted D_2O of same volume. Prior (1 1/2 hrs) to the DA injections, the animals were pretreated with 50 mg/kg of nialamide administered i.p. The number of rotations, sniffs, and grooms were assessed by observation. Rotations were recorded as a ratio of left over right turns (L/R). Results for the L/R index are illustrated in the table and show that old animals exhibited lowered L/R indices to both AMP and DA as the drug doses were increased [analysis of variance, AMP, age x dose, $F(4,48)=2.95$ $p<.05$; DA, age x dose, $F(3,33)=3.61$ $p<.05$]. These results indicate that neither the application of an indirect (AMP) nor a direct (DA) dopamine agonist will increase rotation in these senescent animals despite the use of a wide range of doses. It is suggested that the loss of striatal DA receptors previously found in the senescent animal by several laboratories contributes to decrements seen in this behavior. Neither AMP nor DA produced increases in sniffing or grooming behavior as a function of drug dose. Thus, age differences in these behaviors could not be determined.

AMP Dose(ug)	L/R Index($\bar{X}$±sem)		DA Dose(ug)	L/R Index($\bar{X}$±sem)	
0	8.6± 5.6	8.0±3.7	0	10.1±2.5	10.1±3.6
0.5	22.7±12.2	8.9±3.6	5	27.2±5.6	13.1±2.7
5.0	33.3±10.3	7.6±1.2	25	32.7±7.5	7.4±3.1
7.0	23.7± 7.2	5.7±1.3	50	25.5±9.2	14.4±3.7
10.0	40.7±11.6	11.9±4.4	--	---	---

62.12 INFLUENCE OF AGE AND ENDOGENEOUS NOREPINEPHRINE ON OXIDATIVE METABOLIC RESPONSES TO ELECTRICAL STIMULATION OF RAT CEREBRAL CORTEX IN SITU. A.L. Sylvia, S.I. Harik, J.C. LaManna and M. Rosenthal. Dept. Physiology, Duke Univ. Med. Ctr., Durham, N.C. 27710 & Dept. Neurology, Univ. Miami Sch. Med., Miami, FL 33101

In this study, we test two hypothesis: (1) that there are age-related differences in the ability of the cerebral oxidative metabolic system to respond to stimulus-provoked increases in energy demand; and (2) that such differences may be related to changes in the cerebral concentration of norepinephrine (NE) with age. The latter hypothesis evolved from the observation that the rate of re-reduction of Cyt. a,a_3 following oxidation by stimulation was slowed in cerebral hemispheres of mature rats depleted of NE by ipsilateral 6-hydroxydopamine lesion of the nucleus locus ceruleus (LC) (Br. Res. 204, 87, 1981). Cyt. a,a_3 redox responses to stimulation were monitored by reflection spectrophotometry in young mature (6 mo.) and aged rats (28 mo.) two weeks after unilateral LC lesion or sham operation. In control (C), sham operated (S) and lesioned (LC) hemispheres of young mature and aged animals, cortical stimulation invariably produced transient oxidations of Cyt. a,a_3. While the rates of the oxidation and re-reduction responses were consistently rapid and similar in control and sham operated hemispheres of young mature animals, they were significantly and uniformly slower in (C),(S), and (LC) hemispheres of aged rats. In young mature animals, the stimulus evoked Cyt. a,a_3 cortical responses recorded in LC lesioned hemispheres were also significantly slower than those recorded in (C) and (S) contralateral hemispheres. The temporal kinetics of the responses resembled those observed in all hemispheres of aged rats. There were no significant age-related differences in cortical NE concentrations in either control or sham operated hemispheres. However, the cortical NE content in LC lesioned hemispheres was decreased by approximately 90% compared to (C) and (S) contralateral hemispheres in both age groups studied. These data demonstrate that cerebral oxidative metabolic capabilities do vary with age. The observation that stimulus evoked cortical responses in NE-depleted hemispheres of young mature rats closely resemble those normally obtained in aged animals, suggest the involvement of noradrenergic mechanisms responsible for the age-related changes in metabolic responsivity to direct cortical stimulation. However, the finding that NE-depletion did not additionally alter the Cyt. a,a_3 metabolic responses normally obtained in old rats, implies that these metabolic changes are independent of NE concentration.

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62.13 CHANGES IN NEUROTRANSMITTER SYNTHETIC ENZYMES AS A FUNCTION OF GENOTYPE AND AGE IN MICE. S.B. Waller*, D.K. Ingram*, M.A. Reynolds* and E.D. London. Laboratories of Neurosciences and Behavioral Sciences, National Institute on Aging, Gerontology Research Center, Baltimore City Hospitals, Baltimore, Maryland 21224.

Effects of genotype and age on neurotransmitter synthetic enzymes (choline acetyltransferase, CAT; glutamic acid decarboxylase, GAD; and tyrosine hydroxylase, TH) were studied in C57BL/6J and A/J mice that were 4, 18 or 24 months (mo) of age. Enzyme activities were assayed radiometrically in the frontoparietal cortex, striatum, hippocampus and cerebellum. All differences noted were significant at $p < 0.05$.

Genotype. TH activity was higher in the C57BL/6J strain compared to the A/J strain in all brain regions of all age groups except the striatum at 4 mo, hippocampus at 18 mo and cortex at 24 mo where there were no strain-related differences.

CAT activity was higher in the C57BL/6J mice except at 24 mo, when cerebellar activity was greater in the A/J strain.

GAD activity of A/J mice was higher than in C57BL/6J mice in the cortex and striatum at 4 mo and the cortex at 18 mo. In contrast, GAD activity was lower in the cerebellum and striatum of 24 mo old A/J mice than in C57BL/6J mice.

Age. Striatal TH of both strains was not affected by age. TH activity increased in A/J mice between 4 and 18 mo in the cortex, cerebellum and hippocampus and between 4 and 24 mo in the cortex and cerebellum. TH in C57BL/6J mice increased between 4 and 18 mo in the cortex and cerebellum and between 4 and 24 mo in the cerebellum, but declined in the cortex between 18 and 24 mo.

GAD activity declined between 4 and 24 mo in the A/J striatum and C57BL/6J cortex, and increased between 18 and 24 mo in the AJ hippocampus and between 4 and 24 mo and 18 and 24 mo in both striatum and hippocampus of C57BL/6J mice.

Between 4 and 18 mo and 4 and 24 mo, CAT activity increased in the cortex of both strains, the A/J cerebellum, and the C57BL/6J hippocampus. Between 18 and 24 mo, CAT activity was elevated in the A/J cerebellum and the hippocampus of both strains. Striatal CAT increased in C57BL/6J mice between 4 and 24 mo.

In conclusion, age-associated alterations in activities of neurotransmitter synthetic enzymes vary with the genetic strain under investigation. Increases in enzyme activity may represent age-related changes in affinities for substrates or cofactors. These findings underscore the importance of parallel studies using several inbred strains or hybrid strains in aging research.

62.14 NEURON NUMBER DOES NOT DECLINE IN CORTICAL BARRELS OF AGING MICE. C.A. Curcio* (SPONS: P.D. Coleman) Dept. of Anatomy, Univ. of Rochester Sch. of Med. and Dent., Rochester, N.Y. 14642

Decline in neuron density is an important feature of the aging human cortex. To determine if there is absolute loss of cortical neurons with age one needs to know both cell density and tissue volume. The barrel is a morphological and functional unit of rodent somatosensory cortex whose discrete nature makes it a suitable system in which to address this question in an experimental animal. Cells have been counted in one identified barrel, C3, in male C57Bl/6 mice aged 4, 12, 22, 26, 30, and 33 months.

Neuron density in C3 was determined from a computer model of the barrel (Curcio and Sloan, J. Neurosci. Meth., in press) based on 3-dimensional reconstruction of 3 1-micron sections cut in a plane tangential to the pial surface. Barrel volume was determined from the cross-sectional area, also measured from tangential sections, and the height of layer IV, measured from frontal sections through the opposite hemisphere. Data were collected blind with respect to age and were evaluated with ANOVA statistical methods.

The number of neurons in C3 did not decline with age ($F = 1.12$, $p > 0.25$). The mean number for all 25 barrels studied was 1698 (s.d. 201). Barrel volume also showed no age-related trends.

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62.15 STRIATAL AND CEREBRAL CORTICAL CALMODULIN IN AGED C57BL/6J MICE. J.A. SEVERSON AND C.E. FINCH. DEPT. OF PSYCHIATRY AND DEPT. OF PHYSIOLOGY AND BIOPHYSICS, USC SCHOOL OF MEDICINE, LOS ANGELES, CA 90033.

The nigro-striatal dopaminergic (DA) pathway exhibits age-related declines in several neurochemical parameters, including losses in DA receptor binding sites and DA-sensitive adenylate cyclase. The multifunctional calcium-binding protein, calmodulin (CaM), is of interest with age due to its putative role in coupling the DA receptor to striatal adenylate cyclase and because CaM varies directly with DA receptor number and DA-sensitive adenylate cyclase activity in young rodents.

Striata and cerebral cortices from male C57BL/6J mice, 3, 9 and 28 mo., were analyzed for CaM by phosphodiesterase activation assay. Striatal CaM levels increased 50% between 3 and 28 mo., but cortical CaM was unchanged. Striatal CaM levels increased with age in soluble and particulate fractions. However, in both regions, the percent of CaM in the soluble and particulate fractions was constant with age. In all ages, on a percent basis, striatal CaM in the particulate fraction was increased equally by the presence of Ca^{2+} during homogenization and was decreased equally by EGTA.

The data indicate that striatal CaM and DA receptor binding sites are regulated similarly with age. Possibly, mechanisms that maintain striatal DA receptors at a level found in young mice deteriorate more rapidlywith age. The ubiquity and multifunctionality of CaM complicates the interpretation of these data. However, the interaction of CaM with the DA receptor-adenylate cyclase complex suggests that CaM may increase to facilitate DA receptor coupling with adenylate cyclase as compensation for fewer DA receptors during aging.

62.16 REGIONAL DISTRIBUTION OF BRAIN PHOSPHATASE ACTIVITY IN AGING RAT. J.L. Fuchs, D.A. Deane*, M.L. Hendrix*, and G.S. Lynch. Dept. of Psychobiology, Univ. of Calif., Irvine, Calif. 92717

A growing body of evidence points to an important association of phosphatases with glial and vascular elements in the brain. In light of studies showing age-related changes in both astrocytes and blood vessels, it becomes of interest to investigate possible effects of aging on the distribution of brain phosphatase activity. Accordingly, we have used histochemical techniques to identify 5'-nucleotidase, alkaline phosphatase, ATPase, and acid phosphatase throughout the brain of young adult (3-4 mo), middle-aged (12-14 mo), and old (24-31 mo) rats. The most marked changes were found in 5'-nucleotidase and alkaline phosphatase.

5'-Nucleotidase activity in rat brain increased substantially during the period from young adulthood to middle age, with little change thereafter. The greatest increases were seen in regions of forebrain, diencephalon and midbrain. Radioassay of brain homogenates revealed 63% more 5'-nucleotidase activity per mg protein in the forebrain of middle-aged and old rats than in the young adults. 5'-Nucleotidase activity in most of the 7 other body organs assayed showed a slight decline with age. Thus the increase is not generally characteristic of aging body tissues, and probably represents aging processes particular to brain.

In brain sections stained for alkaline phosphatase activity, young adult rats appear to have a considerably finer network of blood vessels; the older groups show fewer, but in some instances, thicker vessels. The differences probably reflect changes in enzyme activity of vascular endothelium, and were not seen in unstained sections from brains perfused with India ink. The alkaline phosphatase data suggest functional changes in brain vasculature, a findin, which would be compatable with reports of structural aging in blood vessels.

These experiments indicate that aging has a selective effect on phosphatases which have been linked to brain elements showing structural changes during aging. Research is now in progress to test whether changes in these enzyme activities can be used to predict the severity of structural alterations in the aging brain.

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62.17 AGING REDUCES ACETYLCHOLINE AND CARBOHYDRATE METABOLISM *IN VITRO*
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In vivo, the synthesis of whole brain acetylcholine (ACh) declines by 45 and 65% in 10 and 30 month old mice compared to 3 month old mice, respectively. We now report that this decrease is associated with an age-related reduction in oxidative metabolism and an impaired release of ACh.

Age-related changes in carbohydrate metabolism were assessed with brain slices from two strains of mice (C57Bl and Balb/c). $^{14}CO_2$ production was measured from labelled substrates: [U-^{14}C]-glucose (an indicator of overall glucose oxidation), [3,4-^{14}C]-glucose and [1-^{14}C]pyruvate (indices of flux through pyruvate dehydrogenase with low or high concentrations of pyruvate, respectively). When incubation were in low K^+ buffer, $^{14}CO_2$ production from [U-^{14}C]glucose decreases (% decline in C57Bl-Balb/c strains, respectively) by 19-23% (10 month) and 35-42% (30 month) compared to 3 month old mice of their corresponding strains. High K^+ (31 mM) stimulated $^{14}CO_2$ production 2-3 times more in the aged brain (10 and 30 month old mice) than in the young brain (3 month old mice). Thus in high K^+ incubations glucose oxidation declined from the 3 month control value by only 8-6% (10 month) and 13-17% (30 month) in the C57Bl and Balb/c strains, respectively. An age-related decrease in $^{14}CO_2$ production from [1-^{14}C]pyruvate and [3,4-^{14}C]glucose paralleled the reduction with [U-^{14}C]glucose, which suggests that aging alters flux through pyruvate dehydrogenase.

Aging decreased the synthesis of ACh from [U-^{14}C]glucose in vitro. In low K^+ buffer, synthesis declined 28 and 39% in 30 month old mice compared to 3 month old mice in C57Bl and Balb/c strains, respectively. However, in high K^+ buffer, ACh synthesis decreased only 9 and 25% in the two strains. Since these in vitro changes in ACh synthesis and carbohydrate oxidation were small compared to the decline in ACh synthesis in vivo, we examined the release of [^{14}C]ACh from aged brain slices that had been preincubated with [U-^{14}C]glucose. Aging had no effect on the non-Ca^{2+}-dependent-release nor on the low K^+-Ca^{2+}-dependent-release. The high K^+-stimulated-Ca^{2+}-dependent-release decreased 37 and 79% (Balb/c) and 48 and 79% (C57Bl) in 10 and 30 month old mice compared to their 3 month old controls, respectively. This decrease paralleled the decline in the in vivo synthesis of ACh with aging. The data presented implies that decreases in oxidative metabolism, ACh synthesis and in the release of ACh contribute to a reduction in cholinergic function in the senescent brain.

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62.18 SENESCENT CHANGES IN BALANCE, COORDINATION, AND CEREBELLAR MICROANATOMY.
S. Zornetzer[a], F. Bloom, R. Mervis[b], and J. Rogers. Salk Institute, La Jolla, CA 92037 and [b]Dept. Pathology and Neuropathology, College of Medicine, Ohio State University, Columbus, OH 43210.

Recent studies have shown pathologic electrophysiological changes in rat cerebellum with age (1-3). The present experiments investigate their behavioral and anatomical correlates. On a task requiring balancing and walking on progressively narrower planks in order to obtain food, 25 month-old male Sprague-Dawley rats (N=11) are significantly impaired even on a plank three times the size of that successfully negotiated by 6 month-old cohorts (N=13). For example, on a 38 mm wide plank, 25 month-old subjects fall an average of 1.17 ± 0.35 times in 10 trials. Rats 6 months-old average 0.15 ± 0.07 falls per 10 trials on a 13 mm wide plank, and never fall on 25 or 38 mm planks. Likewise, the 25 month-old subjects are significantly worse in balancing on and matching speed with a rotating rod even when the rod diameter is two-fold greater than that used by the younger animals. On 51 and 102 mm diameter rods, old rats fall at running speeds of 1.7 ± 0.5 and 3.2 ± 0.8 cm/sec, respectively, while young rats fall at 5.9 ± 0.3 and 10.2 ± 1.0 cm/sec. On both tests of coordinated locomotor activity, 25 month-old subjects clearly divide into two subpopulations, one moderately impaired compared to 6 month-old rats and the other grossly impaired. This dichotomy is of particular interest in current research where subjects of the behavioral experiments described above are compared on three parameters of cerebellar microanatomy: dendritic atrophy of Purkinje cells in Golgi sections, axodendritic synaptology of Purkinje cells in electron microscopic sections, and Purkinje and granule cell counts in light microscopic sections. Data from these anatomical and behavioral assays complement the age-related pathology already revealed by our electrophysiological studies of senescent cerebellum (1,2); the correlation and cross-validation of such changes could provide sequelae of neural aging based on a multidisciplinary series of measurements.

1. Rogers et al. Neurobiology of Aging, 1:3-11 (1980).
2. Rogers et al. Neurobiology of Aging, in press.
3. Marwaha et al. Brain Research, 201:85-97 (1980).

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62.19 A QUANTITATIVE GOLGI STUDY OF DENDRITIC SPINE TYPES IN AGING RATS.
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Dendritic spines are small, thorn-like appendages that characteristically extend from pyramidal cells in all areas of the cerebral cortex. The density of dendritic spines is influenced by a wide variety of phenomena. Although variations in the morphologic appearance of dendritic spines has been qualitatively reported, few quantitative accounts of these structurally different spines exist.

We have previously distinguished two categories of dendritic spines and reported a difference in density of spine types as a function of age. The first category includes all spines with a thin stalk and a terminal expansion resembling a lollipop (type L). The second category of spines includes those with no terminal expansion, thus having a nubbin configuration (type N). The functional meaning of these spine types is unknown, but they probably represent differences in the electrophysiological input to the neuron.

In this particular study, retired breeder, Sprague-Dawley male rats from GIBCO laboratories were placed at 14 months into either isolation (1 rat to a 30X 20X 28 cm cage) or remained in standard colony (3 rats in a cage of the same size). All other variables were identical. After 6 months the rats were sacrificed and the brains placed in a Golgi-Cox solution. Segments for counting spine densities came from apical, oblique, and basal branches of layers II, III, Va, and Vb pyramidal neurons of the occipital cortex.

Our results show that the density of type N spines is consistently greater on neurons from isolated rats regardless of cortical layer or dendritic segment counted. This difference was most prominent in layer II on neurons whose apical shaft diameter was between 1.1 - 2.0 um.

The type L spine density was, in general, not affected by either of the two housing conditions with one exception. In layer Vb, the type L spine density was consistently greater on segments counted from isolated rats.

It is interesting to note that in conditions such as aging, isolation, and deafferentation, spines without a terminal expansion (type N) selectively increase in density.

62.20 EFFECTS OF AGING, ENVIRONMENT AND/OR CASTRATION ON LAYER III AND LAYER V DENDRITES IN FELINE AUDITORY CORTEX.
Anne S. Kaplan and Arnold B. Scheibel. Brain Research Institute and Departments of Anatomy and Psychiatry, UCLA, Los Angeles, CA. 90024.

Dendritic branching parameters were measured in Golgi-stained auditory cortex (mid-ectosylvian g.) from three groups of cats: OLD = 12-17 years old, castrated pets; YNG1 = 1½-2 years, intact cage-raised; YNG2 = 1½-2 years, castrated pets. Five Layer III (LIII) pyramids from each of four cats in each group were chosen with strict, unbiased criteria. Five Layer V (LV) pyramids were also chosen from the same cats in groups OLD and YNG2. The basilar dendrite system of each of these cells was drawn (in 2-D) at 600X, with a camera lucida system. Several measures were then applied to the drawings, including: concentric ring analysis (D. Sholl, J. Anat. 1953); total dendritic length (as sum of intersections of all rings); number, average length, and total length of each branching order; and number, average length, and total length of terminal (EBR) and bifurcating (XBR) branches.

In both layers, the total dendritic length was greater in YNG2 than in the other groups (LIII: YNG2 = 2950μ, OLD = 2267μ, YNG1 = 2155μ; LV: YNG2 = 3112μ, OLD = 2194μ). In LIII, this difference is largely accounted for by (A) higher average length of EBR in YNG2 (104μ vs. 92 & 91μ); and (B) shorter and fewer XBR in YNG1 (14μ, 48 XBR) as compared to OLD (18μ, 57 XBR) and YNG2 (18μ, 63 XBR). In LV, the difference between YNG2 and OLD is not due to longer EBR (130 vs. 123μ) so much as to the greater numbers of EBR (14 vs. 9) and XBR (25 vs. 18) in YNG2.

There were no differences between groups in either layer in the number of primary branches, which averaged 4.2 in LIII and 5.7 in LV. For most other measures, the pattern in LIII was YNG2 > OLD ≥ YNG1; for LV it was YNG2 > OLD.

Since the hormonal and environmental status of YNG2 and OLD was similar, the differences between these groups may indeed be age-related, or, at least, related to the diseases of aging. In LIII, this aging process seems to be one of dendritic dying-back, i.e., shortening of more distal processes. In LV, there may in addition be loss of whole branches, but net loss of entire dendrites does not appear to occur in aging cat auditory cortex.

The fact that YNG1 differed greatly from YNG2, and slightly or not at all from OLD, suggests either that castration and/or environmental stimulation may counteract aging effects in older cats, or, that the absence of these factors may cause young brains to appear old. A shift in the balance between growth and retraction (Buell & Coleman, Science 1979) may be involved. Further, the effects of these factors may be limited to cortex, as the caudate nuclei of these same OLD cats show senescent losses when compared to cats similar to YNG1 (McAllister, et al., this meeting).

63.1 CENTRAL CONTROL OF AVIAN VOCALIZATION: NEURONAL RECORDINGS FROM SINGING BIRDS. James McCasland and Masakazu Konishi, Division of Biology 216-76, California Institute of Technology, Pasadena, CA 91125

Discrete telencephalic nuclei HVc and RA have been implicated by lesion studies in the control of vocalization of songbirds (Nottebohm, F., in Prog. in Psychobiol. and Physiol. Psych. Vol. 9, 1980). We confirm the role of HVc in vocalization by presenting neuronal recordings taken from HVc of singing birds. However, we do not find clear support for the concept of hemispheric dominance of song control, also developed by Nottebohm et al., who showed strikingly asymmetric effects following unilateral lesions of HVc or RA, or unilateral section of the hypoglossal nerve innervating the avian vocal organ, the syrinx. It was concluded from their studies that left-side song control nuclei played a dominant role in song production.

Recordings of multiple-unit activity were obtained from three species of birds, using chronically implanted coaxial electrodes. Comparisons with preoperative songs ensured that no deficits had resulted from implantation procedures. Lesions were used to verify electrode locations.

Recordings from within the HVc boundary show greatly increased neuronal activity clearly corresponding to the timing of song elements, whereas control recordings from outside the HVc boundary show no such changes in activity. Thus HVc either generates or relays temporal cues for song. Recordings from the same electrode locations show activity associated with calls, which unlike songs in these species do not require learning from other birds.

The simplest neural correlate of hemispheric lateralization would be the presence of activity during vocalization in the dominant side, and absence of activity in the other side. Multi-unit recordings from right and left HVc of the same bird show a striking correspondence in onset time and duration of increased neuronal activity for any given vocalization. Since electrode locations vary widely, many cells must participate in vocal control simultaneously in both hemispheres, so that any simple use-disuse mechanism of dominance at the HVc level must be ruled out.

Since the effects of lesioning HVc can only be assessed by measuring the motor output of the syrinx, such lesions cannot distinguish between lateralization at the HVc level and that at more peripheral levels. The intrinsic syringeal musculature in lateralized species is more massive on the left side than on the right. Thus the mechanisms underlying this peripheral asymmetry may be a profitable focal point for future investigations of dominance in the vocal control system. (Supported by a grant from Pew Memorial Trust)

63.2 RELATION OF SYNAPTIC AND MECHANICAL COUPLING BETWEEN MOTOR UNITS AND MUSCLE SPINDLE AFFERENTS. J. Munson, J. Fleshman, G. Sypert, and J. Zengel. Dept. of Neuroscience, Univ. of Fla. Coll. of Med., Gainesville, Fla. 32610.

Two group Ia afferents from the same muscle may produce EPSPs of very different amplitude in the same homonymous motoneuron (Mendell & Weiner, J. Physiol. 255). Following the suggestion of Binder & Stuart (J. Neurophysiol. 43) we tested whether the afferent-to-motoneuron synaptic coupling strength is related to the motor unit-to-afferent mechanical coupling strength. In barbiturate-anesthetized cats, micropipette electrodes were used to (1) record EPSPs generated by single MG group Ia or group II spindle afferents in MG motoneurons, and (2) stimulate MG motor units by passing brief current pulses. Tetanic contractions of single motor units were produced by 330 ms, 40 Hz trains repeated 1/s. Afferent discharge during the final 200 ms of the tetanus was compared with the 200 ms preceding the next tetanus; 30-120 repetitions of the tetanus were analysed for each case.

Motor unit contraction decreased Ia discharge rate in 84% of 118 cases, while increasing the rate in 10% (for group II: 62% and 22% of 37 cases). Overall, a rank correlation of .37 ($p < .0002$) was found between 40 Hz tetanic tension and the motor unit's ability to alter Ia discharge rate. In pooled data, no correlation was found between the amplitude of the EPSP produced by an afferent in a motoneuron and the ability of the corresponding motor unit to alter the discharge rate of that afferent.

To search for more subtle effects, we studied the interactions of single motor units with pairs of Ia spindle afferents. Overall there was no relation between the relative sizes of EPSPs produced by two afferents and the relative effectiveness of the motor unit in altering their discharge rates. However, for fast-twitch motor units we found a weak positive rank correlation between the ratio of the amplitudes of EPSPs generated by two afferents in a motoneuron and the ratio of the suppression of the respective afferents by the motor unit ($r=.23$, $p < .04$). A very weak tendency may exist for spindle afferents to project strongly to fast twitch motor units with which they are more strongly coupled mechanically. For slow twitch motor units, we found a weak negative rank correlation between the ratio of the amplitudes of the EPSPs generated by two afferents in a motoneuron and the ratio of the suppression of the respective afferents by the motor unit ($r=-.41$, $p < .05$). A very weak tendency may exist for spindle afferents to project more weakly to slow twitch motor units with which they are coupled more strongly mechanically.

We conclude that the cat's MG muscle is not strongly characterized by "reflex localization" (Cohen, J. Neurophysiol. 16). Supported by NS 15913 and Medical Research Service of the VA.

63.3 GROUP III MUSCLE AFFERENTS WHICH EXCITE GAMMA MOTONEURONES IN THE CAT. P. H. Ellaway*, P. R. Murphy* and Anita Tripathi*. (SPON: P. L. Gildenberg). Dept. of Physiology, University College London, London WC1E 6BT, England.

Some mechanoreceptors in muscle supplied by the small (Gp III) myelinated axons have low thresholds to stimuli such as applied pressure, taps and contraction. We find that activity in these receptors excites discharges in gamma motoneurones to muscle spindles of the same or adjacent muscles.

Experiments were performed on decerebrated cats usually with a spinal cord section at T8-10. The left hind limb and tail were denervated extensively except for the muscle (triceps surae or flexor digitorum/hallucis longus) under study. Functionally single, tonically firing, gamma motoneurones were isolated by dissection of a cut fascicle of the peripheral nerve to the same group of muscles. Gamma motoneurones were identified by their axonal conduction velocities (range 15-45 m/sec).

Gamma motoneurones could be excited, inhibited or not affected by electrical stimulation of another muscle nerve at a strength sufficient to excite Gp III (5-30 m/sec) axons. The most powerful and striking effect was a short duration (5-20 msec) excitation which could take the form of one or two driven spikes. Central delays as short as 2 msec indicate close coupling and do not exclude the possibility of a monosynaptic connection.

The type of receptor which might elicit the reflex excitation was investigated by comparing sensory responses of Gp III afferents and reflex responses of gamma motoneurones to different stimuli. At the peak, or during relaxation, of an isometric muscle twitch elicited by electrical stimulation of the muscle nerve some gamma motoneurones showed a short duration driven response similar to that caused by electrical stimulation of Gp III axons. On recording the afferent discharges from triceps surae, 15 out of 38 receptors with Gp III axons discharged one or more spikes at fairly constant latency during or after the peak of a twitch contraction. Among these contraction sensitive units 8 out of 10 tested had low thresholds to firm pressure, taps and squeezing applied to the muscle. These stimuli also excited gamma motoneurones.

The majority of the 23 Gp III units not responding to contraction had high thresholds to mechanical stimuli. Our evidence indicates that it is the lower threshold Gp III mechanoreceptors, sensitive to non-noxious stimuli, which can provide a potent excitation of gamma motoneurones via a spinal segmental pathway.

Supported by the Wellcome Trust.

63.4 LONG-TERM POST-STIMULUS REDUCTION IN AXON EXCITABILITY WHEN TESTED WITH SUBMAXIMAL ELECTRICAL STIMULI IN VIVO OR IN VITRO. F.A. Potts* and R.R. Young. Lab. of Clinical Neurophysiology, Mass. General Hospital, Boston, MA 02114.

The duration of the relative refractory period of large myelinated axons in mammalian mixed nerves varies with factors such as temperature and axon diameter but, when tested with supramaximal stimuli, rarely exceeds 5 msec under ordinary conditions. However, when the second ("test") stimulus is submaximal, activating 50% of the alpha motor axons or less, decreased excitability of individual large motor or sensory axons persists for 150-200 msec after the first stimulus and is maximal at about 50 msec. We have demonstrated this in normal rat and human nerve-muscle preparations in situ and in excised nerves in vitro in a special chamber at 37°C. With paired stimuli activating 1, 25 or 50% of the alpha motor axons, the compound muscle action potential following the second stimulus is about 40% as large as that following the first stimulus at inter-stimulus intervals of 50 msec. There are also 2 "superexcitable" phases, at 10-15 msec and 250 to 600 msec, when the second stimulus produces a larger response than the first.

Mechanisms underlying the unexpectedly prolonged period of subnormal axon excitability, which may include afterhyperpolarization-longlasting increases in potassium conductance, remain to be definitively elucidated. However, such long-term changes significantly affect the ratio of the number of axons stimulated by the second to the number stimulated by the first of two equal stimuli when their strength is at or below firing threshold for many large axons in a mixed nerve. Two "equal submaximal stimuli", separated even by 50 msec, are not equally effective stimuli. Experiments using paired submaximal stimuli, such as the ordinary H-reflex recovery curves, should be interpreted in light of these fluctuations in excitability which occur far beyond the usually accepted limits of the relative refractory period.

The shape of the "recovery curve" for large axons in a mixed nerve bears a remarkable resemblance to the shape and timing of what are proported to be "H-reflex excitability curves".

63.5 SENSORY PROPERTIES OF THE INFERIOR OLIVE OF THE CAT. R.S. Gellman*, A.R. Gibson and J.C. Houk. Neuroscience Prog. and Physiology Dept., Northwestern Univ. Med. Ctr., Chicago, IL 60611.

Recent anatomical studies have documented an ordered and precise organization of the projection of the inferior olive (IO) upon the cerebellar cortex. The results reported here demonstrate a corresponding degree of organization of the sensory properties of the IO. We have recorded from 270 units in 11 cats anaesthetized with pentobarbital. The locations of recording sites were confirmed histologically. Cells in the IO had extracellular action potentials displaying secondary spikes, low spontaneous activity (1-2/sec), and did not follow stimuli above about 8 Hz.

The dorsal accessory nucleus (DAO) (N=101) shows a clear somatotopy, which agrees well with the known anatomy (Berkley and Hand, J. Comp. Neur. 180, 253). Penetrations stepping from medial to lateral successively yield units that respond to stimulation of the contralateral (5% bilateral) face, forelimb, hindlimb and tail. Most units have small receptive fields confined to one area of the body, though units on the borders between regions occasionally showed mixed properites. An approximately equal number of cells responded to superficial stimuli (tap, touch, air puffs) as to deep stimuli (squeeze, limb movement). All but 2 cells in the DAO had purely excitatory responses. Mean latencies to electric shocks were short (chin=13 ms; forelimb=15 ms; hindlimb=24 ms).

In the medial accessory nucleus (MAO) (N=139) 30% of cells responded to inputs from more than one body area, frequently bilateral. Receptive fields (N=90) were excitatory (56%), inhibitory (27%), or contained excitatory and inhibitory subareas (17%). Cells with similar properties tend to cluster in small areas that are consistent between cats. A third of the cells could not be driven by sensory stimuli, and many were driven only by hard squeeze. Latencies were long (up to 300 ms) and variable. Medial parts of the MAO had units driven by clicks (latency 45 ms) or light flashes (latency 45 ms) or both.

In the principal nucleus we recorded few cells (N=30). Only ½ of these were driven by sensory stimuli, and most of these received input from more than one body area. Latencies were intermediate (mean forelimb=25 msec).

Although the DAO and MAO differ dramatically in their response properties, both are highly organized and consistent in detail from cat to cat. The character of DAO receptive fields suggests a possible role in the detection of mechanical contact with the external environment. The mixed properties of MAO units suggest a more complex function.

63.6 MECHANISM OF POSTURAL CONTROL IN CRAYFISH LIMBS. Jack D. Marrelli* (SPON: J. Larimer) Dept. of Zoology, The Univ. of TX., Austin, TX. 78712.

The myochordotonal organ of the crayfish spans the merus-carpus joint of the cheliped (claw). Its sensory neurons are in series with a small receptor muscle. The discharge of these neurons is influenced by the tension generated in this muscle by merus-carpus joint movements.

We examined mychordotonal organ action on the joint flexor muscle for postural control by first producing sinusoidal disturbances of the resting joint. We then compared the flexor responses in the intact case with the flexor responses when the sensory neurons were severed. Joint motion was imposed over an arc of 35 degrees at a frequency of 0.2 Hz. Joint velocity ranged from -10 degrees/sec to +10 degrees/sec. The averaged flexor activity (n=100) was compared to the velocity of the joint during the imposed perturbation and was found to be closely proportional in both phase and amplitude to the velocity of the joint movement.

When the sensory neurons were cut there was no flexor discharge at joint velocities below 2 degrees/sec. Above 2 degrees/sec, the flexor discharge as a function of joint velocity could be described by a linear regression line with slope 2 (PPS/degrees/sec. When the sensory neurons were intact the relation between flexor discharge and velocity was almost identical at velocities above 2 degrees/sec. At velocities below 2 degrees/sec, however, the flexor discharge was greatly enhanced by myochordotonal action. In this region a regression line describing the flexor discharge as a function of the joint velocity had a slope of 10 (PPS/degrees/sec).

These data demonstrate that the myochordotonal organ contributes an intense but very limited excitatory influence upon the flexor muscle when the flexor is stretched from rest by joint extension. We suggest that these myochordotonal organ properties have two major advantages for postural control. First, if the objective of a postural support system is to remain in a predetermined position in spite of fluctuating external forces, then mychordotonal organ high sensitivity and rapid response to the earliest sign of imposed movement is desirable. Secondly, because high sensitivity reflexes proportional to velocity would tend to promote instability, some mechanism would be required to limit the velocity sensitivity. The mychordotonal organ provides an upper limit of response of 2 degrees/sec.

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63.7 AN AUTORADIOGRAPHIC ANALYSIS OF THE CINGULO-PONTINE PROJECTION IN THE RAT. K. Sripanidkulchai and J. M. Wyss. Department of Anatomy, Univ. of Alabama in Birmingham, Birmingham, AL 35294.

The manner in which the limbic cortical areas are able to influence those areas of the brain whose function is better understood, has been a matter of interest for many years. One route by which they might influence the motor output of the organism is via the projections of the cingulate cortex to the ventral pontine gray matter. In 1969 Domesick (Brain Research 12:296-320) presented evidence based on degeneration techniques, which clearly demonstrated both the existence of this pathway, and its topographical organization. The present study used the autoradiographic technique to further investigate this topography. In each of thirty-five rats, a single, iontophoretic injection (apx 10-30 nl) of a mixture of ^{3}H proline, ^{3}H leucine and ^{3}H lysine (20-60 mCi per ml) was made via a 10 µm tip glass pipet into one or more of the divisions of the mesial cortex. The results corroborate the basic finding of Domesick, in that anterior areas of the cingulate project most heavily to the rostromedial ventral pontine gray whereas the more posterior retrosplenial cortex projects predominantly to the lateral portion of the rostral levels of the pontine gray. Within this basic framework, the present data allow us to give a more complete analysis of these projections. Orthogradely transported label from retrosplenial injections remains predominantly confined to the lateral half of the rostral pontine gray and is organized according to the anterior-posterior (medial to lateral in the pons) and dorsoventral (dorso ventral in the pons) position of the cells of origin. Very light labeling of more medial portions of the pons probably reflects the presence of fibers passing on into the dorsal brainstem. Injections in the anterior portion of the cingulate cortex (area infraradiata) demonstrate the rather more complex organization of these efferents and the bilaterality of this projection. Finally we have been able to demonstrate in the present data that these cingulo-pontine projections do not overlap the projections from either the somatosensory or motor cortex. Rather they are positioned around these projection areas on the medial, ventral and lateral borders.

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63.8 MOTOR CORTEX STIMULATED VIBRISSAE MOVEMENT. MAPS OF INCREASED (14C)2 DEOXYGLUCOSE UPTAKE IN SOMATOSENSORY CORTEX, CORTICOSPINAL TRACT & SUBCORTICAL STRUCTURES OF THE RAT, F.R. Sharp, K. Evans* and G.K. Bigley*. Dept. of Neurosciences, UCSD Sch. Med., San Diego, CA 92103.

Electrical stimulation of motor cortex 'vibrissae area' produced contralateral movements restricted to the vibrissae in 9 awake rats. (14C) 2-deoxyglucose (2DG) was injected and cortex stimulated with a bipolar electrode at 5 trains/sec for 45 minutes. Autoradiographs of stimulated animals were compared to sham-operated controls and structure boundaries identified from Nissl stains.

Stimulation increased 2DG uptake in a 2.0mm diameter region about the stimulating electrode in motor cortex. Posterior to this region a columnar increase of 2DG uptake occurred in the barrel field of SmI cortex (Durham, et al. JCN,178:629-644). Subcortical regions which increased 2DG uptake ipsilateral to cortical stimulation included: ventroanterolateral (VL) and ventromedial n. thalamus; reticular n. thalamus-ventral; caudate and putamen-mid, dorsolateral region; globus pallidus-two dorsal segments; substantia nigra; subthalamic nucleus; zona incerta; entopeduncular nucleus; deep mesencephalic nucleus (n. cuneiformis); pontine nuclei-medial; and the medial corticospinal tract. A few animals had increased 2DG uptake in ipsilateral centrolateral (CL) and parafasicular n. of thalamus. Contralateral increases of 2DG uptake occurred in: lateral nucleus of cerebellum; a small region in posterior cerebellar hemisphere; a restricted portion of the midcerebellar hemisphere in granular and molecular layers; and an anterior paravermal region.

Structures related to motor and sensory vibrissae function appear to be activated mono-and polysynaptically. The motor nucleus for vibrissae movement-the facial nucleus, did not seem to increase 2DG uptake. However, the medial corticospinal tract in the pons did increase 2DG uptake ipsilateral to cortex stimulation. The results will be related to known anatomical connections.

63.9 CONVERGENCE OF SPINAL AND CEREBELLAR INPUT IN THE THALAMIC VENTRAL TIER OF A PROSIMIAN, GALAGO. J.C. Pearson*, H.M. Murray and C.H. Phelps. Depts. of Anatomy, Wright State Univ. Sch. of Med., Dayton, OH 45435, and Univ. of New Mexico, Sch. of Med., Albequerque, NM 87131.

A previous report in Galago acknowledged the ventral intermediate nucleus (Vim) as being separate from the ventral posterior lateral nucleus (VPL) based on criteria that (1) spinal input to Vim is widely separated from that to VPL, and (2) cell bodies in the Vim are scattered in organization as compared to the clustered arrangement of those in VPL. In order to more precisely define the extent of the area acknowledged as Vim, and to further examine the suggestion that Vim should be considered as a separate entity from VPL, the thalamic termination of cerebellar fibers were examined in this prosimian with particular emphasis on the possible convergence of cerebellar and spinal input in the ventral tier. In the present study the Fink and Heimer method was employed to examine the termination of cerebellothalamic fibers in six adult lesser bushbabies after lesions were placed stereotaxically in the caudal parts of the lateral cerebellar nucleus. Sections containing cerebellothalamic degeneration were compared with slides from fourteen Galagos which had received spinal hemisections at cervical, thoracic and lumbar cord levels. Results indicate a direct overlap of cerebellar and spinal input in the area of Galago ventral tier previously identified as Vim. This area is located medial and slightly dorsal to the rostral extension of VPL. In this area of convergence cells are scattered in appearance and slightly larger than neurons in VPL. Spinal degeneration is sparse in Vim compared to the moderate termination of cerebellothalamic fibers. Cerebellar terminals are also located more dorsally and rostrally in the region identified as the caudal portion of the ventral lateral complex (VL_C). Based on this evidence, the Vim in Galago may correspond to the VPL_O as recently described in monkey. However, the hodological and cytoarchitectural differences between Vim and VPL outlined above, and the relatively substantial nature of the Vim area in Galago ventral tier appear to support the designation of this area as the ventral intermediate nucleus in this prosimian.

63.10 "RECALCULATION" OF VISUALLY-CUED MOTOR OUPUT DURING REACTION TIME DELAYS EVOKED BY IMPOSED JOINT DISPLACEMENTS. M.J. Eastman* and W.G. Tatton, Playfair Neuroscience Unit, University of Toronto, Toronto, Ontario.

Three macaques were trained to make fast flexion or extension movements of the wrist (mean velocities of approximately 400°/sec) in response to randomly-distributed arrays of yellow and red light emitting diodes (LEDS) positioned so as to encompass their full visual fields. The visually-cued movements were initiated from a neutral wrist zone of 5° in width, signalled by a coextensive array of green LEDS, to mechanical stops placed at 45° of flexion and extension. The monkeys were required to maintain the neutral position for a random interval of 4.5 to 6.0 seconds prior to delivery of the flex (yellow) or extend (red) cue. A torque motor assembly coaxial with the wrist joint, unexpectedly imposed step loads of random duration, onset and magnitude independent of or at a series of randomly-ordered delays (0,20,40,60,80,100,120 msecs) following the onset of the visual cues. EMG was recorded from flexor carpi ulnaris and extensor digitorum communis together with wrist position and velocity. EMG reaction times (RTs) for unperturbed movements were distributed from 120 to 260 msec. Most movements showed EMG RTs of 140 to 160 msec and highly reproducible trajectories as measured by crossing time distributions for 12, 24 and 36° positions from the neutral zone. Step loads that displaced the wrist in a direction opposite to the cued movement delayed the minimum and modal EMG RTs by 70 to 80 msec as measured from the onset of the step loads. Despite the delay in EMG output the distributions for crossing times were shifted by less than 30 msec. The trajectory compensation for the imposed displacements was achieved by increased initial movement velocities resulting from appropriate increases in the magnitude of agonist EMG output despite variation in the timing and size of the imposed displacements. The input/output relations for the reflex responses in the stretched muscles (i.e. EMG output for a given displacement velocity) showed alterations of "gain" (maximum 300%) beginning between 60 and 80 msec after the visual cue. The "gain" increase was initiated 75 to 80 msec prior to the onset of the visually-cued agonist EMG activity. The "gain" increases were not accompanied by increases or decreases in the background EMG activity which was constant for both the agonist and antagonist muscles during the reaction time interval. The findings will be considered in the context of the CNS mechanisms underlying the interactions between programmed motor output and somatosensory input during the RT preparation for a visually-cued movement. (Supported by MRC grant 5218)

64.1 COMBINED INTRACELLULAR RECORDING AND LIGHT AND EM ANALYSIS OF NEURONS OF THE RAT GLOBUS PALLIDUS. W.M. Falls and M.R. Park, Dept. of Anatomy, Michigan State Univ., East Lansing, MI 48824.

Globus pallidus (GP) neurons in rats were impaled with intracellular microelectrodes containing 4% HRP in 0.1 M tris buffer (pH 7.6) and a low concentration of KCl (0.5M). The response to caudate-putamen stimulation is a monosynaptic IPSP of latency 5.1 to 9.8 msec (n=22). Comparison with extracellular controls shows no initial transmembrane depolarization. Dividing latency into straight-line distance between recording and stimulating sites and allowing 0.7 msec for synaptic delay yields conduction velocities of 0.4 to 0.8 m/sec. All recovered neurons were of a single morphological type and were distributed throughout GP. The dendritic fields remain completely within GP, spanning up to 600 µm mediolaterally, stretching for at least 800 µm rostrocaudally, and often extending over 1mm dorsoventrally. Medium to large somata (10x20 to 15x30µm) give rise to 3-5 short (20µm) proximal dendrites (2.5-4µm in diameter) and sparsely branching distal dendrites which may run for more than 800µm without appreciable change in diameter (1-2µm). Dendrites emit a few widely scattered spines. Labeled axons most frequently course medially to enter a fiber bundle traversing GP. Some axons emit collaterals within 50µm of the soma which arborize within the dendritic field of the parent cell and contain several boutons en passant. Cell bodies, dendrites and axons of 2 labeled neurons were examined in serial sections. Most dendrites and cell bodies are covered by closely packed axonal endings. At least five types of axonal endings are observed. The most numerous are small (0.5-1.5µm) type 1 endings containing large pleomorphic vesicles. These interdigitating terminals form symmetrical contacts on cell bodies and all along the lengths of dendritic shafts and have been shown to be of striatal origin by Chang et al, (1980). Synapsing less frequently and only on distal dendrites are small (1-2µm) type 2 endings filled with small oval vesicles and forming asymmetric contacts. The bulbous type 3 ending (2-4µm) is the least frequently encountered ending. It contains small, loosely packed pleomorphic vesicles and makes multiple symmetrical contacts with distal dendritic shafts and vesicle-free spines. Dome-shaped type 4 endings (0.8-1.2µm) are presynaptic only to proximal dendrites, are filled with pleomorphic vesicles and each makes multiple symmetrical contacts. Large type 5 endings (3-4µm) make symmetrical contacts only on cell bodies and contain small oval and numerous large dense core vesicles. Labeled axons and their collaterals are unmyelinated within GP. Labeled boutons en passant correspond to type 4 axonal endings and contact unlabeled proximal dendrites. They have never been observed to synapse on labeled proximal dendrites of the parent cell. (Supported by USPHS Grant NS 14866).

64.2 HIGH-MAGNIFICATION GOLGI STUDY OF AGED CAT CAUDATE. A.B. Scheibel, A.S. Kaplan, and J.P. McAllister. Depts. of Anatomy and Psychiatry; Brain Research Institute; and Mental Retardation Research Center, UCLA, Los Angeles, CA 90024.

Prior data from our laboratory (Scheibel & Tomiyasu, in press) indicate that substantial age-related changes occur in the dendrospinous systems of the medium spiny cells in the aging (89-101 yr.) human caudate nucleus. These cells, when compared to those from young adults (23-35 yr.), show dramatic decreases in number of dendritic spines, in dendrospinous area, and in dendritic area exclusive of spines.

These findings have now been duplicated in old (15-18 yr.) vs. young adult (19 mo.) cats. Five Golgi-impregnated medium spiny Type I neurons were selected from the head of a caudate of each of two old and two young adult cats. The longest, and most complete dendrite of each of these 20 cells was indicated by computer analysis (McAllister, et al., this meeting). All subsequent measures were done blindly. A terminal branch was chosen from each of the indicated dendrites. A 50µ-long segment, beginning 25µ from the start of the branch, was drawn at 8800x apparent magnification, using a modified camera lucida system. (R. Coss, personal communication). Planimetric analysis of these drawings yielded the following results:

MEASURES PER 50µ-LONG DENDRITIC SEGMENT

	YOUNG	OLD	SIGNIF.
Total area (μ^2)	118.8 ± 9.3	88.9 ± 1.5	*
Dendrite area (w/o spines) (μ^2)	77.2 ± 5.3	62.4 ± 4.2	*
Average branch width (µ)	1.55 ± 0.11	1.25 ± 0.08	*
Number of spines	52.9 ± 3.5	32.5 ± 10.3	*
Area of all spines (μ^2)	41.6 ± 4.0	26.5 ± 5.7	*
Average area per spine (μ^2)	0.79 ± 0.03	0.87 ± 0.09	-

mean ± S.D.; sig: * = $p<.05$; - = N.S.

Additionally, spines from old animals showed a much wider range of morphological patterns compared to those from the young adults. The medium spiny cells are now known to be the main receptive element for caudatopetal afferents from cortex, thalamus, and midbrain. Their axons also constitute the major efferent channel from the caudate. Marked decreases in the synaptic surface area of this important cell system must certainly restrict caudate function, with consequent impairment of motor performance and the inception of voluntary activity.
(Supported by USPHS Grants AG 01754 and AG 01428.)

64.3 MORPHOLOGICAL AND ELECTROPHYSIOLOGICAL CHARACTERISTICS OF IDENTIFIED MEDIUM-SIZED SPINY NEURONS IN THE CAUDATE NUCLEUS OF KITTENS AND ADULT CATS. R.S. Fisher, M.S. Levine, C.D. Hull, and N.A. Buchwald. Mental Retardation Research Center and Brain Research Institute, School of Medicine, UCLA, Los Angeles, CA 90024.

Intracellular neuronal records of evoked responses to cortical (Cx), thalamic (Th), and nigral (SN) electrical stimulation were obtained from neurons in the caudate nucleus (Cd) of kittens and adult cats. After evoked response records were obtained, neurons were marked for subsequent anatomical identification by intracellular iontophoresis of horseradish peroxidase (HRP). After transcardial perfusion with aldehyde fixatives followed by 10% sucrose, brains were cryoprotected by immersion in 30% sucrose. Coronal serial frozen sections (100µm thick) of each brain were processed for peroxidase activity with either DAB or TMB chromagens. Intact marked neurons were correlated with their electrophysiological records. To date, we have identified 7 cells in 4 kittens (16-35 days old) and 10 cells in 4 adult cats as medium-sized spiny neurons. The resting membrane potentials of these neurons were lower in kittens than in adults ($\bar{x}$ = 31mV vs. 44mV) but action potential amplitudes were equivalent in both age groups ($\bar{x}$ = 23mV vs. 25mV). All tested neurons responded to ipsilateral Cx stimulation. In kittens, only 1 of 3 tested neurons responded to Th stimulation while 3 of 3 responded to SN stimulation. In adults, 5 of 5 tested cells responded to Th stimulation and 6 of 6 to SN stimulation. In identified adult Cd neurons, evoked responses always consisted of an EPSP followed by a long-duration IPSP (50-200 msec duration). In contrast, identified neurons in young kittens, as reported previously (Morris, et al, Br. Res., 1979) responded to stimulation only with EPSPs. IPSPs rarely followed these initial EPSPs. Morphologically, kitten neurons had smaller dendritic field radii than adult neurons (200-300µm vs. 400-700µm) and fewer apparent dendritic spines. Dendritic varicosities were present in the neurons of younger kittens. Axons of both kitten and adult Cd neurons were thin, collateralized, highly convoluted and could occasionally be followed for long distances.

The reasons for the lack of the IPSP in neonatal kittens could not be totally accounted for by our morphological data. They may be related to delayed maturation of inhibitory interneuronal or axonal collateral systems synapsing on medium-sized spiny neurons.

(Supported by USPHS Grants HD 05958 and MH 15345)

64.4 QUANTIFICATION OF MORPHOLOGICAL ALTERATION OF "MEDIUM" SPINY NEURONS IN CAUDATE NUCLEUS OF AGED CATS. M.S. Levine, J.P. McAllister C.D. Hull, A.M. Adinolfi, and N.A. Buchwald. Mental Retardation Research Center, School of Medicine, UCLA, Los Angeles, CA 90024.

These experiments utilized computer-assisted quantitative techniques to assess alterations in somatic and dendritic morphology of "medium" spiny neurons in the caudate nucleus of aged cats. To date, we have examined tissue from 4 adult cats (55 cells, 1-2 yrs) and 2 aged cats (30 cells, 15-18 yrs.). Tissue was impregnated using the Rapid Golgi method. Spiny neurons were drawn with a camera lucida. Three dimensional analyses of aspects of somatic and dendritic morphology were performed with the aid of a PDP-11/40 computer. Neuronal somata diameter and area decreased slightly in aged cats. The short and long axes of these somata decreased from 14.5 ± .5 (group mean ± s.e.) to 12.2 ± .5µm and 23.3 ± 1.3 to 21.4 ± 4µm in adult and aged neurons, respectively. Similarly, somatic area decreased from 362 ± 9µm^2 to 300 ± 27µm^2. Caudate neurons in aged cats showed marked reductions in dendritic length. Total dendritic length decreased from 3765 ± 335 to 1897 ± 207µm, while average dendritic length decreased from 777 ± 81 to 493 ± 29µm. The average radius of the dendritic field decreased from 220 ± 19 to 133 ± 4µm. The decreases in dendritic length measurements ranged from 25 to 50% for different parameters. The number of dendrites per neuron decreased from 5.12 ± .07 to 4.30 ± .10. Average number of branches per dendrite did not change (9.97 ± .44 to 9.80 ± .74 for adult vs. aged caudate neurons, respectively). These relationships were confirmed by comparing the same measurements from only the longest (optimally impregnated) dendrite of each cell, a procedure that helps to correct for branches that are truncated as they pass out of the section. Counts of dendritic spines indicate spine loss on distal dendritic segments (1.056 ± .163 to .390 ± .190 spines/µm). Spine density increases slightly on proximal segments in aged cats (.008 ± .022 to .019 ± .033 spines/µm). Analysis of branch ordering patterns indicated that branch ordering was not markedly rearranged in aged cats. However, length decreases occured at all orders and were greatest in the higher order segments. An analysis of length of free endings and closed endings indicated that virtually all dendritic length decreases in aged cats occured in free endings (113 ± 3 to 67 ± 0.4µm). Closed endings branch lengths were similar for both groups (28 ± 2 for adults vs 27 ± 2µm for aged cats). These results indicate that medium spiny neurons in the cat caudate nucleus undergo marked decreases in dendritic length and spine density (on distal segments) during aging. Decreases in dendritic length appear to be confined to free endings. In contrast, the number of branches per dendrite and dendritic branch ordering patterns remain relatively unaltered.
(Supported by USPHS HD 05958 and HD 04612)

64.5 AN AUTORADIOGRAPHIC STUDY OF NEOSTRIATAL EFFERENT PROJECTIONS IN KITTENS. A.M. Adinolfi, M.S. Levine and J.A. Cospito. Dept. of Anatomy and Mental Retardation Research Center, University of California, Los Angeles, California 90024.

This study traces autoradiographically the general topography and terminations of striopallidal and strionigral pathways in kittens. Five kittens (2 at 2 days, 2 at 28 days and 1 at 68 days of age) received single injections into the left caudate nucleus of equal mixtures of tritiated leucine and proline reconstituted in sterile saline (0.3-06µl for total radioactive concentrations of 22-45 µCi.) Animals were sacrificed by vascular perfusion with 10% neutral formalin at 24 hrs, 48 hrs, and 6 days depending on their age. Frozen sections were cut at 50 µm in the transverse plane. Every third section was mounted and dipped in Kodak NTB-2 emulsion, exposed for 6 wks, developed in Kodak D-19, and stained with thionin. Injection sites within the caudate head were mapped in bright and dark field illumination. Total caudate volume increased by 2.8 from 2-68 days. While injection sites were comparable in size, they occupied 26% of caudate volume at 2 days and 9% at 68 days. In the youngest animals labelled amino acids diffused further within the caudate nucleus and spread into the rostral putamen. In all kittens, labelled fibers from the caudate head project ipsilaterally as discrete bundles through the anterior limb of the internal capsule and course in a ventrocaudal direction within the capsule. Fibers enter the rostral globus pallidus near the anterior commissure and terminate diffusely. Silver grains in linear configurations were considered as axonal bundles and those in random or diffuse array as axonal terminals. Further caudally, discrete bundles are found among capsular fibers dorsomedial to the entopeduncular nucleus. The medial two-thirds of this nucleus contains a moderate to heavy projection but only scattered terminals are found in the lateral entopeduncular and pallidal regions at these levels. Labelled efferent projections proceed in a caudal direction along the ventral border of the subthalamic nucleus. Fiber bundles traverse this nucleus but no terminals are found in this region. At midbrain levels, the strionigral pathway occupies the medial portion of the cerebral peduncle and enters the substantia nigra. Terminal fields are labelled heavily in the rostromedial part of the pars reticulata. The caudal and lateral parts of the pars reticulata and the pars compacta contain fewer labelled terminals. No neostriatal projections are found caudal to midbrain nigral levels. This study concludes that caudate neurons in newborn kittens project prominently and exclusively to ipsilateral pallidoentopeduncular and nigral regions. (Supported by USPHS grant no. HD-05958.)

64.6 DEVELOPMENT OF SPONTANEOUS AND EVOKED UNIT ACTIVITY IN THE VENTRAL ANTERIOR AND VENTRAL LATERAL THALAMIC NUCLEI (VA-VL) IN CATS. S.W. Kiefer, M.S. Levine, C.D. Hull, and N.A. Buchwald. MRRC and BRI, UCLA School of Medicine, Los Angeles, CA 90024.

We have analyzed the development of neurons in the VA-VL complex, a thalamic region receiving major afferents from both the basal ganglia and the cerebellum. VA-VL cells are also reciprocally connected with the pericruciate cortex. Animals were anesthetized with a Halothane-N_2O respiratory mixture, paralyzed with flaxedil and artificially respirated. Extracellular single unit data were obtained from 14 cats in four age groups (1-10 days, n=4; 11-20 days, n=4; 31-40 days, n=3; adult > 1 year, n=3). For each cell, spontaneous firing and responsiveness to stimulation of ipsilateral pericruciate cortex (Cx), caudate nucleus (Cd), and contralateral deep nuclei of the cerebellum (Cbl) were determined. Spontaneous activity has been analyzed in 262 VA-VL neurons. In 1-10 day old kittens VA-VL cells fired infrequently ($\bar{x}$ ISI=2,490 msec). Spontaneous firing increased with age (11-20 days, $\bar{x}$ ISI=1,670; 31-40 day, $\bar{x}$ ISI=1,210). However, even by 40 days, the rate of spontaneous firing did not equal that of adults ($\bar{x}$ ISI=460 msec). Burst occurence (at least 2 successive intervals < 10 msec) also increased with age. 12% of the units in 1-10 day kittens; 18% in the 11-20 day group, and 65% in the 31-40 day group showed bursting activity. Adult levels (93%) were not reached by 40 days. Evoked responses: 1) Cbl stimulation evoked responses in about 25% of neurons tested regardless of developmental age. About half of these responses were initially excitatory, the remaining inhibitory both in the youngest kittens and in adults, b) Cd stimulation- Responsiveness of VA-VL neurons to Cd stimulation increased as a function of age: 15% (10/66 cells) in the 1-10 day group; 41% (23/56) in adults. In the 1-10 day old kittens 60% of the cells responded with initial inhibition; the others with initial excitation. In adults, the proportion of initial inhibition increased to 78% (18/23), c) Cx stimulation- Responsiveness to Cx stimulation also increased with age: 25% (15/60 units) in 1-10 day old kittens; 77% (44/57) in adult cats. As with Cd, the percentage of initially inhibitory responses to Cx stimulation increased with age: 47% (7/15) in the 1-10 day group showed initial inhibition; in adult cats, 84% (37/44). The data suggest that VA-VL neurons in the neonatal kitten are immature and must go through further postnatal development before achieving adult levels of function.

(Supported by USPHS HD 05958 and HD 04612)

64.7 CONNECTIVITY OF THE CAUDATE NUCLEUS IN NEWBORN KITTENS AND ADULT CATS. N.A. Buchwald, R.S. Fisher, M.S. Levine, R. Gazzara, and C.D. Hull. Mental Retardation Research Center and Brain Research Institute, School of Medicine, UCLA, Los Angeles, CA 90024.

Retrograde and orthograde neuronal transport of lectin-bound horseradish peroxidase (WG-HRP) were used to demonstrate the connectivity of the caudate nucleus (Cd) in 7 newborn kittens and in 4 adult cats. A 2.5% solution of WG-HRP was pressure injected into the left Cd of each animal (.05 - .20µl, .87% NaCl vehicle). After 24-48h animals were killed and perfused with aldehyde fixatives followed by 10% sucrose. The tissue was immersed in 30% sucrose for cryoprotection. Adjacent sets of frontal or parasagital serial frozen sections (100µ thick) were processed for peroxidase activity with DAB, TMB, and BDHC chromagens and counterstained with neutral red.

The location and cytological characteristics of neurons labelled retrogradely (Cd inputs) and fibers/terminal fields labelled orthogradely (Cd outputs) were similar in newborn and adult cats. Labelled neuronal cell bodies were prominent in four anatomical sites: 1) cortex (bilateral in the cingulate, pre- and postsigmoid, proreus, and anterior gyri; ipsilateral input in the posterior sylvian gyrus), 2) ventral thalamus (ipsilateral in the centromedian, parafascicular, central medial, dorsomedial, ventral anterior, and rhomboidal nuclei), 3) ventral midbrain (ipsilateral in all regions of the substantia nigra, ventral tegmental area, and retrorubral fields), and 4) dorsal raphe (bilateral). Smaller aggregations of labelled neuronal cell bodies were found in the contralateral substantia nigra in both kittens and adults. In adults, labelled neurons were also observed in the ipsilateral globus pallidus and basolateral amygdala. No cell bodies were labelled in the entopeduncular nucleus. Orthogradely labelled fibers and terminal fields were evident in three ipsilateral locations: globus pallidus, entopenduncular nucleus and substantia nigra. There was considerable overlap of labelled cell bodies and strionigral terminal fields in the pars compacta. Labelled cell bodies tended to be smaller in kittens than adults.

The density of the WG-HRP reaction product was characteristic of each anatomical location. Cortical neurons (small pyramidal figures prominent in layer 3; scattered through layers 4-6) were very lightly labelled; thalamic and raphe neurons (small-to medium stellate figures) had moderate label density; nigral neurons (medium-to-large fusiform figures) were heavily labelled.

These results are consonant with the major findings of our previous studies of electrophysiological development. Inputs and outputs of the Cd are present and functional throughout postnatal life.

(Supported by grants: HD 05958 and MH 15345)

64.8 AN ELECTRON MICROSCOPIC STUDY OF GOLGI-IMPREGNATED MEDIUM SPINY NEURONS IN CAT CAUDATE NUCLEUS. J.P. McAllister, D.S. Hayes and A.M. Adinolfi (SPON: Russell A. Gazzara). Dept. of Anatomy and Mental Retardation Research Center, UCLA, Los Angeles, CA 90024.

In cats, medium spiny neurons (homologous to Type I spiny neurons in primates) are distributed throughout the caudate nucleus and constitute the largest proportion of neurons impregnated by the Rapid Golgi method. In this study, we describe the cytological features and synaptic organization of Golgi-impregnated medium spiny neurons after gold toning and deimpregnation, according to the method of Fairen, Peters and Saldanha (1977). Single neurons were drawn from Epon-embedded deimpregnated sections before serial thin sectioning for electron microscopy. The selected neurons had ovoid cell bodies and proximal dendrites which were smooth, spine-free and uniform in diameter. These gave rise to intermediate and distal branches, richly covered with short, pedunculated and often, bifurcated spines. Dendritic varicosities were absent and distal branches with free endings tapered slightly to their terminations. In thin sections of medium spiny neurons, metablic gold is concentrated beneath the plasma membrane. These fine particles outline the periphery of the perikaryon and proximal dendrites. Cell bodies are characterized by a pale nucleus containing a prominent nucleolus and surrounded by scant cytoplasm with few formed organelles. Occasional small arrays of rough endoplasmic reticulum extend into the proximal dendrites and are situated among parallel microtubules. Somatic and dendritic surfaces are contacted symmetrically by few axon terminals containing pleomorphic vesicles. Most of the proximal surfaces are apposed by astroglial processes. Intermediate and distal spiny dendrites were followed in serial thin sections. Gold particles are much more coarse and, except for large mitochondria, tend to fill smaller dendrites. These particles also fill the thin stalks and expanded heads of the spines. When present, the spine apparatus is not obscured by the gold. Small dendrites and dendritic spines are contacted asymmetrically by axon terminals containing spherical vesicles. Less often, the dendritic profile or thin stalk of a spine is contacted symmetrically. Initial segments and preterminal portions of axons have been followed for short distances and are contacted symmetrically. This study confirms the distribution of symmetrical and asymmetrical synaptic contacts on spiny neurons in cat neostriatum. (Supported by USPHS Grant HD 05958).

64.9 TWO SPATIALLY SEPARATE STRIOPALLIDAL AXONAL ARBORIZATIONS ARE LABELED BY SMALL AMINO ACID INJECTIONS IN RAT NEOSTRIATUM. K.D. Phelan* and C.J. Wilson* (SPON: D. Tanaka). Department of Anatomy, Michigan State University, East Lansing, Michigan 48824.

In a previous study, single intracellularly labeled neostriatal projection neurons were shown to exhibit two separate axon collateral arborizations in globus pallidus. To examine the topographical organization of the striopallidal pathway in the rat, patterns of axonal distribution of small populations of neostriatal projection neurons were demonstrated autoradiographically following stereotaxic placement of iontophoretic injections of tritiated amino acids in various parts of the neostriatum. Two distinct regions of labeled axonal processes were found in globus pallidus after such injections in ipsilateral neostriatum. In sagittal sections, label was seen over fiber bundles of the internal capsule coursing from the injection site into globus pallidus. As they penetrated the border of globus pallidus, these fibers gave rise to a sharply bounded region of concentrated autoradiographic labeling in the pallidal neuropil. The dorso-ventral and medio-lateral extent of this region varied with the size of the neostriatal injection site. However, its rostro-caudal dimension remained relatively constant (50-100μm) and corresponded with that previously seen for individual striopallidal axons. The position of this labeled zone along the neostriatal-pallidal border depended upon the position of the injection site, being centered on the point of penetration of labeled capsular bundles. Labeled bundles of fibers continued caudally past this region and through a variable region of relatively sparse labeling before forming a second arborization in the pallidal neuropil. Labeling in this more caudal pallidal region was more diffuse and irregular in shape than that seen in the rostral zone. It varied in size according to the size of the injection site, and exhibited a rostro-caudal topographic relationship with the neostriatum. These results suggest that the striopallidal pathway in the rat is more precisely organized than previous suspected, and that there may exist at least two distinct topographical representations of neostriatum within globus pallidus.

(Supported by USPHS Grant 17294).

64.10 A STRIATO-STRIATAL CONNECTION IN RATS. I. J. Bak, C. H.Markham, and E. S. Morgan* Dept. Neurology, Sch. Med., UCLA, Los Angeles, Ca. 90024.

It has been shown in previous studies that herpes simplex virus (HSV) can be used as a tracer in the study of neural pathways in the neostriatum. When HSV is injected into the neostriatum, it is transported retrogradely along afferent nerve fibers and labels their cell bodies outside of the neostriatum. In the present study, particular attention was focused on possible neural connections between left and right striatum.

1 μL of HSV wild type 1 (10,000 plaque forming units per microliter) was injected into the rostral medial part of the head of the left caudate nucleus. Three to four days after microinjection of HSV, the brain was perfused with glutaraldehyde fixative and further prepared for light and electron microscopy.

Injection of HSV into the left neostriatum results in selective labelling of large neurons in the contralateral side of the neostriatum. These large neurons were easily identified not only under the electron microscope but also with light microscopy. There were numerous virus particles (nucleocapsids) in the nucleus and matured virus (virions) in the cytoplasm. There was also a distinct migration of chromatin substance towards the nuclear membrane and a loss of nucleoli.

These large HSV labelled neurons are morphologically characterized by a diameter ranging from 30μm to 50μm. Usually two or three primary dendrites extend from the neuron in opposite directions. The cells contain rich cytoplasmic organelles such as ribosomes, rough endoplasmic reticulum, mitochondria and lysosomal granules. The nuclear membrane usually appears lobulated. Further careful observation reveals that these HSV labelled large neurons are spiny neurons. Approximately 1% of the total neuronal population of the neostriatum is made up of this cell type.

Present results strongly suggest that one class of large striatal neurons terminate in the contralateral side of the neostriatum.

64.11 DIFFERENTIAL DISTRIBUTION OF NEURONES IN THE HEAD OF THE CAUDATE NUCLEUS. Y. Estrada-Palma*, J.A. Roig, I. Zarco-Coronado* and H. Brust-Carmona. Depto. de Fisiología, Div. de Investigación, Fac. de Medicina, Univ. Nal. Autónoma de México, México 20, D.F.

Previous work has suggested an anatomicofunctional organization of the caudate nucleus (CN). However, morphological descriptions do not agree with this suggestion. To elucidate this possible organization of the CN we performed acute experiments in both intact cats and after bilateral injections of 6-Hydroxydopamine (6-OHDA) into the substantia nigra (SN) or dorsal to it (ZI). The extracellular unitary activity was recorded with microelectrodes in one CN or simultaneously in both. We chose only those units appearing spontaneously and being twice as big as the background activity and which remained invariant for 2 min before being fed into a magnetic tape recorder for further analysis by a PDP 11/40 computer. At the end of the recordings the cats received a lethal anesthetic dose and their brains were perfused. Thereafter histological sections were stained (Nissl). Microscopically we counted in each mm the neurones observed along the microelectrode tract and measured their greatest somatic diameter. The best analyzed region corresponded to the A 16-17 coordinates (Jasper and Ajmone Marsan Atlas). In the dorsal regions the potentials were small and iterative at almost constant intervals. In the ventro-lateral regions they were big and appeared in bursts. The dorsal units were hardly affected by electrical stimulation of the n. centralis medialis (NCM), the SN, or the radial nerve, while the ventral ones were modified. The response pattern was usually one of inhibition-excitation for the SN and of excitation-inhibition for the NCM. The analysis of the field potentials, obtained with the same microelectrode, seem to support these observations. At the medial tracts (L 3-4 mm) we found in each mm starting at the dorsal border of the CN the following mean diameters: 1st = 12.86μ, 2nd = 13.64μ, 3rd = 13.59μ, 4th = 13.24μ. At the lateral tracts (L 5 mm) the mean diameters were: 1st = 13.70μ, 2nd = 16.08μ, 3rd = 16.25μ, 4th = 16.44μ. The differences (t test) between the dorsal and ventral regions were statistically significant ($P < 0.05$). In the cats with SN lesions the greatest diameters were also observed in the ventral regions. Furthermore, the mean diameter observed in the second mm was of 15.71μ which differs statistically from those observed in the intact animals as well as the ZI lesioned. These data indicate a differential neuronal distribution according to size and sensibility to electrical stimulation of other cerebral structures, shedding more light on the proposed anatomicofunctional organization of the CN.

64.12 CROSSED CONNECTIONS OF THE SUBSTANTIA NIGRA IN THE RAT. C.R. Gerfen, Wm.A. Staines*, G.W. Arbuthnott* and H.C. Fibiger. Div. of Neurological Science, U.B.C., Vancouver, B.C., Canada.

The existence of crossed multisynaptic pathways that allow for the interdependent control of activity in the two substantia nigrae have been inferred from a number of recent investigations (see Cheramy et al., Nature 289 (1981) 537). This prompted a reexamination of the connections of the substantia nigra (SN) with an emphasis on crossed inputs to and crossed projections from that nucleus. Male albino rats received 20-50 nl pressure injections of a 1% wheat germ agglutinin conjugated horseradish peroxidase (WGA-HRP) solution into the SN or into surrounding areas as controls. Following a 24 hr survival period the animals were anaesthetized and processed according to the tetramethyl benzidine (TMB) protocol of Mesulam (J. Histochem. Cytochem. 26 (1978) 106) for the visualization of HRP.

The pattern of anterograde transport of WGA-HRP after SN injections, confirming for the most part previous reports, demonstrated ipsilateral nigral efferent projections to the striatum, globus pallidus (GP), subthalamic nucleus (SUT), thalamic nuclei including the paralamellar mediodorsal (MD), ventromedial (VM), and parafascicular (Pf) nuclei, central grey, midbrain reticular formation, superior colliculus (SC) and parabrachial area including the pedunculopontine nucleus (PB-PPN). Additionally, the nigral projections to the paralamellar MD and VM thalamic nuclei and to the SC were demonstrated to be bilateral. Previous reports of HRP injection into the SC have indicated that only 2-3 contralateral SNr neurons provide crossed nigro-tectal fibers (Beckstead et al., J. Neurosci. 1 (1981) 121). However, in the present study WGA-HRP injections into the rostral ventral lateral SC, the area in which terminal labelling was seen contralateral to SN injections, labelled as many as 200 contralateral SNr perikarya.

Confirming previous reports, SN WGA-HRP injections retrogradely labelled neurons located in the following ipsilateral brain areas: 1) prefrontal cortex, 2) motor cortex, 3) striatum, 4) GP, 5) central nucleus of the amygdala, 6) anterior hypothalamic area (2-3 neurons), 7) SUT, and 8) dorsal raphe. Additionally, labelled perikarya were observed in the ipsilateral Pf thalamic nucleus, in the contralateral posterior lateral hypothalamic area (LHp) and in the ipsi- and contralateral PB-PPN area. These nigral afferents were confirmed with complimentary WGA-HRP injections into each of these brain regions. While bilateral PB-PPN innervation of the SN has been reported in the cat there has been no previous demonstration of a crossed nigral afferent system from the contralateral LHp area.

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64.13 THE PALLIDOHABENULAR AND PALLIDOTHALAMIC PATHWAYS ARISE LARGELY FROM TWO DIFFERENT CELL POPULATIONS WITHIN PRIMATE GLOBUS PALLIDUS. L. De Bellefeuille*, A. Parent and R. Boucher. Lab. of Neurobiology, Fac. Med., Laval Univ., Québec, Canada, G1K 7P4.

The double fluorescent retrograde labelling technique (Van der Kooy et al., '78) was used in an attempt to disclose the exact sources at pallidal levels of the projections to the habenula (Hb), and to the ventral anterior (VA)-ventral lateral (VL) thalamic nuclei in primate. A total of four squirrel monkeys (Saimiri sciureus) were used. Injections of a mixture of DAPI-Primuline (DP) in quantities ranging from 0.2 to 0.6 µl were made in Hb, whereas Evans blue (EB) was injected in similar quantities within the VA-VL nuclei on the same side of the brain. The animals were allowed to survive from 6 to 7 days and the brain sections were examined with the help of a Leitz Ploemopak fluorescence microscope.

After these injections a multitude of DP-labelled neurons was found within the anterior lateral hypothalamic area which stands out as the main source of forebrain afferents to Hb. A lesser number of DP cells occurred in the substantia innominata beneath the globus pallidus (GP). No EB-labelled cells were disclosed in these two peripallidal sites. Within the GP itself the DP cells were much less numerous than the EB cells and both were differently distributed. The DP (Hb-projecting) cells were most abundant in the rostral pole of the internal segment of GP (GPi) where they merge indistinctly with the large population of DP cells present in the adjoining lateral hypothalamus. As we proceed caudalward, the DP cells become less numerous and are mostly found at the periphery of GPi where they surround a multitude of EB (VA/VL-projecting) cells which are uniformely distributed within the core of the internal pallidum. The EB cells were extremely abundant all along the rostrocaudal extent of GPi except within the rostral and caudal poles of this structure where only DP cells can be found. Only a few double-labelled cells were observed in the present experimental material. They occurred just caudal to the rostral pole of GPi where there was a certain intermingle of DP and EB cells.

These findings suggest that the primate GPi is organized according to a complex pattern basically consisting of various concentric cell layers. The VA/VL projecting neurons being located within the core of GPi are nearly completely surrounded by a shell of Hb-projecting cells. Furthermore, the entire GPi is enveloped by a neuronal network consisting of large and strongly-reactive acetylcholinesterase (AChE)-containing neurons. However, these AChE cells do not project significantly to either Hb or VA/VL but instead are seemingly related to peripallidal limbic structures, particularly the nucleus basalis (Parent et al., '81), which project to neocortex.

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64.14 THREE DIMENSIONAL QUANTITATIVE MORPHOLOGY OF SUBTHALAMIC NEURONS INTRACELLULARLY LABELED WITH HRP. C. Hammond*, J. Yelnik*, J.M. Deniau*, M. Kalia, and S.T. Kitai (SPON: A. Foley). Dept. of Anatomy, Michigan State University, E. Lansing, MI and Lab. Physiol. CTR Nerv and U3 INSERM France.

Responses of rat subthalamic nucelus (STN) neurons following cortical stimulation were intracellularly recorded and subsequently labeled with HRP. After fixation, the brains were cut serially (50µm) in sagittal plane and processed by the $CoCl_2$-DAB procedure. Sections containing labeled neurons were post-fixed in osmium and section embedded in plastic. Morphology of the neuron is described by means of algebraic and geometric parameters following light microscopic examination and three-dimensional computer reconstruction. Twenty-two labeled neurons were identified as Golgi type I (projection) neurons with branched axons. The somata were ovoidal in shape and had a few somatic spines. Four dendritic stems (3.70 ± 0.75) arose from the soma and gave rise to 25.0 ± 4.1 dendritic tips. The dendritic field had the shape of a flat ellipsoid, and was 1000µm long, 600µm wide and 250µm tall. Its long axis was parallel to the rostral-caudal axis of the nucleus. The total dendritic length was 6000 µm (6148 ± 692) and the longest dendrite measured 750µm (753 ± 158µm). Some dendrites extended beyond the borders of the nucleus and terminated in the cerebral peduncle, internal capsule and zona incerta. The dendrites were thin and had spines and protrusions of various sizes and shapes. The axon emerged from the soma or a proximal dendrite and divided within or close to the STN into two branches, one going rostrally and the other caudally. The caudal-going branch of one neuron was followed into the substantia nigra (SN), where it divided into two branches which gave rise to several thin collaterals running dorso-ventrally. The rostral-going branch of another neuron gave rise to a collateral terminating within the dendritic field of the parent cell before dividing into two branches, running rostrally. These observations suggest that rat STN neurons could receive afferent inputs other than those terminating within the nucleus. Axons of the STN neurons are collateralized within the nucleus and the main axon branches into two major axons before or immediately after they emerge from the nucleus.

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64.15 EM-AUTORADIOGRAPHY OF NIGROTHALAMIC TERMINALS IN THE CAT. I.A. Ilinsky, K. Kultas-Ilinsky, S. Warton*, and K.R. Smith. Depts. of Anatomy and Surgery, St. Louis Univ. Sch. of Med., St. Louis, MO 63104.

The ultrastructural features and synaptic sites of nigrothalamic afferents were described in a degeneration study in the cat by Kultas-Ilinsky et al. (Exp. Brain Res., 1978). Degenerative changes in synaptic boutons, however, limit the possibilities of ultrastructural analysis as well as identification of the degenerating boutons with their counterparts in the normal neuropil. Thus an EM-autoradiographic study was undertaken to overcome these difficulties. 0.2-0.4 µl of 2,3,4,5[3]H-leucine (ICN) in concentration 70 µCi/µl was injected into the pars reticularis of substantia nigra in seven cats. The animals were allowed to survive for 4-6 days, then they were perfused and the tissue was processed for EM and LM autoradiography as described previously (Kultas-Ilinsky et al., Brain Res., 1980). The exposure time for EM-autoradiography was 4-6 months.

A large number of labelled boutons and myelinated fibers were found in the ventral medial nucleus of the thalamus (VM). The diameter of labelled myelinated fibers ranged from 0.5 to 1.4 µm. The mean of the largest and smallest diameters of labelled boutons were 2.93 µm and 1.39 µm respectively. Some terminals, however, reached a length of 3.7 µm. The boutons were characterized by large numbers of long mitochondria, very distinct neurotubules and a population of synaptic vesicles of extremely variable shapes and sizes. Preliminary measurements indicate that the mean diameter of synaptic vesicles is in the range of 40 nm with ratio of longest to shortest diameter being 1.46. In all labelled boutons 3-4 large (103 nm) dense core vesicles were observed also. Synaptic junctions were of the symmetrical type with a relatively long contact area. Labelled nigral boutons formed synaptic contacts on large and small diameter dendritic branches of thalamocortical projection neurons as well as on vesicle-containing dendrites which presumably belong to local circuit neurons. In several instances the same labelled nigral bouton was observed contacting both types of dendrites. Some of the nigral boutons seemed to be of the en passant type. Labelled terminals were found either singly in the neuropil or in complex synaptic arrangements in glomeruli. Quantitative analysis of the density of nigral boutons on the dendrites of projection and local circuit neurons is currently in process. Supported by a grant from American Parkinson Disease Association.

64.16 A BASAL GANGLIA-THALAMIC-TELENCEPHALIC PATHWAY IN PIGEONS. Cheryl A. Kitt and Steven E. Brauth, Department of Psychology, University of Maryland, College Park, Maryland 20742.

One of the major pathways by which the mammalian basal ganglia influence motor functions is via projections to nuclei of the thalamic ventral tier. Ventral tier nuclei, in turn, project upon portions of the frontal cortex including the motor and premotor cortices. Previous studies (Brauth, Ferguson and Kitt, 1978; Reiner, Brauth, Kitt and Karten, 1980) have suggested that similar pathways may exist in birds via the thalamic nucleus dorsointermedius posterior (DIP). In the present study the afferent and efferent connections of DIP were traced by horseradish peroxidase histochemistry and autoradiography.

HRP injections centered within DIP labeled neurons within the ipsilateral paleostriatal complex (avian basal ganglia), ipsilateral dorsal reticular nucleus (RSd) and bilaterally within the lateral cerebellar nucleus (CL). Within the paleostriatum, large cells of the paleostriatum primitivum (PP) and ventral paleostriatum (VP) were labeled as well as occasional small neurons of the lobus parolfactorius (LPO). Previous studies have considered the PP comparable to the mammalian globus pallidus, VP comparable to the mammalian substantia inominata and LPO comparable to portions of the mammalian striatum such as the caudate nucleus and nucleus accumbens.

The results of the autoradiography studies indicate that DIP fibers enter the telencephalon via the lateral forebrain bundle. Silver grain accumulations were found in the region ventral to PP including the VP and nucleus intrapeduncularis (INP). DIP efferents pass through the paleostriatum augmentatum (PA) to terminate within a small crescent shaped field of neurons lying in the rostromedial portion of the neostriatum intermedium (NI).

These results suggest that DIP neurons, like those of the mammalian ventral tier, may provide a pathway by which the basal ganglia influence motor functions in birds. The neostriatum intermedium (NI) has been shown to project to a more caudal field of neurons within the lateral neostriatum, the neostriatum intermedium pars lateralis (NIL)(Ritchie and Cohen, 1977). The NIL, in turn, projects to the avian archistriatum. While no direct counterpart to the mammalian motor cortex has been identified in birds, the avian archistriatum is the source of a long descending telencephalic efferent system called the occipitomesencephalic tract (OM). In pigeons, OM fibers reach the lateral reticular formation and lateral pontine nucleus, structures which, in mammals, are in receipt of descending projections from the motor cortex.

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64.17 AUTORADIOGRAPHIC STUDY OF THE EFFERENT FIBERS OF THE ENTOPEDUNCULAR NUCLEUS AND SUBSTANTIA NIGRA IN THE CAT.

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Following injections of ^{3}H-leucine in different parts of the entopeduncular nucleus (EP) and the substantia nigra (SN) it was observed that both structures project to a common terminal area. This area corresponds to the ventral anterior, ventral lateral and ventral medial thalamic nuclei and the nucleus tegmenti pedonculopontinus (TPP). Furthermore, a specific group of efferent fibers arising from each structure (EP and SN) was also disclosed: the EP sends axons to the lateral part of the lateral habenular nucleus and to the centro-median-parafascicular complex and the pars reticulata of the SN projects to the superior colliculus.

Projections to the thalamic nuclei (VA-VL-VM) follow a topographical pattern which suggests that the EP and the pars reticulata of the SN may be regarded as being morphologically interrelated. The pars compacta of the SN projects profusely to the striatum and more so in a restricted area corresponding to the rostral part of this structure.

(Supported by an MRC grant).

64.18 ACETYLCHOLINESTERASE-RICH PROJECTION FROM THE BASAL GANGLIA TO NEOCORTEX IN THE CAT.

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In the brains of cats prepared according to the DFP pharmacohistochemical procedure for the demonstration of acetylcholinesterase (AChE)-producing neurons, the entopeduncular nucleus (EN) was found to be composed of small to medium-sized (maximum diameter: 28.2 ± 0.3 um) neurons staining only weakly for AChE. In contrast, the globus pallidus (GP) comprises a mixted neuronal population. There is (1) weakly-stained cells (27.3 ± 0.4 um) which are morphologically very similar to those in EN, and (2) large (40.3 ± 0.6 um) and intensely reactive AChE cells which are topographically related to similar multipolar AChE cells located in various peripallidal limbic structures, particularly the substantia innominata (SI). Large (40.2 ± 0.5 um) and strongly stained AChE neurons are also scattered throughout the putamen (PUT) amongst a multitude of smaller and weakly reactive cells. The large and strongly-stained AChE cells in PUT are morphologically similar to the voluminous AChE neurons present in GP and often there is no clear boundary between these two cell populations.

In an attempt to find out if some of these AChE neurons project toward neocortex, multiple WGA-HRP injections were made within the auditory cortex (AI and AII) in 15 cats and the brain sections processed according to the combined HRP-AChE method (Mesulam, '76). These injections resulted in a retrograde labelling of numerous large and strongly-stained AChE cells within GP and of a smaller number of similar neurons in PUT.In PUT the AChE-HRP labelled cells were found most abundantly along the medial border, and in the caudoventral aspect of this structure. These double-labelled PUT cells often appear continous with those in GP. No HRP material could be detected in the smaller and weakly reactive AChE cells present in EN, GP or PUT after cortical injection. In contrast, HRP injections into the various subcortical target structures of the basal ganglia (including substantia nigra, VA/VL thalamic nuclei and EN) label only the smaller pale cells and not the large and intensely reactive AChE cells within the basal ganglia (Parent et al., '80).

These findings suggest that in regard to its output elements the feline basal ganglia may be subdivided into two fundamental cellular compartments: 1. the parvocellular neurons which stain only weakly for AChE, project to the main subcortical target structures of the basal ganglia, and appear to represent the typical "striatal" elements, 2. the magnocellular neurons which stain strongly for AChE, project widely upon neocortex and appear to represent the "limbic" elements of the basal ganglia.

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64.19 ORGANIZATIONAL FEATURES OF THE CORTICOSTRIATE PROJECTION IN THE MONKEY.

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Previous anatomical studies in the monkey have revealed that two areas of the cerebral cortex with reciprocal interconnections project in part to the same specific subdivisions of the neostriatum. These results imply that the topography of a given corticostriate projection can be understood on the basis of the intrahemispheric association connections of the cortical area. The present investigation was undertaken to ascertain the extent of communality in the corticostriate projections of three non-adjacent cortical areas known to have substantial reciprocal corticortical connections with each other.

Injections of tritiated amino acids (^{3}H - leucine and ^{3}H - lysine) were made in the posterior one-half of the cingulate gyrus (area 23), the posterior parahippocampal area (area TF) or the inferior parietal lobule (area 7) in 9 rhesus monkeys. Conventional autoradiographic methods were used to reveal the distribution of label. The sections were studied and charted with darkfield illumination and light microscopic procedures. Prior to analyzing the corticostraite projections, it was confirmed that areas 23, TF and 7 are indeed interconnected, as previous results had indicated. The corticostriate projections of these areas to the head, body and tail of the caudate nucleus were then examined. It was observed that all three cortical areas project to widespread parts of the caudate nucleus, and overall, have a unique topography. However, at numerous locations a communality was observed in the terminal labeling of corticostriate projections. This was most conspicuous in the dorsal and lateral parts of the head of the caudate nucleus, in the body and in the middle and dorsal parts of the tail. In three other monkeys control injections of labeled amino acids in the foot region of area 4, the posterior orbitofrontal cortex (area 13) or the medial temporal polar cortex (area TG or 38) revealed few if any corticocortical projections from these areas to areas 23, TF and 7. Additionally, there was little or no communality in their corticostriate projections.

The results further establish the fact that corticostriate and corticocortical projections are correlated closely. They imply that a given part of the caudate nucleus receives corticostriate projections from multiple areas of the cerebral cortex and that these areas are interconnected with each other via corticocortical connections. One interpretation of this organization might be that the information carried in the corticostriate projection is more related to corticocortical circuits than to specific cortical loci. (Supported by grant NS 14944 to G.W. VH.)

64.20 ORIGINS OF SUBCORTICAL PROJECTIONS TO THE NEOSTRIATUM OF THE OPOSSUM.

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While studies in several eutherian forms have detailed the origins of thalamic and mesencephalic projections to the neostriatum, similar information is not presently available for any example of the marsupial radiation. Accordingly, we have utilized retrograde axon transport techniques to examine the origin of neostriatal afferents in the opossum. Furthermore, since several of these systems are reported to be topographically organized, rostral, middle and caudal portions of both the caudate and putamen were targeted for injection. At thalamic levels placements in the body of the caudate resulted in moderate retrograde labelling throughout the intralaminar complex (parafascicular posterolateral, parafascicular and paracentral nuclei) and one midline cell group, nucleus centralis thalami. Comparable putamen placements resulted in a similar distribution but with fewer numbers of reactive cells. Caudal caudate or putamen injections were marginally effective in labelling thalamic cells. However, the few reactive cells which were observed occupied the appropriate loci. Injections in the head of the caudate produced a considerably more extensive distribution of labelled thalamic cells. As expected, this included the intralaminar complex and nucleus centralis. Moreover, it appeared that almost every neuron in these locations contained at least some reaction product. In addition, labelled thalamic cells appeared in the anterior and posterior paraventricular, intermediodorsal, interanterodorsal, mediodorsal and rhomboid nuclei. A few reactive neurons were also observed in the anterior thalamic complex. At mesencephalic levels only the rostral caudate (head) injections resulted in labelling of the nucleus raphe dorsalis and ventral tegmental area. In contrast, all caudate and putamen injections yielded retrograde labelling in the substantia nigra. On the basis of mid caudate and mid putamen injections we suggested that most neurons in the pars compacta and a few cells in the adjacent p. reticulata projected to the caudate, while certain cells in the p. lateralis (well developed in the opossum) together with a few neurons in the adjacent p. reticulata projected to the putamen (Hazlett, '80). Subsequent placements at the rostral and caudal extremes of both neostriatal subdivisions further confirm the presence of a medial to lateral nigrostriatal topography and provide additional evidence suggesting an anterior to posterior representation and gradient. (Supported by the National Science Foundation - Grant #BNS 80-22312.)

64.21 NEUROCHEMICAL CHARACTERIZATION OF THE NIGROTEGMENTAL PROJECTIONS IN THE RAT. J.A. Childs* and K. Gale (SPON: F.G. Standaert). Department of Pharmacology, Georgetown University, Schools of Medicine and Dentistry, Washington, D.C. 20007.

Complete cerebral hemitransection placed rostral to substantia nigra (SN) did not prevent the contralateral circling behavior induced by intranigral microinjection of muscimol (50 ng); under these conditions, specific GABA receptor binding in SN was unchanged. In contrast, transections placed caudal to SN caused a marked loss (50%) of GABA receptors in SN, and, at the same time, reduced glutamic acid decarboxylase activity in the caudal mesencephalic tegmentum by 20%.

To determine whether the projections from SN to the caudal mesencephalon might be GABA-containing, ^{3}H-GABA was microinjected in combination with a GABA-transaminase inhibitor (gamma-vinyl-GABA, 2ug) into SN and the transport of the ^{3}H-GABA was followed over 1-5 hours. Of the radioactive GABA remaining in the brain, the highest concentration (outside of the injection site) was found in the ipsilateral caudal mesencephalic tegmentum, at the midcollicular level, just ventral to the region of the central grey. When ^{3}H-GABA was injected into this region, a significant portion appeared in the ipsilateral SN.

These data suggest that some of the descending efferent projections from the SN to the caudal mesencephalic tegmentum may be GABAergic. These pathways may be subject to GABA-receptor-mediated inhibitory influences in SN.

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65.1 PROPERTIES OF CALCIUM UPTAKE AND SEROTONIN RELEASE IN RAT PLATELETS. L. Toll and S.H. Snyder. Johns Hopkins University, Sch. of Med., Dept. of Neuroscience, Pharmacology and Psychiatry, Baltimore, MD 21205.

It has been shown that under certain conditions extracellular calcium is required for serotonin release from platelets (Bennett, W.F., Belville, J. and Lynch, G., 1979, Neuroscience 4:1203). Studies were undertaken to determine if factors which block serotonin release from platelets could do so by inhibiting stimulus induced calcium influx. In this manner platelets were used as a model system to study the properties of stimulus induced calcium entry and neurotransmitter release.

Rat platelet rich plasma was incubated with [^{3}H]5-HT to allow incorporation into platelets. After washing, platelets were incubated with ^{45}Ca in the presence of thrombin. Thrombin stimulation of ^{45}Ca entry and [^{3}H]5-HT release were then measured simultaneously by filtration. Both processes occured rapidly, being virtually complete by 3 min at 30°C. At 0°C or in the presence of EDTA the level of ^{45}Ca uptake and [^{3}H]5-HT release is similar to that in the absence of thrombin. Both thrombin induced ^{45}Ca uptake and [^{3}H]5-HT release were inhibited by divalent cations with the order of potency being $Cd^{2+} > Ni^{2+} > Mn^{2+} \geq Co^{2+} > Ba^{2+} = Mg^{2+}$. This is similar to the divalent ion potency in inhibiting depolarization induced Ca^{2+} entry into nerve terminals.

Serotonin release from platelets is inhibited by agents which increase intracellular cAMP levels (Friedman, F. and Detwiler, T.C., 1975, Biochemistry, 14:1315) We have found that phosphodiesterase inhibitors theophylline, isobutylmethylxanthine and papavarine as well as dibutyryl-cAMP and PGE_1, all of which increase intracellular cAMP levels, inhibit both ^{45}Ca entry and [^{3}H]5-HT release in a dose dependent manner. Theophylline, dibutyryl-cAMP and PGE_1, require preincubation to produce inhibition, indicating the accumulation of cAMP is necessary. Papavarine inhibits instantaneously indicating a more direct blockage of ^{45}Ca entry. ^{45}Ca uptake was also inhibited by dinitrophenol and FCCP, uncouplers of oxidative phosphorylation, as well as by mitrochondrial ATPase inhibitors oligomycin and dicyclohexylcarbodimide. This indicates the requirement for energy for thrombin induced calcium flux. Of these compounds only oligomycin inhibited release of [^{3}H]5-HT. The other compounds probably interfere with serotonin storage in intracellular vesicles and thus, allow its unstimulated efflux from the platelets.

65.2 EVIDENCE THAT LECTIN-BINDING SITES ARE PRESENT ON ISOLATED HYPOTHALAMIC GRANULES CONTAINING LHRH. A. Barnea, and G. Cho*. Depts. Ob-Gyn and Physiology, Univ. of Texas Southwestern Med. Sch., Dallas, TX 75235.

Luteinizing hormone releasing hormone (LHRH), stored in granule-like particles within hypothalamic neurons, is known to be released into the hypophysial portal system. It is reasonable to assume that fusion of the LHRH granules with the neuronal plasma membrane is an important step in this release process. Since glycoproteins, particularly their terminal carbohydrate moeities, have been implicated in membrane-membrane interactions, we wished to ascertain if tightly-bound carbohydrates are present on the surface of hypothalamic granules containing LHRH. LHRH granules, isolated from hypothalami of adult male rats by means of hypo-osmotic shock and differential centrifugation, were passed through columns of Sepharose to which a lectin (a carbohydrate-binding protein) has been covalently attached. LHRH was eluted from the columns, and the amount present in the unbound and bound fraction was measured by RIA. Using this procedure, we evaluated the binding of LHRH granules to two immobilized lectins: Concanavalin A (Con A; a lectin binding molecules having α-D-mannopyranosyl and sterically related residues) and wheat germ agglutinin (WGA; a lectin binding molecules having N-acetyl-glucosaminyl residues). When LHRH granules were chromatographed on columns of Con A-Sepharose, 80% of the total LHRH was recovered in the unbound fraction and 20% in the bound fraction. The total recovery of LHRH from the columns was 96 ± 2% (mean ± SE; N = 21). When the unbound fraction was passed again through another column, 32% of the total LHRH was bound to the columns. Synthetic LHRH, added to the LHRH granules before chromatography, did not bind to the Con A-Sepharose. To examine the specificity of the binding of the LHRH granules to Con A, LHRH granules were chromatographed on columns of Con A-Sepharose, WGA-Sepharose, or Sepharose-4B. Of the total LHRH recovered from the columns, 21.4 ± 1.6% (N = 9) was bound to Con A-Sepharose, 10.5 ± 0.5% to WGA-Sepharose, and none was bound to Sepharose-4B. These findings are indicative that LHRH granules bind to immobilized lectins, and that the degree of binding is dependent on the binding specificity of the lectin. We propose that tightly-bound carbohydrates (lectin binding sites) are present on the surface of the LHRH granules and that such binding sites may be involved in the release of LHRH from hypothalamic neurons.

65.3 MONOVALENT ION REQUIREMENTS FOR THE MgATP-STIMULATED RELEASE OF LHRH FROM ISOLATED HYPOTHALAMIC GRANULES. G. H. Burrows and A. Barnea. Depts. Ob-Gyn and Physiology, Univ. of Texas Southwestern Med. Sch., Dallas, TX 75235.

We have previously demonstrated that MgATP stimulates the release of luteinizing hormone releasing hormone (LHRH) from isolated hypothalamic granules by a mechanism which requires KCl. In the present study, we have examined the concentration dependence and specificity of the KCl requirement for the MgATP-stimulated release of LHRH by replacing first the K^+ and then the Cl^- with other monovalent ions. LHRH granules were isolated from homogenates of adult male hypothalami by differential centrifugation and then incubated in buffered media containing the various salts with or without MgATP. LHRH released into the medium was separated from that remaining in the granules by affinity chromatography and then quantified by radioimmunoassay. When LHRH granules were incubated in the absence of KCl, LHRH release was not stimulated by MgATP. However, when KCl was included in the incubation medium, LHRH release was stimulated by MgATP. Moreover, the magnitude of the stimulation was found to be a saturable function of KCl concentration and maximal stimulation was observed at 80 mM KCl. When granules were incubated in the presence of 60 mM LiCl, NaCL, KCl, CsCL, choline chloride or tetraethylammonium chloride, the MgATP-stimulated release of LHRH was 12.1, 8.5, 9.1, 8.5, 5.3, or 0%, respectively. Inclusion of anion transport inhibitors (SITS, phloretin, probenecid, or pyridoxal phosphate) in the incubation mixture did not inhibit the stimulation of LHRH release by MgATP. The finding that the MgATP-stimulated release of LHRH can occur in the presence of each of the monovalent anions tested or in the presence of anion-transport inhibitors, is indicative that an active site selective for monovalent anions is not involved in the MgATP-stimulated release of LHRH. On the other hand, the findings that the MgATP-stimulated release of LHRH cannot occur in the presence of each of the monovalent cations tested and that the stimulated release was inversely related to the crystal radius of the cation, are indicative that an active site selective for monovalent cations is involved in the MgATP-stimulated release of LHRH from isolated hypothalamic granules.

65.4 REGULATION OF NOREPINEPHRINE UPTAKE BY THE INTERACTIONS OF SYNAPTOSOMES WITH DIVALENT CATIONS AND ATP. E.D. Hendley, S.R. Whittemore* and Y.H. Ehrlich. Depts. Physiol. Biophys, Psychiatry and Biochem. Univ. Vermont Coll. Med., Burlington, VT 05405.

The high affinity uptake of norepinephrine (NE) in brain has been thoroughly characterized pharmacologically, and has been shown by us and others to be subject to rapid and reversible alteration during altered behavioral states in rats and mice. Other studies in our laboratories have demonstrated alterations in the phosphorylation of synaptosomal proteins following similar behavioral manipulations. In the present study we have investigated the possibility that membrane protein-phosphorylation/dephosphorylation systems play a role in the regulation of transmitter uptake. This was done by measuring the high affinity uptake of ^{3}H-l-NE under phosphorylating conditions (in the presence of Ca^{+2} Mg^{+2} and ATP), conditions previously shown to cause phosphorylation of specific neuronal membrane proteins, and under non-phosphorylating conditions (omission of Ca^{+2}, Mg^{+2} and/or ATP). High affinity uptake of NE was determined by adding synaptosomes (P_2) of rat cerebral cortex to prewarmed media, and incubating them for 1 min at 30°C in Krebs-bicarbonate Ringer (KRB) containing ^{3}H-l-NE at 5 concentrations from 0.1 to 1.0 uM. Cocaine, 10 uM, was used to define specific uptake. Samples were filtered under vacuum and ^{3}H-l-NE uptake was measured by liquid scintillation counting. The kinetic constants, apparent Km and Vmax, were determined by regression analysis of Lineweaver-Burk plots. Under these assay conditions, control uptake exhibited an apparent Km of 0.18 uM and Vmax of 83 fmol/mg/min. When ATP, 200 uM, was added, uptake rates markedly increased; Vmax increased 2-fold and Km also increased. Under non-phosphorylating conditions uptake markedly decreased as Km was elevated with no change in Vmax.

In a second series, phosphorylating conditions were maintained during 5 min preincubation at 30°C in normal KRB, following which P_2 was pelleted and resuspended in isotonic sucrose, and then NE uptake was determined as described above. This procedure led to marked increases in uptake rates (elevated Km and Vmax) as seen previously with addition of ATP during the one min uptake measurement. Preincubation for 5 min in the absence of Ca^{+2} and Mg^{+2} led to decreased uptake rates, however, this decrease was caused by reduced Vmax with no change in apparent Km. Inclusion of γ-P^{32}-ATP during these preincubation or incubation procedures revealed that varying conditions which result in altered uptake rates also cause changes in the pattern of phosphorylation of specific proteins. Ongoing studies are examining the possible functional relationships between these systems. The changes in uptake rates observed here under phosphorylating conditions are similar to those we observe in vivo following stress, aggression, electroconvulsive shock and genetic hyperactivity. (USPHS MH 25811)

65.5 HIGH-AFFINITY GLUTAMATE AND GABA UPTAKE IN HUMAN BRAIN: NEUROCHEMICAL CHARACTERISTICS AND ULTRASTRUCTURAL CORRELATES. William O. Whetsell, Jr. and Robert Schwarcz. Division of Neuropathology, University of Tennessee and Memphis V.A. Hospital, Memphis, Tenn. 38163 and Maryland Psychiatric Research Center, Baltimore, Md. 21228

Following a recent report on the effects of freezing and tissue storage on glutamate uptake in rat brain (Schwarcz, Life Sci., 28, 1147, 1981), uptake of both glutamate and GABA was examined in human brain tissue collected at autopsy. The brains of all subjects (age range 38-85; no history of neurologic or psychiatric disorders) were dissected and quick-frozen at -80°C within 7 hr post mortem. Tissues were subsequently thawed and P_2-fractions prepared according to conventional differential centrifugation steps. Uptakes were routinely determined at $3x10^{-7}$M glutamate and 10^{-6}M GABA, respectively. Portions of the P_2-fractions were also examined by electron microscopy.

The data demonstrated the presence of strictly sodium-dependent uptake systems for both amino acids. Accumulation of both glutamate and GABA was abolished by sonicating the P_2-suspension prior to incubation, indicating a dependency of the processes on intact brain elements. Frozen brain samples could be stored up to 3 months (but not longer) without appreciable changes in experimental data. Regional differences in glutamate uptake were pronounced (N=7-9): cortical areas=hippocampus>caudate=putamen=thalamus=cerebellum>hypothalamus>globus pallidus=substantia nigra>medulla - the last showing less than 15% of the uptake found in cortical specimens. Kinetic analysis of high-affinity glutamate uptake in caudate tissue (N=5) demonstrated a K_m of 2.6±1.3μM and a V_{max} of 166±53 pmol/min/mg prot - in excellent agreement with figures obtained from frozen and thawed rat striata. Pharmacological assessment of both glutamate and GABA uptake substantiated these similarities: the selectivity of the uptake processes (a wide range of drugs did not interfere at 1mM) and the absolute and relative potencies of a spectrum of specific uptake inhibitors were virtually identical in human caudate and rat striatum.

Ultrastructural examination of P_2-preparations from the frozen human caudate tissue clearly demonstrated the presence of synaptosomes identical to those observed in fresh rat striatum.

Our combined neurochemical and morphological data support our previously expressed view that uptake measurements in quick-frozen and thawed human brain samples 1) are technically feasible 2) reflect functional properties of glutamatergic and GABAergic nerve terminals in the human brain and 3) may provide a tool for examination of these mechanisms in neuropathological states.

This work was supported by USPHS grant 16941 and a grant from the Wills Foundation.

65.6 PHOSPHATIDYLSERINE ENHANCEMENT OF [^{3}H] GABA UPTAKE BY RAT WHOLE BRAIN SYNAPTOSOMES. Andrew Y. Chweh* and Steven W. Leslie. Department of Pharmacology and Neurosciences Program, University of Alabama in Birmingham, Birmingham, AL 35294.

[^{3}H] GABA binding to purified lipids has been examined in an organic solvent-aqueous partition system. In addition, [^{3}H] GABA binding capacity in the partition system was compared with the ability of the lipids to alter sodium-dependent [^{3}H] GABA uptake into synaptosomes isolated from rat whole brains. For the binding study, three ml aliquots of $CHCl_3$-CH_3OH (2:1, v/v) containing 0.5 mg of each lipid and 1.0 ml of Ringer's solution (NaCl, 116 mM; KCl, 2.5 mM; $CaCl_2$, 1.0 mM, Tris base, 2 mM; pH adjusted to 7.4 with concentrated HCl) containing 0.1 μCi of [^{3}H] GABA with or without drug (nipecotic acid or bicuculline) and/or cations (Ca^{2+}, Na^+) were added to 15 ml capped culture tubes. Tubes were vortexed thoroughly for 1 min. at room temperature. After centrifugation at 500 g for 5 min. 200 μl of a lower organic phase was evaporated and counted for the radioactivity. [^{3}H] GABA was found to bind to all of the lipids studied in the organic solvent-aqueous partition system (phosphatidic acid, phosphatidylethanolamine, phosphatidylinositol, phosphatidylserine, gangliosides and sulfatide), although phosphatidylserine exhibited by far the greatest binding capacity. [^{3}H] GABA uptake into synaptosomes was enhanced by phosphatidylserine (0.1 mM) (48.0 percent), but was not altered by any other lipid. Phosphatidylserine enhancement of [^{3}H] GABA uptake required the presence of sodium and was blocked by nipecotic acid (10 μM). These results suggest that phosphatidylserine may play a role as a GABA carrier in the sodium-dependent GABA uptake process in the presynaptic nerve end. (Supported by NIAAA grant AA05132-02).

65.7 IN VITRO RELEASE OF ENDOGENOUS DOPAMINE AND 3,4-DIHYDROXYPHENYLACETIC ACID FROM RAT STRIATUM. R.S. Flint*, J.M. Murphy, M.T. Ciancone* and W.J. McBride (SPON: M.B. Waller). Depts. of Psych. & Biochem., Inst. Psych. Res., Indiana Univ. Sch. of Med., Indianapolis, IN 46223.

The release of endogenous dopamine (DA) and 3,4-dihydroxyphenylacetic acid (DOPAC) from 300x300 μm prism-shaped tissue slices of the rat striatum was studied. The tissue slices were suspended in G50 coarse Sephadex in polypropylene colums. After a suitable washout period with physiological medium, the release, induced by 55 mM K^+ or 100 μM veratridine, of endogenous DA and DOPAC was measured in the superfusate by HPLC procedures. Initial studies using 55 mM K^+-stimulation in the presence of 2.5 mM Ca^{+2}, resulted in the release of DA (54 ± 6 pmole/mg prot/min) as well as DOPAC (31 ± 3 pmole/mg prot/min). Addition of an MAO inhibitor ($8x10^{-6}$M pargyline) to the medium throughout the superfusion reduced the stimulated, Ca^{+2}-dependent amount of DOPAC released by 93% while increasing the amount of DA released to 62% above control values. Addition of pargyline, only at the time stimulated release (induced by 55 mM K^+ plus 2.5 mM Ca^{+2}) occurred, did not alter the amount of DA and DOPAC released. When 10^{-5}M cocaine was included throughout the superfusion (which should prevent uptake nearly 100%), there was no effect on the K^+-stimulated, Ca^{+2}-dependent release of DA or DOPAC. When α-methyl-β-tyrosine, a tyrosine hydroxylase inhibitor, was present during the superfusion, the amount of stimulated release of both DA (20% of control) and DOPAC (10% of control) was reduced. One hundred μM veratridine (in the absence of Ca^{+2}) stimulated the release of DA and DOPAC to the same extent as that found for 55 mM K^+ plus 2.5 mM Ca^{+2}. The peak release of DA occurred 5 min after changing the superfusion medium to one containing 55 mM K^+ plus 2.5 mM Ca^{+2} whereas the peak release of DOPAC occurred 10 min after changing the medium. Addition of 16 mM Mg^{+2} to the medium had no effect on the K^+-stimulated, Ca^{2+}-dependent release of either DA or DOPAC. In fact, substituting 16 mM Mg^{+2} for 2.5 mM Ca^{+2} markedly stimulated the release of both DA (21 ± 5 pmoles/mg prot/min) and DOPAC (23 ± 7 pmoles/mg prot/min) above baseline levels, although the amount released was less than that obtained for Ca^{+2} (40% of control for DA and 74% of control for DOPAC). The data indicate that (1) both DA and DOPAC are formed within dopaminergic terminals in the in vitro system; (2) little or no uptake of released DA occurred in the superfusion columns; (3) 55 mM K^+-stimulated release of DOPAC occurs nearly as well in the presence of 16 mM Mg^{+2} as in the presence of 2.5 mM Ca^{+2}; and (4) approximately half the DA releasable pool can be released by Mg^{+2}, suggesting the possible existence of a nonclassical release mechanism in the DA terminals. (Supported by MH00203).

65.8 THE EFFECT OF AN IN VIVO ADMINISTRATION OF PHENCYCLIDINE ON HIGH AFFINITY CHOLINE UPTAKE IN RAT HIPPOCAMPUS AND STRIATUM. G.C. Haggerty*, J.A. Richter and R.B. Forney* (SPON:J.I. Nurnberger), Dept. of Pharm. & Tox. & Inst. Psych. Res., Indiana Univ. Sch. of Med., Indianapolis, IN 46223.

Phencyclidine (PCP) has been reported to interact with the cholinergic nervous system primarily in an antagonistic manner. Since other anticholinergic drugs such as atropine and scopolomine have been reported to increase high affinity choline uptake (HACU) in whole brain (Atweh et al., Life Sci. 17: 1535-1544, 1975) we wanted to determine if the anticholinergic properties of PCP extended to a similar effect on HACU.

Experiments were carried out in male Wistar rats 250-350g. They were injected with saline, 7.0, 26.0 or 54.4 mg/kg PCP i.p. 30 min before decapitation. Synaptosomes (P_2 fraction) were prepared from hippocampus and striatum and incubated with 0.5 μM ^{3}H-choline at 30°C. Preliminary studies demonstrated that uptake was linear with time and protein concentration. Blanks were incubated in sodium-free medium so that the values reported are for sodium dependent high affinity choline uptake.

TREATMENT mg/kg PCP	HACU HIPPOCAMPUS pmoles/4 min/mgP	HACU STRIATUM pmoles/3 min/mgP
0	21.3	38.0
7.0	23.1	33.7
26.0	21.8	34.0
54.4	19.6	28.1

Analysis of variance demonstrated that PCP caused a significant decrease in HACU in the striatum but no significant change in the hippocampus. In parallel studies atropine (40 mg/kg i.p.) caused the expected increase in HACU in both brain regions. These data suggest that PCP does not act similarly to classical anticholinergic agents on this parameter. Behavioral studies in our laboratory have shown that at 30 min after a 54.4 mg/kg dose of PCP, rats are very ataxic and exhibit extreme rigidity of the body and often fine tremors. On the other hand, atropine caused hyperactivity and irritability at the dose used. Further studies will look at the temporal effects of the high dose of PCP on HACU for comparison with long-term behavioral effects.

65.9 ENHANCED IN VITRO NOREPINEPHRINE (NE) UPTAKE BY ISOLATED HYPOTHALMIC STORAGE VESICLES OF SPONTANEOUSLY HYPERTENSIVE RAT (SHR). J.H.Rho*, K. Hough*, B. Newman and N. Alexander. Clin. Pharmacol., Dept. of Med. and Dept. of Anatomy, Sch. of Med. and Pharm. Univ. of Southern California, Los Angeles, CA 90033

The in vitro uptake of ^{3}H-NE by storage vesicles from the hypothalamus of age-matched SHR and Wistar-Kyoto (WK) rats has been studied. An improved isolation technique for controlling the hypo-osmotic lysing of the synaptosomes, adjustments of the suspending medium and alterations of the centrifugation procedures have been devised. The result has been an improved procedure for the isolation of biochemically active NE storage vesicles from rat hypothalamus and rat whole brain. Differences between samples do not exceed 4%. Electron-microscopic examination of these samples confirms the concentration of synaptic vesicles.

The uptake mechanisms of storage vesicles of both SHR and WK rats exhibit a marked temperature dependence and the absolute requirement for ATP-Mg^{++} (90%). A complete inhibition of NE uptake by reserpine or dicyclohexylcarbodiimide (DCCD) was also documented. NE uptake is linear for 5 min. at 30°C, is saturable and does not level off for in excess of 20 min. Although the kinetics of the NE uptake by the hypothalamic samples of the two rat strains were the same, the amount of uptake was consistently different. The average ^{3}H-NE uptake by 15 hypothalamic samples of SHR was 46.75 p mol per mg protein of storage vesicles (5 min incubation) while that of age-matched WK was 35.21 p mol per mg protein. These differences are highly significant ($p < 0.0005$).

65.10 IN VITRO RELEASE OF ENDOGENOUS MONOAMINES AND AMINO ACIDS FROM NINE CNS REGIONS. W.J. McBride, R.S. Flint*, M.T. Ciancone* and J.M. Murphy. Depts. of Psych. & Biochem., Inst. Psych. Res., Indiana Univ. Sch. of Med., Indianapolis, IN 46223.

The K^+-stimulated, Ca^{+2}-dependent release of norepinephrine (NE), dopamine (DA), serotonin (5-HT), GABA, glutamate (Glu), aspartate (Asp), glycine (Gly), taurine (Tau) and alanine (Ala) was measured in the superfusate of tissue slices prepared from cerebral cortex (CTX), striatum (STR), hippocampus (HIP), hypothalamus (HYPO), midbrain (MB), thalamus (THAL), nucleus accumbens (ACC), pons-medulla (PM) and spinal cord (SC) of the rat. Each region was chopped into 300x300 µm prism shaped sections and suspended in G50 coarse Sephadex in polypropylene columns. After a suitable washout period in physiological medium, the release of endogenous monoamines and amino acids, induced by 55 mM K^+ in the absence and presence of 2.5 mM Ca^{+2}, was measured. Portions of each fraction were assayed by HPLC for monoamine content (reported as pmoles/mg protein/min) while another aliquot was used for determining amino acid values (reported as nmoles/mg protein/min) by GLC procedures, except for Tau which was measured spectrofluorometrically. NE, DA and 5-HT were released from all 9 regions. The release of NE was greatest from the HYPO (2.7 ± 0.3) and ACC (1.8 ± 0.6), while the release of DA was highest from the STR (33 ± 5) and ACC (8 ± 2). The release of 5-HT was nearly uniform (0.1 - 0.3) from all regions studied with the exception of the HYPO which exhibited the highest amount released (0.9 ± 0.3). The release of GABA was highest from the HYPO, MB and THAL (0.7 - 1.1). The release of Glu varied over a wide range and had its highest value in the STR (2.1 ± 0.6). Compared to the amount of Glu released, the amount of Asp released was small (0.09 - 0.17 in STR, CTX and THAL). There was no stimulated release of Gly, Tau or Ala observed for any of the CNS regions studied with two exceptions. The two exceptions were that a significant release of endogenous Gly was detected from the spinal cord (0.03 ± 0.01) and pons-medulla (0.14 ± 0.01). The finding that there was no K^+-stimulated, Ca^{+2}-dependent release of taurine under conditions where GABA, and other transmitters, are released is evidence against a transmitter function for this amino acid. The selective release of Gly from only the SC and PM is in accord with the evidence supporting a transmitter function for this amino acid in these 2 regions. The high release of Glu and DA in the STR; GABA, NE and 5-HT in the HYPO; and DA in the ACC support a significant transmitter role for these compounds in these regions. (Supported in part by MH00203 and NS13925).

65.11 HIGH AFFINITY BINDING OF [^{3}H] DESIPRAMINE TO RAT BRAIN LABELS NORADRENERGIC UPTAKE SITES. M. Rehavi*, F. K. Goodwin, P. Skolnick*, and S. M. Paul*. (SPON: J. Axelrod). Clinical Psychobiology Branch, NIMH, Laboratory of Bioorganic Chemistry, NIAMDD, Bethesda, MD 20205.

High affinity binding sites for [^{3}H] imipramine have been characterized in rat brain, human brain, and human platelets. Recent evidence has shown that these high affinity binding sites for [^{3}H] imipramine selectively label serotonin uptake sites in brain and platelets (Paul et al., Life Sci. 26:953, 1980; Langer et al., Science 210,:1133, 1980; Paul et al., Life Sci. 28:2753, 1981). Since secondary amine tricyclic antidepressants were found to be relatively weak inhibitors of [^{3}H] imipramine binding, we set out to determine whether "high affinity" binding sites for [^{3}H] desipramine are also present in rat brain. In the present study high affinity binding sites (K_D = 1.5 nM) for [^{3}H] desipramine have been demonstrated and characterized in membranes of rat brain. The binding of [^{3}H] desipramine was found to be saturable, reversible, heat sensitive and regionally distributed in the brain. High concentrations of [^{3}H] desipramine binding sites were found in the septum, cerebral cortex, and hypothalamus and low concentrations in the medulla, cerebellum and corpus striatum. A good correlation ($r = 0.81$, $p < 0.001$) was observed between the potencies of a series of drugs in inhibiting high affinity [^{3}H] desipramine binding and their ability to block norepinephrine uptake into synaptosomes. In general, the secondary amine tricyclic antidepressants desipramine and nortriptyline were much more potent than the tertiary amine antidepressants. In 6-OHDA-lesioned rats there was a marked decrease in [^{3}H] NE uptake and [^{3}H] desipramine binding with no significant alterations in [^{3}H] 5HT uptake and [^{3}H] imipramine binding.

Our results indicate that high affinity binding of [^{3}H] desipramine is pharmacologically and biochemically different from the high affinity binding of [^{3}H] imipramine in rat brain and that there is a close relationship between the high affinity binding sites for [^{3}H] desipramine and the uptake site for norepinephrine. Labelling of norepinephrine uptake sites with [^{3}H] desipramine may be a valuable tool for studying the mechanism of presynaptic transport of norepinephrine.

65.12 XYLAMINE, A NITROGEN MUSTARD, SELECTIVELY INHIBITS NE UPTAKE BY A Na+ DEPENDENT MECHANISM. R.W. Ransom* and A.K. CHO* (SPON: S. BUTCHER). Dept. of Pharmacology, UCLA School of Medicine, Los Angeles, CA 90024

In this laboratory xylamine (XYL)(N-2'-chloroethyl-N-ethyl-2-methylbenzylamine) has been shown to be a long-lasting inhibitor of norepinephrine (NE) transport across the plasma membrane of noradrenergic neurons. We have further studied XYL's selectivity and mechanism of action in efforts to develop it as a specific labelling agent for the NE uptake carrier. The incubation protocol used in these experiments is designed to measure XYL's irreversible inhibitory actions. Rat cortex is finely minced and exposed to XYL for 10 min at 37°C in Krebs bicarbonate buffer. Washed P_2 fractions are then prepared from control and drug treated minces and used as the source of synaptosomes for determining [^{3}H]NE uptake. Using this incubation protocol XYL exhibits an ID_{50} of 0.1 µM as an NE uptake inhibitor. This effect can be blocked by coincubating cocaine (10 µM) with XYL. When Na^+ is removed from the Krebs buffer and replaced by either Li^+ or choline, uptake inhibition is also prevented. This Na^+ dependency and cocaine protection indicate that XYL requires interaction with the carrier to inhibit NE uptake. XYL's selectivity was evaluated in studies of its ability to inhibit choline uptake into striatal synaptosomes and 5-HT uptake into cortical synaptosomes. While NE uptake is inhibited maximally at 1 µM XYL, neither choline or 5-HT uptake was inhibited at 10 µM XYL. XYL is also effective when administered in vivo and maximal uptake blockade in synaptosomes prepared from injected rat occurs after a 40 mg/kg (i.p.) dose. After this treatment choline transport and 5-HT transport into striatal and cortical synaptosomes, respectively, were unaffected. These results indicate XYL's selectivity and show that it is not an indiscriminate alkylating agent.

65.13 GABA UPTAKE BY GRANULE CELLS OF THE RAT OLFACTORY TUBERCLE. N.R. Krieger, J.R. Megill* and P. Sterling. (SPON: M. Selzer). Depts. of Pharmacology and Anatomy, University of Pennsylvania School of Medicine, Philadelphia, PA 19104.

The rat olfactory tubercle is suited to neurochemical studies by its laminar organization and rich complement of transmitters and related enzymes. It contains high concentrations of GABA and its synthetic enzyme, glutamic acid decarboxylase (GAD). Using techniques of laminar localization we previously demonstrated that GABA and GAD are most concentrated in the deeper polymorphic layers of the tubercle and relatively absent from the superficial plexiform and pyramidal layers (J. Neurochem. 33 (1979) 299-302). Here we report that the granule cell population (the islands of Calleja) of the polymorphic layer of the tubercle accumulates ^{3}H-GABA.

^{3}H-GABA (34.5 Ci/mMole; 1.5 µl) was injected into the tubercle, and an hour later the rat was perfused with a mixture of paraformaldehyde and glutaraldehyde. The tissue was osmicated, dehydrated and embedded in epon. Serial sections were prepared as EM autoradiograms with Ilford L-4 emulsion.

Silver grains were sparse over the pyramidal and polymorphic cell bodies, but numerous over the granule cell bodies in the islands of Calleja. Grain densities (grains/µm^3) for the granule cells were 0.41 compared to 0.024 and 0.089 respectively for the pyramidal and polymorphic cells. Within the island, all the granule cells appeared to be labeled.

These results, combined with our previous demonstration of the presence in this region of endogenous GABA and GAD, suggest that the granule neurons of the rat olfactory tubercle are gabaergic. Supported by NIH grant MH 31820.

65.14 A PROSPECTIVE METHOD FOR THE QUANTITATIVE DETERMINATION OF NEUROTRANSMITTER COMPARTMENTATION. James V. O'Fallon and Joseph W. Harding. Department of Veterinary and Comparative Anatomy, Pharmacology and Physiology, Washington State University, Pullman, WA 99164.

The Na^+, K^+-ATPase generates and maintains the membrane potential in nervous tissue. The Na^+ gradient that is established by this enzyme has been linked to the transport of compounds, including neurotransmitters and amino acids, across synaptic membranes. It has been further suggested that the Na^+, K^+-ATPase may be a trigger for a neurotransmitter release [e.g., Vizi, Neuroscience 3, 367-384 (1978)]. We have recently presented evidence that 20 µM ouabain, a specific inhibitor of the Na^+, K^+-ATPase, induces the release of cytoplasmic neurotransmitters exclusively [O'Fallon et al., J. Neurochem. 36, 369-378 (1981)].

In the present report advantage was taken of our observation that 20 µM ouabain induced the release of cytoplasmic transmitters in the development of a method to quantify the release of transmitters from neuronal-vesicular, neuronal-cytoplasmic, and glial compartments. In the method, 20 µM ouabain was used in parallel with two other neuroactive agents, 60 mM K^+ and 20 µM veratridine. The effects of these three agents on neurotransmitter release can be summarized by three simultaneous equations which contain the three transmitter compartments as variables. Coefficients for the fractional amount of transmitter released from each compartment by each agent were obtained from model transmitter-region combinations. After the coefficients had been empirically established, the equations were applied to the data obtained on the release of eight transmitters from eight mouse brain regions. The results provided a quantitative evaluation of compartmentation for each transmitter in each brain region examined. This methodology that was developed in this work should be generally applicable to studies involving both compartmentation and transport of neurotransmitters.

65.15 INHIBITION OF γ-AMINOBUTYRIC ACID TRANSPORTER BINDING BY THE POTENTIAL PHOTO-AFFINITY LABEL 4(4'-AZIDOBENZOIMIDYLAMINO)BUTANOIC ACID. G. Tunnicliff* (SPON: H. C. Stanton). Laboratory of Neurochemistry, Indiana University School of Medicine, Evansville, Indiana 47712.

The sodium-dependent binding of [^{3}H]γ-aminobutyric acid to synaptic membranes from rat cerebral cortex exhibited a high affinity component (K_d = 1.2 µM) and a low affinity component (K_d = 35 µM). Concentrations of γ-aminobutyric acid above 40 µM produced an inhibition of binding (substrate inhibition).

4(4'-Azidobenzoimidylamino)butanoic acid (ABBA) competitively inhibited [^{3}H]γ-aminobutyric acid binding to both the high affinity and low affinity binding systems. The K_i values were 11.2 µM and 49.1 µM respectively. Nipecotic acid was more effective in inhibiting both binding systems. K_i values were 4.3 µM and 10 µM. Similar results were obtained for the inhibition of [^{3}H]γ-aminobutyric acid uptake by cortical synaptosomes in the presence of ABBA or nipecotic acid. This supports the idea that sodium-dependent binding is indeed binding to the γ-aminobutyric acid transporter.

ABBA contains an azide group which could make the molecule a useful photo-affinity label for the transporter. Moreover, since ABBA possesses a benzene ring this compound might possibly cross the blood-brain barrier and thus could have important in vivo effects on the uptake of γ-aminobutyric acid.

65.16 EFFECTS OF ACUTE ADMINISTRATION OF PHENCYCLIDINE ON SERUM PROLACTIN CONCENTRATIONS IN THE RAT: DOPAMINERGIC OR CHOLINERGIC? D.F.Kirksey* and C.M. Kuhn. Department of Pharmacology, Duke Univ. Sch. Med., Durham, NC 27710.

Recent studies have suggested that phencyclidine (PCP) may produce its clinical symptomotology via direct or indirect interactions with the central dopaminergic and/or cholinergic systems. PCP reportedly inhibits: 1) amine uptake systems (Smith et.al., Biochem. Pharmacol. 26: 1435, 1977), 2) acetylcholine receptor-regulated ion conductances (Tsai et.al., Mol. Pharmacol. 18:159, 1980), 3) cholinesterase activity (Maayani et.al., Eur. J. Pharmacol. 18: 167, 1974), and 4) ^{3}H.QNB binding to central muscarinic receptors (Aronstam et.al., Mol. Pharmacol. 18: 179, 1980). In the present experiments the pharmacological effects of acute administration of PCP on basal and elevated serum prolactin concentrations in the rat were compared with effects of cholinergic agents and a variety of indirect- acting dopaminergic agonists, agents which facilitate release or block reuptake of neurogenically released dopamine.

Adult male rats (175-200g), sham-injected with saline for 3 consecutive days prior to each experiment, were divided equally into experimental groups and injected i.p. with either saline (0.9%), reserpine (5 mg/kg), or alpha-methyl-p-tyrosine (150 mg/kg). Alpha-methyl-p-tyrosine (AMPT) or reserpine was injected 90' and 300'prior to sacrifice, respectively. One hour prior to sacrifice animals received a second injection of either saline, PCP (10 mg/kg), amphetamine (5 mg/kg), methylphenidate (10 mg/kg), cocaine (20 mg/kg), pilocarpine (10 mg/kg), or atropine (20 mg/kg). All rats were decapitated 5 hours after initial injection of reserpine groups and trunk blood collected, allowed to clot at 4°C, and centrifuged. Serum was collected and assayed for prolactin by radioimmunoassay kits supplied by NIAMDD.

Basal levels of serum prolactin (15 ± 5 ng/ml) were reduced slightly but significantly by PCP, methylphenidate, and amphetamine. Reserpine pre-treatment produced marked increases in serum prolactin levels (51 ± 13 ng/ml) which were completely antagonized by PCP, amphetamine, methylphenidate, and cocaine. In contrast, the cholinergic drugs, atropine and pilocarpine, exhibited no effects on either basal or elevated prolactin levels. Pre-treatment of animals with AMPT, which inhibits dopamine synthesis resulted in an approximate four-fold increase in serum prolactin levels (69 ± 20). PCP and the indirect-acting dopaminergic agonists were all ineffective in reducing these AMPT-induced prolactin elevations. These data suggest that the pharmacological effects of PCP on prolactin release are mediated through an enhancement of tuberoinfundibular dopaminergic neuronal transmission rather than effects on cholinergic systems.

65.17 THE GADOLINIUM ION: A POTENT BLOCKER OF CALCIUM CHANNELS AND CATECHOLAMINE RELEASE FROM CULTURED CHROMAFFIN CELLS. J. M. Trifaró* and G. W. Bourne (SPON: B. Esplin). Dept. Pharmacol. & Ther., McGill Univ., Montreal, Canada.

Gadolinium (Gd^{3+}) is trivalent ion of the lanthanide series which has a high charge to density ratio but a similar ionic radius to Ca^{2+}. Therefore, the effects of gadolinium ions on [^{3}H]noradrenaline output and ^{45}Ca fluxes during resting conditions and stimulation were investigated in cultured bovine chromaffin cells.

Chromaffin cells isolated from bovine adrenal medullae were plated on collagen-coated dishes (10^6 cells/dish) in DMEM containing 10% fetal calf serum as previously described (J. M. Trifaró & R. W. H. Lee, Neuroscience, 1980, 5, 1533-1546). Seven day-old cells were used in all studies and noradrenaline stores were labelled by incubating for 5 min with amino acid-free DMEM containing 10^{-7}M [^{3}H]noradrenaline (specific activity = 1 µCi/0.1 nmole) as described elsewhere (R. L. Kenigsberg & J. M. Trifaró, Neuroscience, 1980, 5, 1547-1556).

Exposure of chromaffin cells to 0.05 mM Gd^{3+} produced 80 ± 5% and 81 ± 4% inhibition of the release of [^{3}H]noradrenaline in response to 10^{-4}M acetylcholine and 56 mM K^+ respectively. Doubling of the extracellular concentration of Gd^{3+} (0.1 mM) produced a 87 ± 3% and 100 ± 2% inhibition of the secretory responses to acetylcholine and K^+. Gd^{3+} (0.05 mM) also produced a parallel shift to the right in the dose-response relationship between the extracellular Ca^{2+} concentration and [^{3}H]noradrenaline output during acetylcholine stimulation, this observation suggests a competitive antagonism of the Ca^{2+} effects by Gd^{3+} in stimulus-secretion coupling.

Gd^{3+} (0.05 mM) was an effective inhibitor (92.3 ± 0.7%) of the uptake of ^{45}Ca into chromaffin cells induced by a depolarizing concentration (56 mM) of K^+. Furthermore, Gd^{3+} also inhibited the increase in both Ca^{2+}-Ca^{2+} exchange mechanism and [^{3}H]noradrenaline output observed in chromaffin cells upon the re-introduction of Ca^{2+} into a Ca^{2+}-free incubation medium.

The present results, which were obtained using low concentrations of Gd^{3+}, indicate that gadolinium is a powerful inhibitor of the Ca^{2+} movements which are required for triggering amine release from chromaffin cells by different secretagogues. Moreover, the results also suggest that Gd^{3+} might be a useful tool for release and electrophysiological studies in other Ca^{2+}-dependent systems.

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66.1 OLFACTORY BULBECTOMY AND CHRONIC AMITRIPTYLINE TREATMENT. J.A. Jesberger* and J.S. Richardson. Depts. of Pharmacology and Psychiatry, University of Saskatchewan, Saskatoon, Sask., S7N 0W0.

Bilateral abalation of olfactory bulbs produces a number of behavioral and biochemical changes that are normalized by chronic antidepressant therapy. We have used this preparation as an animal model of depression to examine the biochemical events associated with chronic treatment with the tricyclic antidepressant (TCA), amitriptyline (AMI). Recent investigations have shown that chronic treatment with TCA's will result in a decrease in the density of high affinity binding sites for the beta-adrenergic antagonist ^{3}H-dihydroalprenolol (^{3}H-DHA) and the TCA, ^{3}H-imipramine in several regions of the brain. We have investigated the binding of these two ligands to brain membranes from sham and bulbectomized male Sprague Dawley rats that have received either saline or AMI for 28 days (10 mg/kg i.p.) followed by a 5-day drug free period. Behavioral testing (stepdown passive avoidance and emotionality rating) was conducted after the 5-day drug washout period. The animals were then sacrificed, trunk blood was collected and the brain was excised and dissected. Regions dissected out and investigated were the hypothalamus, midbrain, hippocampus and pons medulla.

Olfactory bulbectomy resulted in an increase in the number of trials for acquisition of the stepdown passive avoidance task, increased irritability scores and elevated 11-hydroxycorticosteroid levels, all of which were returned to near sham values by AMI treatment. Membrane preparations from sham-operated amitriptyline treated animals exhibited a decreased binding for the beta-adrenergic antagonist ^{3}H-DHA relative to that of saline treated shams. Lesioned-saline treated animals did not show any significant decrease in the binding of ^{3}H-DHA relative to saline treated shams nor did the AMI treated lesioned animals exhibit any altered binding relative to that found in saline treated lesioned animals. Chronic treatment with amitriptyline resulted in a decrease in binding for ^{3}H-imipramine in sham-operated animals. Binding studies of ^{3}H-imipramine in bulbectomized subjects are in progress.

These studies suggest that olfactory bulbectomy indirectly or directly alters the noradrenergic response potential to chronic AMI treatment.

66.2 STUDIES ON THE EFFECTS OF INTRAVENTRICULAR INFUSIONS OF (-)-NICOTINE ON BEHAVIOR MAINTAINED UNDER FIXED RATIO SCHEDULES, V. J. DeNoble* and L. Carron*. Behavioral Pharmacology Laboratory, Philip Morris Research Center, Richmond, VA 23261.

Intraventricular infusion of (-)-nicotine produces a prostration syndrome that is prevented by intraventricular pretreatment with derivatives of nicotine but not by anticholinergic drugs (Abood et al., 1979). Present studies investigated 1) intraventricular infusions of (-)-nicotine on behavior maintained under fixed ratio (FR) schedules of food reinforcement, 2) effect of repeated infusions and, 3) effect of nicotinic-cholinergic antagonists on prostration.

In exp. 1 eight male albino rats, each implanted with a cannula in the lateral ventricle were maintained under FR 16 until response rates were stable (<10% variance in responses/sec for 5 consecutive sessions). Sessions were two consecutive 15-min periods with a 5-min time-out (TO) in between. All infusions were separated by 5 days. Rats were first infused with saline (5µl) and repetitive 15-min sessions were run until response rate was at preinfusion levels. Latency to complete the first FR following infusions was recorded. Next, rats were infused with 5µg of (-)-nicotine. This was repeated with the rats maintained under FR 32 and 64. In exp 2 (N=6) rats were maintained under FR 32 and tested as follows: 1) saline infusion, 2) (-)-nicotine (10µg) 3) saline/presession injection (sc) of mecamylamine HCL (0.05, 1.5 and 3.0) or hexamethonium Cl (0.5, 1.0, 1.5 mg/kg/sc) 4) (-)-nicotine/presession injection of the antagonists, and 5) (-)-nicotine retest.

Results of exp 1 showed that latency to complete the first ratio under FR 16 following a saline infusion was $\bar{X}$ = 0.8 min (.02 SE) whereas the latency following (-)-nicotine was $\bar{X}$ = 10.9 min (1.6 SE). Latencies were inversely related to FR size (FR 16, 11.0 min (7.6 SE), FR 32, 7.7 min (1.5 SE), FR 64, 4.3 min (0.9 SE). To determine if this inverse relationship was due to repeated (-)-nicotine infusions independent of the FR schedule the rats in exp 1 were tested with (-)-nicotine twice under FR 32. No significant difference was found between the latencies. Latency following a 10µg infusion of (-)-nicotine (exp 2) was $\bar{X}$ = 13.0 min (1.4 SE). Mecamylamine had no effect during saline infusions, however, it did produce a dose dependent decrease in the latency following a (-)-nicotine infusion (0.05, 1.5 and 3.0 mg/kg/sc; latency - 6.8, (1.2 SE), 2.0, (0.7 SE), 0.6, (0.1 SE) min. Hexamethonium injections had no effect. These results suggest that: 1) duration of the effect of intraventricular infusions of (-)-nicotine extended beyond the observed prostration, 2) duration of suppression varies as a function of the FR size, 3) there is a lack of tolerance with repeated infusions and 4) mecamylamine but not hexamethonium antagonized the effects of (-)-nicotine on FR responding suggesting that the effects are mediated by central nicotinic-cholinergic receptors.

66.3 THE EFFECTS OF STRYCHNINE, 5-METHOXY-N,N-DIMETHYLTRYPTAMINE AND CLONIDINE ON ACOUSTIC AND ELECTRICALLY-ELICITED 'STARTLE' RESPONSES IN THE RAT. R.L. Commissaris* and M. Davis (SPON: M. Bowers). Dept. Psychiat., Yale Univ. Sch. Med., New Haven, CT 06508.

Although many neuropharmacological agents have been shown to alter the acoustic startle response, the site(s) of action for many of these agents has not been defined. This study used electrical stimulation within the startle circuit in combination with selected drug treatments as a technique to localize the site(s) of action for these agents.

Previous work has suggested that the primary acoustic startle circuit in the rat is: auditory nerve, ventral cochlear nucleus, nuclei of the lateral lemniscus, nucleus reticularis pontis caudalis (RPC), spinal interneuron, lower motor neuron, muscles. Electrical stimulation of sites within this circuit produces a response similar to the acoustic startle response (Gendelman and Davis, Neurosci. Abstr. 5: 494, 1979). In the present study male rats (350-400 g) received bilateral, single-pulse (100 µA per electrode, 1 msec duration) stimulations of the RPC alternating with acoustic noise bursts (115-db, 4-20 kHz band-width, 90 msec). The interstimulus interval between the two types of stimuli was 10 sec. Both acoustic and RPC-elicited 'startle' responses were measured for forty minutes following intraperitoneal administration of strychnine (1.0 mg/kg), 5-methoxy-N,N-dimethyltryptamine (5-MeODMT; 4.0 mg/kg), clonidine (80 µg/kg) and saline. These drugs were chosen because previous studies in which they were injected directly into the lumbar region of the spinal sub-arachnoid space (intrathecal administration) have indicated that their excitatory (strychnine, 5-MeODMT) and inhibitory (clonidine) effects on acoustic startle are mediated at least in part through the spinal cord (i.e., "downstream" from the RPC). As expected, strychnine and 5-MeODMT increased and clonidine decreased acoustic startle. Consistent with the spinal actions of these agents in modulating acoustic startle, RPC-elicited 'startle' was also increased by strychnine and 5-MeODMT and decreased by clonidine.

These results indicate that the 'startle' elicited by electrical stimulation of the RPC can be altered by treatments which act in the spinal cord to modulate acoustic startle. Furthermore, these results suggest that the 'startle' response produced by electrical stimulation of various sites within the acoustic startle circuit may be used to elucidate the site(s) of action for agents which modulate the acoustic startle response in the rat.

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66.4 COCAINE POTENTIATES KETAMINE-INDUCED LOSS OF RIGHTING REFLEX AND SLEEP TIME. Christina VanderWende, Marie T. Spoerlein and Judy Lapollo*. College of Pharmacy, Rutgers.The State University, Box 789, Piscataway, N.J. 08854.

Ketamine has dissociative anesthetic properties and unlike the parent compound, phencyclidine (PCP), is still used as an anesthetic in the human. However, it does have post anesthetic sequelae which resemble the psychotomimetic effects of PCP. Because of these mind-altering effects, ketamine has now gained acceptance as a street drug. There is the potential that ketamine will be used in combination with other drugs such as cocaine, amphetamine and caffeine which are at times used as adulterants of ketamine powders. We have found that cocaine modifies ketamine-induced loss of the righting reflex and sleep time.

Cocaine caused a shift to the left of the dose-response curve of ketamine for the loss of righting reflex in a dose-dependent manner. Cocaine administered simultaneously with ketamine reduced the ED_{50} to 48(30-76) and 86(61-120) mg/kg when given in doses of 30 and 15 mg/kg, respectively, as compared to 115 mg/kg in the ketamine controls. Sleep time with 150 mg/kg of ketamine was significantly increased by 56% when administered simultaneously with 30 mg/kg of cocaine. This effect of cocaine was not a generalized effect with CNS depressants since cocaine had no effect on loss of righting reflex or sleep induced by pentobarbital, phenobarbital or hexobarbital. Conversely, Metrazol (55 mg/kg) antagonized ketamine sleep as would be anticipated for a CNS stimulant.

Since ketamine has a cocaine-like effect on catecholamines (CA), we examined the possibility that CA systems may underlie the cocaine potentiation of ketamine. Mice were treated with alpha-methyl-p-tyrosine (∝MPT), 400 mg/kg, to deplete the CA's. At various times after the pretreatment, the cocaine effect was again reexamined. Although the sleep time with ketamine (100 mg/kg) was significantly increased with ∝MPT itself, the administration of cocaine had no further effect on sleep. Thus, the effect of cocaine was blocked by ∝MPT. Attempts to further study the CA involvement using adrenergic and dopaminergic receptor modifying drugs were more difficult to interpret. Generally DA agonists increased while DA blockers antagonized the effect of cocaine, although they, in themselves, potentiated ketamine. The results with alpha and beta agonists and antagonists were less clear.

66.5 EFFECTS OF INTRASEPTAL GABAERGIC AND SEROTONERGIC AGENTS ON MURICIDE. M. Potegal, B. Yoburn* and M. Glusman, N.Y. State Psychiatric Institute, New York, NY 10032.

Electrical stimulation of the septal region of hamsters, rats, cats and monkeys inhibits various forms of aggressive behavior (Potegal, et al Physiol Behav., 24:863, 1980). The neurotransmitter systems mediating this effect have not yet been established although it has been shown that dopamine, norepinephrine, acetylcholine and glutamate are not specifically involved (Albert & Chew, Behav Neural Biol., 30:357, 1980). A GABA involvement is suggested by the moderately high concentration of the GABA synthesizing enzyme glutamate decarboxylase (GAD) in lateral septum and the effectiveness of the GABA agonist muscimol in altering the muricidal (mouse-killing) behavior of rats when injected intracranially (Mandel, et al, 1978). Serotonin is a possibility because raphe lesions dramatically facilitate muricide while systemic administration of the agonist quipazine and antagonist metergoline alter the threshold for septal muricide-inhibitory electrical stimulation (Gibbons et al, Soc. Neurosci. Abstr. 6: 366, 1980).

In our first experiment 5 muricidal male Long-Evans hooded rats were implanted with a chronic intraseptal cannula. In each of 8 sessions a drug was injected intraseptally after a baseline muricide latency had been obtained. Mice were presented at 5,20, 40 and 60 min following administration of counterbalanced muscimol (0.5, 0.05, 0.005 µg), thiosemicarbazide (a GAD inhibitor; 7.5, 2.5, 0.75 µg) or saline. Muscimol significantly facilitated muricide in a dose-dependent manner while thiosemicarbazide (TSC) had a significant dose-dependent inhibitory effect. Both drugs increased activity moderately. Muscimol increased irritability; TSC had no effect on irritability.

In a second experiment the effects of these GABAergic agents on muricide and eating were compared to those of quipazine and metergoline in 3 rats. The respective effects of muscimol and TSC on muricide were replicated while quipazine and metergoline appeared to have no major effects. None of the drugs affected eating. These results suggest that GABA may suppress the aggression-inhibitory neuronal elements of the septum resulting in a disinhibition of muricide. Muscimol-induced increases in irritability may indicate that this effect occurs across different types of aggression. Serotonin-receptive neural tissue is not directly involved at the septal level but may be activated through septal-raphe pathways. The lack of differential effects of muscimol and TSC on activity and eating suggest that septal GABAergic systems may have a specific role in aggressive behavior. (This research was supported in part by a grant from the Harry F. Guggenheim Foundation.)

66.6 LOCUS OF ACTION OF ANTIPSYCHOTIC DRUGS. A. D. Sherman* and F. Petty. Dept. of Psychiatry, Univ. of Iowa, and Veterans Adm. Med. Cent., Iowa City, IA 52242.

Inhibition of the conditioned avoidance response (CAR) by neuroleptics correlates well with their antipsychotic potency. Stereotactic injection of chlorpromazine (CPZ, 1.0µg in 0.2 µl) delayed acquisition of a one-way CAR when injected into the amygdala, septum, or caudate, but was without behavioral effect in the anterior neocortex, hippocampus, globus pallidus, n. accumbens, thalamus, hypothalamus, substantia nigra, entorhinal cortex, or lateral geniculate body. Haloperidol (HDL) also inhibited CAR acquisition in the three CPZ-sensitive areas.

Atropine, given intraperitoneally, diminished the CAR-inhibitory effect of intracranial CPZ in septum and caudate, but not in amygdala. IP atropine blocked the CAR-inhibition of HDL in caudate, but not in septum or amygdala. Atropine had no effect on CAR-inhibition of either drug injected IP.

When CPZ at the ED_{50} for CAR inhibition (2.3 mg/kg) was injected IP, tissue levels of CPZ in septum, caudate, and amygdala, but not in other areas, demonstrated a significant correlation with behavioral effects.

A series of putative neurotransmitters, dopamine (DA), norepinephrine (NE), serotonin (5-HT), γ-amino butyric acid (GABA), glutamic acid (GLU), aspartic acid (ASP), and acetylcholine (ACh) were then injected into the septum, caudate, and amygdala at a dose of 1 µg in 0.2 µl. In the amygdala, both GABA and ACh delayed acquisition of the CAR. This effect was also found with injection of NE or ASP into the septum, and with injection of GABA, ASP, or ACh into the caudate.

These findings demonstrate the significant interaction of neuroleptics and the acetycholine system in the caudate, an interaction that was also present for CPZ but not HDL in the septum. This, plus the higher potency for CAR inhibition found for HDL vs. CPZ in the amygdala support the hypothesis that the anti-avoidance actions of neuroleptics may derive from an action in the amygdala.

The data also suggest a potential role for the amino acid neurotransmitters, and support the hypothesis of Janssen (Int. J. Neuropsychiat. 4:10, 1967) that GABA is involved in the action of neuroleptics. The finding that ASP in the septum inhibits CAR is of particular interest as this putative neurotransmitter has not previously been thought to play a role in the mechanism of action of psychotropic drugs.

66.7 ^{3}H BENZODIAZEPINE BINDING SITE DISTRIBUTION FOLLOWING ICSS. M. Sano*, F. Gimino*, S.Steiner, A. Lippa* and B. Beer*. Behavioral Physiology Lab., City College of New York, New York, N.Y. 10031.

As a means of examining the relationship between classical central reward mechanisms and more recently discovered brain mechanisms of anxiety, the techniques of ICSS and high affinity radioactive binding prodedures have been conducted concurrently. The C-T monophasic pulse pair procedure is well established as a tool for delineating site-specificity of reward mechanisms in discrete brain regions. This technique has been coupled with a radioactive binding assay using ^{3}H Flunitrazepam as a ligand to characterize the distribution of benzodiazepine binding sites in specific brain regions. These binding sites appear to be related to behavioral measures of anxiety.

Albino Sprague-Dawley rats were implanted with an indifferent skull electrode and with 2 bipolar electrodes bilaterally placed in discrete ICSS reward sites. Animals were shaped at one site for a maximum of 15 daily sessions at a variety of current intensities. The crude homogenates of several brain areas including cortex, hippocampus and diencephalon were incubated with ^{3}H Flunitrazepam alone or in the presence of clonazepam to determine non-specific binding. The results evaluate binding in tissue after exposure to ICSS. Kinetic analysis will permit the evaluation of B max and KCl values in specific brain regions.

66.8 EVIDENCE THAT ANTI-ACETYLCHOLINE RECEPTOR ANTIBODIES DO NOT INTERACT WITH HYPOTHALAMIC BUTX SITES IN VIVO AND IN VITRO. P.F. Strang-Brown and B.J. Morley. The Boys Town Institute for Communication Disorders in Children, Omaha, NE 68131.

The cross-reactivity between whole brain BuTX binding sites and antibodies to peripheral receptors is negligible (Speth et al., Soc. Neurosci. Abst., 3:339, 1977; Morley and Kemp, Soc. Neurosci. Abst., 5:1013, 1979, Morley and Kemp, Brain Res. Rev., 2, 1981) but Block and Billiar (Brain Res., 117:381, 1979) demonstrated cross-reactivity between antisera to peripheral receptors and rat brain hypothalamic BuTX sites. Recently, Tarrab-Hazdai and Edery (Exp. Neurol., 67:670, 1980) have reported that intraventricular injections of anti-Torpedo IgG into rabbits resulted in a depression of water and food intake. The concentration of BuTX binding was also shown to be decreased substantially in the hypothalamus but not in the hippocampus or cerebral cortex. These latter two studies suggest that there are regional variations in the cross-reactivity of peripheral antibodies with brain BuTX sites. The following studies tested this hypothesis.

For our studies, purified nAChR from Narcine brasiliensis was injected into rats to produce anti-Narcine antibodies. The titer was calculated to be 5×10^{-8}M. Cannulas were stereotaxically implanted bilaterally into the hypothalami of 20 anesthetized male albino rats. After a recovery period of 5 days, the animals were given daily injections of anti-Narcine antisera or normal rat sera. Injections were proportioned so that 5 µl of sera was injected into each cannula 4 times daily for a total of 40 µl sera daily. Food and water intake and body weight were monitored daily. The hypothalamus, hippocampus, and cerebral cortex were assayed biochemically as described by Morley et al. (Brain Res., 134:161, 1977) for specific BuTX binding. Additionally, the cross-reactivity of Triton extracts of rat hypothalamic ^{125}I-BuTX sites was investigated using anti-Torpedo, anti-Electrophorus, and anti-Narcine antisera in radioimmunoassays.

The chronic administration of anti-Narcine antisera for 7 or 14 days resulted in no effect on either food or water intake in the rats. Results of the biochemical assays of hypothalamus, cerebral cortex, and hippocampus showed no alterations in the concentration of BuTX binding in any area. Peripheral antibodies did not significantly immunoprecipitate BuTX sites in radioimmunoassays.

These results support the hypothesis that there is little or no cross-reactivity between antibodies to peripheral receptors and brain BuTX sites.

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66.9 PSYCHOMOTOR STIMULANTS, SOCIAL ISOLATION AND PLAY IN YOUNG RATS. W. W. Beatty, A. M. Dodge*, L. J. Dodge*, K. White* and J. Panksepp. Depts. of Psychology, North Dakota State Univ., Fargo, ND 58105 and Bowling Green State Univ., Bowling Green, OH 43403.

Brief social isolation (8-24 h) profoundly stimulates play fighting in juvenile rats (Panksepp & Beatty, Behav. Neural Biol. 30: 197, 1980). To begin the study of the neurochemical mechanisms that regulate play the effects of ip injections of d-amphetamine sulfate (0.125-1.0 mg/kg) or methylphenidate HCl (0.5-4.0 mg/kg) were determined. Both agents suppressed play fighting in a dose-dependent fashion regardless of whether the animals were housed in pairs (social housing) or alone (isolation). At the highest doses tested both drugs increased the time the rats spent in social investigation (mutual grooming and sniffing), but only if the animals had been socially isolated. Neither agent affected rearing under the present conditions. Thus, low doses of psychomotor stimulants lead to a redistribution of social behavior, at least when animals have been socially isolated. Play fighting is suppressed while social investigation is enhanced.

66.10 THE EFFECTS OF PARA-CHLOROAMPHETAMINE AND A MONOFLUORINATED ANALOGUE ON THE BEHAVIOR OF RATS AND ON BRAIN AMINE LEVELS. C.H.M. Beck, H. Chow*, W.G. Dewhurst*, G.B. Baker and R.T. Coutts*. Neurochemical Research Unit, Dep't. of Psychology, Dep't. of Psychiatry, Fac. of Pharmacy and Pharmaceutical Sciences, Univ. of Alberta, Edmonton, Alberta, T6G 2E1.

The clinical value of the serotonin depletor para-chloroamphetamine (pCA) as an antidepressant is limited by its toxic effect on serotonin neurons. The monofluorinated analogue of pCA, namely 2-amino-1-(4-chlorophenyl)-3-fluoropropane or FpCA, like pCA reduces brain 5-HT levels, although higher doses are required and the 5-HT concentrations return to normal faster than with pCA (Baker, G.B. et al., Neuropharmacology, 1980, 19:1255). The purpose of the present study was to compare the short term effects of pCA and FpCA on brain amine levels and on open field behavior of rats. Male Sprague Dawley rats received, one hour before recording, injections of pCA (5.2 mg/kg, i.p.) or FpCA (5.6 mg/kg, i.p.) or saline (2 ml/kg, .9%, i.p.) having been pretreated, 24 hours earlier, with saline (2 ml/kg, .9%, i.p.) or reserpine (1 mg/kg, i.p.). Behavioral observations were made prior to the first injection and in the second hour after the second injection. The rats' behavior was recorded continuously by a trained observer during 15 two-minute observation periods interpolated over the one hour sessions. The observer used a checklist of comprehensive, mutually exclusive behavioral categories. In 9 sessions the rat was alone and in 6 sessions the rat was paired with another rat. Immediately after the second observation session at 2 hours after the second injection, the rats were decapitated and their brains fluorimetrically assayed for norepinephrine, dopamine and serotonin. Relative to saline controls, the whole brain levels of serotonin were reduced in rats given pCA (to 54% of controls), and these levels were further reduced (to 28% of controls) by pretreatment with reserpine. The dose of FpCA used did not reduce serotonin levels following saline pretreatment, however, serotonin levels were reduced to 49% of controls in animals given FpCA with reserpine pretreatment. This was a reserpine effect since reserpine alone depleted serotonin levels to a similar degree (to 36% of controls). All reserpine pretreated animals had similar reductions in whole brain levels of norepinephrine (to 33% of controls) and of dopamine (to 58% of controls). Animals given FpCA and pCA with saline pretreatment showed brain norepinephrine levels increased to 114% of controls and increased dopamine levels to 144% of controls. The general effect on behavior of the injections in the experimental groups compared to either saline injections or the effect of no injections at all (predrug) was to increase the likelihood of the rat being immobile.

66.11 "OFF" RESPONDING IN THE SHUTTLEBOX SELF-STIMULATION TEST: EFFECTS OF ANXIOLYTICS AND d-AMPHETAMINE. S. Gerhardt*, J. Prowse and J. M. Liebman. (SPON: M. Roffman). Res. Dept., Pharma. Div., CIBA-GEIGY Corp., Summit, NJ 07901.

In Atrens' shuttlebox self-stimulation procedure (Psychopharmacol. 49:97, 1976), rats interrupt a photocell beam to initiate brain stimulation, then terminate it by interrupting another beam at the opposite end of the shuttlebox. Latency to initiate stimulation (the ON latency) is considered to correlate inversely with reward value of stimulation, while the latency to terminate stimulation (the OFF latency) has been used as an indicator of concomitant, nonspecific performance disruption. We now report that the OFF latency is differentially elevated by anxiolytic agents and, in contrast, reduced by d-amphetamine.

Rats with lateral hypothalamic electrodes were trained to interrupt an infrared beam to initiate continuous biphasic square-wave stimulation. Interruption of a beam at the other end of the shuttlebox terminated stimulation but left the animal free to re-initiate current. Test sessions lasted 10 min, during which ON and OFF latencies were compiled by computer. Drugs were administered i.p. and were given 30 min before testing except for pentobarbital (15 min).

Moderate doses of diazepam (2.5 and 5 mg/kg) and chlordiazepoxide (3 and 10 mg/kg) increased OFF latencies significantly more than ON latencies. A similar effect was noted after pentobarbital (10 mg/kg). At a lower dose, each of these agents reduced ON latencies slightly at the same time that OFF latencies were slightly elevated.

The effects of d-amphetamine (0.1-1 mg/kg) were compared with those of pipradrol (1-10 mg/kg), another psychomotor stimulant, and buproprion (10-100 mg/kg) a dopamine uptake blocker. All three agents reduced the ON latency, as expected from their facilitatory effects on bar-pressing self-stimulation rates. However, only d-amphetamine concurrently reduced OFF latencies. Pipradrol reduced ON latencies with a magnitude comparable to d-amphetamine, yet failed to alter OFF latencies. It is possible that d-amphetamine differs from other stimulant drugs in a manner which may reflect anxiomimetic properties. Alternatively, psychomotor activation caused by d-amphetamine may account for its ability to reduce OFF latencies.

That OFF responding may reflect an aversive component of stimulation is suggested by these results, by informal observations of performance, and by the proximity of electrode placements to the medial hypothalamus. Further pharmacological characterization of OFF responding may aid in understanding the substrates of aversive motivation and anxiety.

66.12 EFFECTS OF VARIOUS CATECHOLAMINE ANTAGONISTS AND NONSPECIFIC MOTOR DISRUPTION ON SHUTTLEBOX SELF-STIMULATION PERFORMANCE: A SYSTEMATIC COMPARISON. J. M. Liebman, J. Prowse* and S. Gerhardt*. Res. Dept., Pharma. Div., CIBA-GEIGY Corp., Summit, NJ 07901.

The shuttlebox self-stimulation procedure developed by Atrens has been proposed as a means of dissociating drug effects on "reward" from nonspecific motor effects. We systematically evaluated this hypothesis by comparing the effects of: (a) several dopamine antagonists, (b) disruption of noradrenergic rather than dopaminergic neurotransmission, and (c) nonspecific disruption of motor performance.

Rats with lateral hypothalamic electrodes were trained to interrupt an infrared beam at one end of a shuttlebox to activate a stimulator that delivered continuous biphasic square-wave brain stimulation. Interuption of a beam at the other end terminated stimulation but left the animal free to re-initiate current. Test sessions lasted 10 min, during which "ON" and "OFF" latencies were compiled by computer. Drugs were administered i.p. 30 min before testing. Changes in ON latencies are thought to correlate with changes in reward value of stimulation. The absence of concurrent changes in OFF latencies is taken to indicate that changes in ON latencies are not attributable to nonspecific motor effects.

In agreement with Atrens et al. (Psychopharmacol. 49:97, 1976), we found that clozapine (1-10 mg/kg) elevated ON latencies more strongly than OFF latencies. This profile was not unique among dopamine antagonists, however, as metoclopramide (1-10 mg/kg) had very similar effects. Haloperidol (0.03-1 mg/kg) also elevated ON latencies more strongly than OFF latencies, but the differentiation between latencies was weaker than with clozapine or metoclopramide. Prazosin (0.3-10 mg/kg), an α-1 adrenergic antagonist, had effects very similar to those of haloperidol. Thus, although various catecholamine receptor antagonists elevated ON latencies more strongly than OFF latencies, they were not readily discriminated from each other using this procedure. Moreover, the same profile was produced by methocarbamol, a muscle relaxant with sedative properties.

Clonidine (0.03-0.3) mg/kg very clearly differentiated ON from OFF latencies, elevating the former markedly while only slightly altering the latter. The reason for clonidine's unusually selective effect on ON latencies remains to be explored further, but such an effect parallels findings from other laboratories.

66.13 ANTAGONISM OF NICOTINE EFFECT BUT NOT TOLERANCE DEVELOPMENT BY HEXAMETHONIUM. Glenn Daniel Todd* and John A. Dougherty. Depts. Psychiatry and Pharmacology, Univ. of Kentucky Medical Center and Veterans Admin. Medical Center, Lexington, Ky.

In a previous study (Soc. Neurosci. Abstr., 5:665, 1979) we found that tolerance to a suppressant effect of nicotine on a fixed-ratio schedule of lever-pressing developed within one hour and was still present 8 hours later. The duration of suppression after the second injection was often 75% shorter than after the first injection (4.1 vs. 14.2 min). To evaluate the mechanism of this tolerance development, the effect of hexamethonium upon the suppression of responding produced by nicotine was examined in 10 Wistar rats. A saline injection (control) or a nicotine injection (200 ug/kg, BASE, i.p.) was given immediately before each of two daily lever-pressing sessions conducted four hours apart. Each session lasted 30-minutes with every fiftieth press reinforced by water presentation, with the rats maintained at 80% of ad lib weight by limitation of water access.

Hexamethonium (1 or 5 mg/kg, BASE, s.c.) given 1 hour prior to the start of the first daily session produced no change in responding in either of the two daily saline control sessions. Hexamethonium, 1 mg/kg, did not affect the suppression of responding produced by the first nicotine injection (13.3 vs. 14.2 min) and did not affect the development of tolerance to the second daily injection of nicotine (4.8 vs. 4.1 min) 4 hours later. Hexamethonium, 5 mg/kg, given 1 hour prior to the first daily nicotine injection did antagonize the suppression of responding normally produced by the first daily nicotine injection (3.4 vs. 14.2 min). However, when the effect of the second nicotine injection was assessed 4 hours later, the development of tolerance was found to be unchanged (4.6 vs. 4.1 min).

The possibility that a prolonged effect of hexamethonium antagonized the suppressant effect of nicotine in the second daily session was ruled out by a study in which 5 mg/kg hexamethonium was given 5 hours before the first daily lever-pressing session. Hexamethonium had no effect on the normal 14-15 min suppression produced by nicotine in the first daily session.

Thus, 5 mg/kg hexamethonium antagonized the suppressant effect of nicotine but had no effect on the development of tolerance to that suppressant effect 4 hours later. Therefore, tolerance occurred to the effect of nicotine even though the effect of nicotine had been antagonized. This suggests that the development of tolerance to the behavioral suppresant effect of nicotine may be independent of cholinergic receptor blockade.

66.14 EFFECTS OF TRIETHYLTIN ON SCHEDULE DEPENDENT AND SCHEDULE INDUCED BEHAVIORS USING A FIXED INTERVAL OR FIXED RATIO SCHEDULE OF REINFORCEMENT. D. L. DeHaven, S. M. Evans*, M. J. Wayner and F. C. Barone. Brain Research Lab., Syracuse Univ., 601 University Ave., Syracuse, NY 13210

The effects of the demyelinating agent triethyltin (TET) on operant responding using a FR-30 schedule of reinforcement and on schedule dependent and schedule induced behaviors using a FI-1 min schedule of reinforcement were assessed. TET (0.5, 1.0 and 1.5 mg/kg IP in 15% ethanol, immediately pre-session) was administered twice a week for two weeks following an established stable baseline of responding during the same regimen of vehicle administration. Response rate under the FR-30 schedule was decreased following 1.5 mg/kg TET. Schedule induced licking and water intake and schedule dependent lever pressing under the FI-1 min schedule were significantly decreased following 1.0 and 1.5 mg/kg TET. Rats on the FI-1 min schedule were also allowed to recover to ad lib feeding body weight and were retested in the same operant chambers on the same schedule. Baseline responding and subsequent vehicle-injection baseline measures were again determined. TET was then administered as described above. Decreases in schedule induced licking and water intake and schedule dependent lever pressing were observed following 0.5 and 1.0 mg/kg TET. Results indicate that operant responding and particularly schedule induced behavior are sensitive to low doses of TET. Schedule induced behavior which occurs in animals at ad lib feeding body weight is most sensitive to the behaviorally toxic effects of TET. (Supported by NIH Grant NINCDS USPHS No. 13543.)

66.15 A SIMPLE AND RAPID METHOD FOR IMPLANTATION OF A CHRONIC CANNULA INTO THE MOUSE CEREBROVENTRICULAR SYSTEM. D. L. Colbern*, P. J. Syapin, and E. G. Zimmermann. Brain Research Institute, UCLA School of Medicine, Los Angeles, CA 90024, and Department of Neurology, USC School of Medicine, Los Angeles, CA 90033.

Cannulation of the lateral ventricle in two strains of mice (Swiss-Webster and C57) was accomplished by modifying a rat implantation method previously described by Brakkee, et al. (Lab. Animal Sci., 29:78-81, 1979). Major advantages of the present technique include the accurate, non-stereotaxic placement of an easily-made polyethylene cannula, and the extremely secure attachment of dental cement to the fragile mouse skull without the use of jeweler's screws. A metal guard placed around the exposed portion of the cannula permits post-operative group housing of mice. Using pentobarbital anesthesia, sequential cannulation of 20 mice by one person routinely takes 4 hours (10-15 min per mouse). Surgeries performed in parallel on a number of mice anesthetized by other means would require less time.

This technique allows quick and painless injection of microliter volumes of chemicals into the cerebrospinal fluid of unanesthetized mice. ACTH 1-24 injected via these cannulae produced expected excessive grooming behavior when compared to saline injections. Repeated injections through the cannulae have been made for 2-3 weeks after implantation. The simplicity of the cannulation procedure and subsequent non-stressful injection make this a valuable technique for evaluating the effects of intracerebroventricularly applied drugs on behavioral and neurochemical parameters.

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67.1 ATTENUATION OF DOPAMINE AGONIST EFFECTS OF CHRONIC PERGOLIDE ADMINISTRATION. D. Jiang*, A. Reches*, H.R. Wagner*, S. Fahn, Dept. Neurology, College of Physicians and Surgeons, Columbia University, New York, NY 10032.

The dopamine agonist properties of pergolide, an N-propyl-ergoline derivative, were studied in the rat. At low dosage (0.1 mg kg^{-1} i.p), pergolide depressed motor activity, while at higher doses (0.5-4.0 mg kg^{-1}) motor activity was increased and stereotypy appeared. Increased motor activity at higher doses was both preceded and followed by a decrease in motor activity. A reduction in the deaminated dopamine (DA) metabolites, 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA), was found in the striatum during periods of increased motor activity. No significant change in metabolite levels was noted during the initial hypoactive phase. During the period of motor hyperactivity, decreases in HVA and DOPAC levels were dose independent between the range of doses investigated (0.5-2.0 mg kg^{-1} pergolide). In rats treated chronically with pergolide (0.5 or 1.0 mg kg^{-1}, i.p., on alternate days for two weeks), the duration of the heightened behavioral responses (hyperactivity and stereotypy) decreased while the enusing hypoactive phase increased in both duration and magnitude. The decrease in the levels of DA metabolites (DOPAC and HVA) was also reduced following chronic pergolide treatment; after two weeks, levels were no longer significantly different from controls. A similar attenuation of behavioral response occurred in rats previously injected unilaterally in the substantia nigra with 6-OHDA. Turning behavior in such rats persisted in excess of 36 hrs following initial pergolide injections but seldom lasted beyond 24 hrs following two weeks of repeated injections. The increase in motor activity, stereotypy and turning behavior at higher drug doses and the decrease in DA metabolite levels (DOPAC + HVA) are consistent with a DA agonist role for this ergot derivative. The attenuation of these reponses with repeated drug exposure suggests the development of tolerance.

67.2 INHIBITION OF DOPA UPTAKE AND METABOLISM IN RAT STRIATUM BY O-METHYLDOPA A. Reches*, and S. Fahn, (SPON: R.E. Barrett). Dept. Neurology, College of P & S, Columbia University, New York, NY 10032.

A major part of DOPA given to parkinsonian patients is methylated by COMT to 3-O-methyldopa (OMD) which is inactive therapeutically. Clinical studies indicate that OMD may antagonize the beneficial effect of DOPA; for example, patients treated with DOPA deteriorate when OMD is added to their regimen. We have therefore studied the effect of OMD on DOPA metabolism. Rats were injected with OMD (400 mg·kg^{-1} i.p.) and 2 h later with DOPA (100 mg·kg^{-1} i.p.); control rats received DOPA only. Rats were sacrificed 15 min after DOPA injection. In OMD pretreated rats DOPA, dopamine, DOPAC and HVA levels in the striatum were significantly lower than control rats: 0.89 ± 0.2 vs. 1.58 ± 0.19, $p< 0.05$; 18.5 ± 2.6 vs. 23 ± 1.25, $p<0.05$; 6.3 ± 0.9 vs. 9.6 ± 0.16, $p<0.02$; and 1.39 ± 0.14 vs. 1.82 ± 0.06, $p<0.05$, respectively. No effect was observed on 5-HIAA levels; 0.616 ± 0.11 vs. 0.663 ± 0.13. (All results were determined by HPLC, and are expressed as mean ng/mg tissue ± S.E.M. of n=5, statistics by two-tailed student t-test.) Control and OMD pretreated rats were injected with NSD-1015 (100 mg·kg^{-1} i.p.) and were sacrificed 20 min later. In OMD pretreated rats the accumulation of DOPA in the striatum was significantly lower compared with the control group: 1.3 ± 0.04 (n=9) vs. 1.65 ± 0.117 (n=9), $p<0.025$. Control and OMD pretreated rats were injected with gamma-butyrylactone (750 mg·kg^{-1} i.p.) and sacrificed 40 min later. Dopamine accumulation in OMD pretreated rats was lower, compared with control group 24.5 ± 1.1 (n=11) vs. 28.2 ± 1.1 (n=10), $p<0.05$. It is concluded that OMD interferes with dopamine synthesis in the striatum, possibly either by decreasing brain uptake of precursors or by inhibiting tyrosine hydroxylation. This work was supported in part by NIH Grant #NS15959-02. A.R. is an NIH Fogarty International Fellow supported in part by Grant # TW02884-01.

67.3 DO CATECHOLAMINE SYNTHESIZING ENZYMES SHARE COMMON GENE CODING SEQUENCES? T.H. Joh, E.E. Baetge, B.B. Kaplan, M.E. Ross, M.J. Brodsky, V.R. Albert*, D.H. Park and D.J. Reis. Departments of Neurology and Anatomy, Cornell University Medical College, New York, N.Y. 10021.

The biosynthesis of epinephrine in adrenal medulla requires three specific enzymes: tyrosine hydroxylase (TH), dopamine B-hydroxylase (DBH) and phenylethanolamine N-methyltransferase (PNMT). These enzymes are regulated together, usually by control of their biosynthesis. Conceivably, these enzymes might be coded for by a single gene, or by several genes that have evolved through gene duplication. To test this hypothesis, we sought to determine whether the three enzymes, as similar gene products, structurally resemble one another.

All three enzymes were purified to homogeneity from bovine adrenal and their molecular weights (MWs) determined by SDS-urea PAGE. MWs of the enzymes are: DBH=75,000; TH= 60,000; PNMT= 30,000; and the trypsin-digested form of TH (tTH)=35,000. Antibodies (Abs) to all three enzymes and tTh were raised in rabbits or by hybridoma techniques.

To determine whether the enzymes can be identified as similar proteins in mRNA translation products, poly(A)mRNA was purified from fresh bovine adrenal medulla,and translated in vitro in a reticulocyte cell-free protein-synthesizing system. Individual enzymes were identified from the translated products by immunoprecipitation and SDS-PAGE. All four Abs precipitated similar proteins. That is, DBH-Ab precipitated DBH and TH; TH-Ab precipitated TH, DBH, and a small quantity of PNMT; PNMT-Ab precipitated PNMT, TH, and a small quantity of DBH;and tTH-Ab precipitated PNMT and TH.

Peptide analysis of purified PNMT and of tTH using Staphlococcus aureus protease digestion and SDS-PAGE for identification of peptides showed that both enzymes produced eight identical peptides. The amino acid composition of all three enzymes indicated that the molar ratios of the major amino acids are similar, the major amino acids being: leu, glu, asp, ala, val, and gly, respectively. Serine is a major amino acid in both DBH and TH, but not in PNMT and tTH.

These results indicate that all three enzymes have protein domains with similar primary structure. This is consistant with the hypothesis that the three enzymes are coded for by a single gene or genes evolved from a common ancestral precurser. (Supported by NIH grant, MH24285 ,HL 18974, and HD 11392).

67.4 MESOCORTICAL DOPAMINE NEURONS: UNIQUE RESPONSE TO HALOPERIDOL EXPLAINED BY ABSENCE OF AUTORECEPTORS. M. J. Bannon and R. H. Roth. Depts. Pharmacology & Psychiatry, Yale University School of Medicine, New Haven, CT 06510.

Haloperidol markedly accelerates dopamine (DA) turnover in the striatum and olfactory tubercle following acute administration. This effect is due to multiple sites of haloperidol action, including (a) the blockade of postsynaptic DA receptors with a subsequent increase in dopaminergic impulse flow through neuronal feedback mechanisms, and (b) the blockade of nerve terminal DA autoreceptors. In the prefrontal cortex, haloperidol elicits a much smaller increase in DA turnover. This difference may be explained by the recent finding that mesocortical DA neurons lack nerve terminal autoreceptors (Mol. Pharmacol. 19: 270-275, 1981).

In the present experiments, acute haloperidol challenge (1 mg/kg, i.p.) to rats dramatically increased both striatal and olfactory tubercle α -methyltyrosine-induced DA depletion (319% and 241% control depletion, respectively) and dihydroxyphenylacetic acid levels (DOPAC; 401% and 243% control DOPAC, respectively). In contrast, in the prefrontal cortex only small changes in α -methyltyrosine-induced DA depletion and DOPAC levels were observed following haloperidol challenge. It has been previously demonstrated that the small haloperidol-induced increase in prefrontal cortical DA synthesis is totally prevented following the blockade of dopaminergic impulse flow (Mol. Pharmacol. 19: 270-275, 1981). These data strongly suggest that the effects of haloperidol on mesocortical DA neurons are dependent on alterations in neuronal impulse flow and do not involve nerve terminal autoreceptors.

Following chronic haloperidol treatment (0.5 mg/kg/day, i.p. X 28 days), significant tolerance to the DOPAC-elevating effect of a haloperidol challenge (1 mg/kg) developed in both the striatum and olfactory tubercle. No tolerance to haloperidol was observed in the prefrontal cortex. In additional experiments with chronically treated rats, no evidence of DA autoreceptor function was revealed in the prefrontal cortex. It is suggested that tolerance to the effects of haloperidol may be mediated in part by nerve terminal DA autoreceptors. In fact, in brain regions where haloperidol tolerance was seen (i.e. striatum and olfactory tubercle), the development of DA autoreceptor supersensitivity was also demonstrated. (Supported in part by USPHS Grants MH-14092, MH-25642 and the State of Connecticut.)

67.5 LONG-TERM EFFECTS OF A SINGLE DOSE OF AMPHETAMINE ON STRIATAL DOPAMINE NEURONS IN IPRINDOLE-TREATED RATS: TIME-COURSE AND TIME-DEPENDENT INTERACTIONS WITH AMFONELIC ACID. L. R. Steranka. Dept. of Pharmacology and Physiology, Northwest Center for Medical Education, Indiana University School of Medicine, Gary, IN 46408.

The administration of a single dose of (+)-amphetamine sulfate (9.2 mg/kg, i.p.) to rats treated with iprindole hydrochloride (10 mg/kg, i.p.) produced marked decreases in the striatal concentrations of dopamine (DA), 3,4-dihydroxyphenylacetic acid (DOPAC), and homovanillic acid (HVA) one week after drug administration. Significant changes were not observed in striatal 5-hydroxyindoleacetic acid (5HIAA) nor in norepinephrine, DA, DOPAC, HVA and 5HIAA concentrations in frontal cortex and a limbic forebrain sample containing primarily nucleus accumbens and olfactory tubercles. In time-course experiments, decreases in striatal DA were apparent by 12 hours after amphetamine plus iprindole administration and persisted for at least 4 weeks. Decreases in striatal DOPAC and HVA followed a similar time course, except decreases in these parameters were observed at 6 hours as well. The administration of amfonelic acid (5 mg/kg, i.p.), a potent DA uptake inhibitor, up to 8 hours, but not at 12 hours, after amphetamine administration prevented the decreases in striatal DA, DOPAC and HVA at one week after the administration of the drug to iprindole-treated rats. These data indicate that the actions of amphetamine which are necessary and sufficient for the production of the long-term decreases in striatal DA, DOPAC and HVA are dependent upon the integrity of the neuronal uptake mechanism for DA and occur within 12 hours after the administration of amphetamine to iprindole-treated rats.

Although amfonelic acid prevented the long-term effects of amphetamine on striatal DA neurons, it did not alter the decrease in DOPAC produced by amphetamine at 6 hours after the administration of amphetamine plus iprindole. This finding suggests that the ability of amfonelic acid to prevent the long-term effects of amphetamine on striatal DA neurons in iprindole-treated rats is not due to a blockade of the entry of amphetamine into the neuron and, thus, suggests that the access of amphetamine to the inside of the neuron is not sufficient for the production of its long-term, possibly neurotoxic, effects on striatal DA neurons.

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67.6 PLASMA HOMOVANILLIC ACID AS AN INDEX OF CNS DOPAMINE METABOLISM: ENHANCEMENT WITH A PERIPHERAL MONOAMINE OXIDASE INHIBITOR. D.E. Sternberg*, G.R. Heninger, and R.H. Roth. Yale University School of Medicine, New Haven, CT 06508.

Plasma homovanillic acid (HVA) may be a useful measure of CNS HVA production by central dopamine (DA) systems. Even though there is a significant peripheral contribution to plasma HVA, experimental manipulations that alter brain HVA also change plasma HVA. In rodents, electrical stimulation or lesions of DA pathways, and pharmacologic treatments that increase or decrease brain HVA, produce parallel changes in plasma HVA levels. The present study was designed to assess whether the relationship between haloperidol induced increases in brain and plasma HVA levels could be strengthened by reducing the contribution to plasma HVA from peripheral sources. Debrisoquin sulfate, a monoamine oxidase inhibitor which does not enter the brain, decreases the production of HVA from peripheral sources and can be safely used in humans. Experiments were conducted in rats to determine an effective dose and time schedule for debrisoquin administration which would reduce the peripheral contribution to plasma HVA and MHPG. A minimum dose schedule of debrisoquin (.5 mg/kg x 3 days, 1 mg/kg x 1 day) produced a significant 27% and 37% decrease in plasma HVA and MHPG, respectively, but no change in brain HVA or MHPG. Using this schedule, rats were pretreated with either debrisoquin or vehicle. Two hours prior to sacrifice, they were administered either vehicle or various doses of haloperidol (0.2,0.1,0.05 mg/kg). A significant plasma HVA increase following haloperidol was observed at a lower dose (0.05 mg/kg) in the debrisoquin pretreated rats. In this group, a 71% increase in brain HVA was accompanied by a 60% increase in plasma. In the vehicle pretreated group, twice the dose of haloperidol (0.1 mg/kg) was required, producing a 131% increase in brain HVA and a 51% increase in plasma. Debrisoquin had no effect on the magnitude of the haloperidol-induced brain HVA increase. These data indicate that debrisoquin pretreatment improves the reliability of plasma HVA as a measure reflecting changes in CNS DA metabolism. Plasma HVA samples obtained in humans following debrisoquin treatment may provide a clinically applicable method for assessing brain DA systems in neurologic and psychiatric illness. (Supported by MH25642, MH14092 and MH30929).

67.7 TETRABENAZINE AND RESERPINE MAY AFFECT DIFFERENTLY THE PHENCYCLIDINE, COCAINE AND AMFONELIC ACID ACTIONS ON SYNAPTOSOMAL DOPAMINE. S. P. Bagchi and M.A. Reilly* Rockland Research Institute, Orangeburg, NY 10962.

We have observed that the stimulants phencyclidine (PCP), cocaine (CO) and amfonelic acid (AA) may enhance the synthesis and release of synaptosomal (P_2) DA (Biochem. Pharm. 29, 2957 (1980); Neuropharmacology (in press)) from phenylalanine (PHE); also, the presence of reserpine may not only block such synthesis stimulation by either PCP, CO or AA but reveal their inhibitory effects on DA formation. We have now tested the influence of tetrabenazine (TBZ), another amine depletor and an antipsychotic, upon the actions of these stimulants for comparative purposes. The methods consisted of incubating rat caudate nucleus P_2 in tris buffer (10 mM; pH 7.4) containing salts, pargyline (0.08 mM), glucose (10 mM), sucrose (0.32 M) and labelled PHE substrate at 37° for 10 min with/without a drug. Postincubation separation of the particulates from the medium was by filtration on a 0.8μm Millipore filter. The separated fractions were then analyzed for labelled DA and for the particulate labelled PHE substrate uptake. The sum of particulate and medium contents indicated the total formation of labelled DA and its synaptosomal release was expressed by dividing the ratio medium/total labelled DA in the presence of a drug by the same ratio from the control incubation. The results indicated that both Res and TBZ inhibited the total formation of labelled DA and stimulated its release; also their effects on the synthesis and release were quantitatively similar over a wide concentration range (22 to 1800 nM). The presence of either PCP, CO or AA stimulated the synthesis as reported before and a coaddition of Res with any of the stimulants blocked such synthesis increase and furthermore revealed their inhibitory effects. The presence of TBZ however maintained the synthesis stimulations by the same drugs. The releasing actions of these stimulants however were not affected differently by Res and TBZ. Tyramine (TA), another releasing agent, also inhibited the synthesis and stimulated the release of labelled DA; but a coaddition of TA with any of the stimulants also failed to block their synthesis enhancing effects. The results suggest, a) TBZ and Res may act on different DA pools, b) the DA synthesis stimulations by either PCP, CO or AA may be due to their actions on synaptic vesicles but not synaptosomal reuptake and c) Res influence on PCP, CO and AA actions may have a specificity beyond its simple releasing action. (Kindly supported by the Office of Mental Health, State of New York).

67.8 Lumbar Elimination Mechanism for Homovanillic Acid Which is Probenecid Insensitive. R. Stanley Burns*, Susan A. Cosgrove* and Michael H. Ebert* (SPON: Chuang C. Chiueh). Lab. of Clin. Sci., NIMH, Bethesda, MD 20205.

The level of homovanillic acid (HVA) in cerebrospinal fluid (CSF) is used as an index of brain dopamine metabolism. The kinetics of HVA in lumbar CSF was studied in the Rhesus monkey.

In 4 animals, 50 μg of deuterium-labeled (d_2-) HVA alone or combined with 5 μCi of ^{14}C-inulin, a marker of bulk flow of CSF, was administered in 100 μl of saline as a bolus injection into the subarachnoid space one vertebral level above an indwelling lumbar sampling catheter. A second group of 3 animals were pretreated with an intravenous infusion of probenecid at a dose (120 mg/Kg over 1 hour followed by 20 mg/Kg/hour X 6 hours) which produces maximal inhibition of acid transport at the choroid plexus. Timed 100 μl CSF samples and sequential blood samples were obtained over the 6 hour post-injection period. Endogenous HVA and d_2-HVA were determined by gas chromatography-mass spectrometry and ^{14}C-inulin by liquid scintillation counting.

The d_2-HVA concentration in lumbar CSF decreased with a mean half-life ($t_{1/2}$) of 40 minutes, while the $t_{1/2}$ of ^{14}C-inulin was 6-7 hours. Endogenous HVA levels remained constant with a CSF:serum ratio of about 10. d_2-HVA was detected in blood at 15 minutes and reached peak levels 90 minutes after intra-lumbar injection. The rate of elimination of d_2-HVA from lumbar CSF was not altered by probenecid pretreatment. The level of HVA in lumbar CSF is determined not only by the delivery rate from the ventricular system, but also by its local clearance rate.

67.9 EFFECTS OF VARYING INTENSITIES OF WHITE LIGHT ON DOPA ACCUMULATION IN RAT RETINAS. Melissa A. Proll*, Cylia W. Kamp and William W. Morgan. Dept. Anat., Univ. Texas Health Sci. Ctr., San Antonio, TX 78284.

It is well established that light activates the retinal dopamine (DA) neurons in rats, but the parameters controlling this response are ill-defined. The objective of this study was to determine the effects of different intensities of white light on the activity of the retinal DA neurons. Dihydroxyphenylalanine (DOPA) accumulation following an injection of NSD 1015 (hydroxybenzylhydrazine), a L-aromatic amino acid decarboxylase inhibitor, was used as an index of DA neuron activity. Groups of six to eight male rats, dark adapted for approximately fifteen hours, were exposed either to no light (other than a photographic red light) or to 0.7, 5.3, 32.2, or 570.3 lux in a specially designed light box with illumination provided by Sylvania Cool White fluorescent bulbs. The bulbs were covered with black plastic, and light intensity was controlled by varying the number of slits cut in the plastic. Light intensity was measured with a Tektronix J16 Digital Photometer. All rats received NSD (100 mg/kg, i.p.) fifteen minutes prior to sacrifice by decapitation. This was immediately prior to light exposure in groups receiving light. Concentrations of DOPA and DA were measured by high performance liquid chromatography with electrochemical detection. DOPA accumulation in rats exposed to no light or to 0.7 lux did not differ significantly from each other. In rats receiving no light, DOPA accumulation was significantly less than that measured in rats exposed to either 5.3 ($p<0.05$), 32.2 or 570.3 lux ($p<0.01$). DOPA accumulation after exposure to only 0.7 lux was also significantly less than the accumulation after 5.3, 32.2 or 570.3 lux ($p<0.05$). DOPA accumulation in rats receiving 5.3, 32.2, or 570.3 lux did not differ significantly. These results suggest that the threshold of white light required for the activation of the retinal DA neurons is between 0.7 and 5.3 lux. Supported by DA 00755 and NS 14855 to WWM.

67.10 DOPAMINE TURNOVER AS ESTIMATED BY SIMULTANEOUS LCEC ASSAY OF DOPAMINE AND DOPAMINE METABOLITES. C.H. Cheng and G.F. Wooten, Depts. of Neurol. and Pharmacol.; Wash. Univ. Sch. Med.; St. Louis, MO 63110

Estimation of the ratio of dopamine (DA) to dihydroxyphenlacetic acid (DOPAC) is a useful index of DA turnover. However amphetamine (Amph), a DA-releasing drug, produces paradoxical changes in the DA/DOPAC ratio (J. Pharmacol. Meth. 5:165-173, 1981) probably as a result of the ability of Amph. to both block DA reuptake and inhibit monoamine oxidase activity. In order to fully characterize the effects of drugs, particularly amph, on DA turnover and release, we have developed a method for simultaneous assay of DA, DOPAC, 3-methoxytyramine (3MT), and homovanillic acid (HVA) using liquid chromatography with electrochemical detection. Brain samples were homogenized in $HClO_4$ 0.1N containing GSH 5mM, centrifuged, and aliquots of the supernatant were injected directly. Samples were separated on a μBondapak C_{18} reverse phase column with the amperometric detector set at 760 mV. The assays were performed at a flow rate of 1.5 ml/min. at ambient temperature. The mobile phase consisted of methanol 10%, acetic acid 1%, EDTA 1mM, and heptane sulfonic acid 4mM. Sensitivity of the assay defined as twice the amplitude of the background noise was 30 pg for DA and DOPAC and 300 pg for HVA and 3-MT. The assay was highly specific as the retention times for each compound were sufficiently different so that each peak was totally resolved on the column. Dihydroxybenzylamine was used as the internal standard. The assay was linear in the concentration ranges that were studied. Control values in striatum (μg/g) were as follows: DA 10.4±0.82, DOPAC 1.57±0.12, 3-MT 0.27±0.07, and HVA 1.03±0.06. Thirty minutes after Apomorphine (0.5 mg/kg SC) striatal DOPAC levels were 59% and HVA 40% of control with no change in DA. One hour after administration of haldol (1 mg/kg SC) DOPAC levels were 356% and HVA 1700% of control, again with no change in DA. One hour after amph (2.5 mg/kg SC) striatal levels of DA were 154% of control, DOPAC 59%, 3-MT 144%, and HVA 61% ($P < 0.05$ for all changes reported).

These data suggest that changes in levels of DA and 3-MT reflect the release of DA induced by amph administration. The reduction of DOPAC and HVA are consistent with the ability of amph to competitively inhibit monoamine oxidase activity.

67.11 PURIFICATION AND CHARACTERIZATION OF RAT STRIATAL TYROSINE HYDROXYLASE. N. M. Richtand* and R. Kuczenski. Departments of Biochemistry and Pharmacology, Vanderbilt University School of Medicine, Nashville, TN 37232.

Recent experiments have shown that the kinetic properties of rat striatal tyrosine hydroxylase (TOH) and its activation by the polyanion heparin are sensitive to the presence of specific monovalent cations (R. Kuczenski, J. Neurochem., in press). For example, activation of TOH by heparin, which proceeds through a decrease in Km of the enzyme for its reduced pterin cofactor, is preferentially prevented by increasing concentrations of KCl or NaCl as compared to TrisCl or tetramethylammonium chloride (TMACl). Thus, heparin can still effect a two-fold decrease in Km of TOH for cofactor in the presence of 0.35M TMACl or TrisCl, but fails to alter the activity of the enzyme in the presence of an equal concentration of KCl or NaCl. These and other data suggest that K^+ or Na^+, but not Tris or TMA^+, may stabilize or promote a conformational state of TOH which has a lower affinity for heparin.

Based on these observations, rat striatal TOH has been purified to apparent homogeneity. The pivitol step in the purification protocol involves differential monovalent cation elution of enzyme activity from a heparin-substituted Sepharose 4B affinity column. Crude extracts of rat striatum are applied to the column, and a substantial portion of contaminating proteins is removed during extensive washing with high ionic strength TMACl. Subsequently, TOH is eluted with a KCl gradient. This procedure yields a 130-fold purification of TOH in a single step with near 50% recovery of enzyme activity.

The purification protocol is notable in two respects. First, it provides a simple procedure for the rapid purification of rat striatal TOH in sufficient yield to allow for physicochemical characterization of the enzyme. Second, the success of the specific monovalent cation elution of TOH from the heparin-substituted Sepharose provides support for the suggestion that monovalent cations can modify the conformational state of the enzyme.

Purified TOH has been examined for protein-bound phosphate, and the kinetic properties of the purified enzyme have been detailed and compared to the enzyme in crude homogenates. The susceptibility of the purified enzyme to kinetic activation by heparin, phospholipids and phosphorylating conditions has been assessed. Finally, studies of the effects of activating conditions on the physico-chemical properties of the enzyme have been initiated.

67.12 EVIDENCE FOR DOPAMINE DEFICIENCY IN THE WEAVER MOUSE. M. J. Schmidt, B. D. Sawyer, * K. W. Perry,* R. W. Fuller, M. M. Foreman and B. Ghetti. The Lilly Research Laboratories, Indianapolis, IN 46285, and Indiana University Medical Center, Indianapolis, IN 46223.

Dopamine (DA) deficiency is a contributing factor in the symptomatology of Parkinson's disease. Weaver (WV) mice display a number of motor impairments reflecting cerebellar abnormalities. However, they also exhibit neurologic changes that might be due to DA deficiency. These mice are hyperactive when stimulated and intermittently exhibit a fine tremor. We undertook a thorough study of the DA system in the WV by using biochemical, pharmacological and anatomical approaches, since preliminary evidence indicated that DA in the whole brain of WV mice was reduced (Lane et al., 1977). WV mutant mice (CBA Aw-J/A) and heterozygous littermates were obtained from Jackson Lab. (Bar Harbor, ME) or raised from stock in our facilities.

Monoamine levels in the olfactory tubercle, frontal cortex and striatum were determined using high-pressure liquid chromatography and electrochemical detection. Dopamine was 27 percent lower in the olfactory tubercle, 77 percent lower in the frontal cortex and 75 percent lower in the striatum of WV mice. Norepinephrine and serotonin were not lower in these brain areas. Tyrosine hydroxylase activity in the striatum was measured with a radiometric assay and was 70 percent lower in WV mice. Examination of mice from 11-270 days-of-age revealed that the DA system failed to develop in WV mice. Motor activity was assessed in individual animals using circular photocell activity cages with minimal illumination. Apomorphine and pergolide, direct DA agonists, increased activity more in WV than in normal littermates. Amphetamine, which releases endogenous stores of DA, was less active in WV mice. These findings provide suggestive evidence that post-synaptic DA receptors in WV mutants might have become supersensitive as a result of lower levels of DA in motor areas of the brain.

Anatomical evidence of DA system abnormalities was found in WV mice. Serial sections were cut from the midbrain of WV and control mice and stained alternatively with gallocyanin for Nissl substance or with hemotoxylin-eosin. The pars compacta of the substantia nigra in WV appeared hypocellular when compared with the corresponding sections from controls. Fewer large neurons were seen in the affected animals.

This study illustrates that WV mice have specific deficiencies in the DA system. As such, they might prove useful as a model for the treatment of Parkinson's disease.

67.13 CONTINUOUS AMPHETAMINES:SWITCH FROM STEREOTYPICAL TO HALLUCINOGEN-LIKE BEHAVIORS CORRELATED WITH FLUCTUATIONS IN CAUDATE BUT NOT ACCUMBENS DOPAMINE RECEPTORS. **G. Ellison, W. Morris, and P. Schwartz. Dept. Psychology and Neuroscience, UCLA, Los Angeles 90024.**

Following implantation with slow-release d-amphetamine pellets rats and monkeys initially enter a phase of motor stereotypies or bouts of staring, then become inactive, and finally enter a late-stage during which behaviors usually elicited only by hallucinogens appear. These stages are correlated with cyclical alterations in caudate dopamine receptors. During the initial (stereotypy) phase, there is a potentiation in caudate dopaminergic receptor tone. This is evidenced both by an increased potency of d-Amp to induce motor stereotypies, and by increased in vivo and in vitro spiroperidol binding in caudate. Spiroperidol accumulation is not increased in nucleus accumbens at this time. During the late-stage, the number of receptors in caudate decreases. Continuously administered d-Amp is especially effective (compared to repeated daily injections) in inducing both of these alterations in caudate, and in producing long-term damage to dopamine terminals in caudate but not accumbens. From these observations we hypothesize that the selective neurotoxic action which amphetamines have on caudate but not accumbens dopamine terminals is correlated with an early proliferation of caudate dopamine mechanisms. Supported by DA 02312.

67.14 EFFECTS OF THE PUTATIVE AUTORECEPTOR AGONISTS, 3-PPP AND TL-99 ON CENTRAL DOPAMINERGIC MECHANISMS. M. Williams, G. E. Martin and D. R. Haubrich. Merck Institute for Therapeutic Research, West Point, PA 19486.

The effects of two putative dopamine (DA) autoreceptor agonists, 3-PPP (Hjorth et al., Abst. Amer. Col. Neuropsychopharm., 1979) and TL-99 (Goodale et al., Science 210: 1141, 1980) were examined together with the reference DA agonists, N-n-propylnor-apomorphine (NPA), apomorphine (APO) and pergolide (PERG) in three dopaminergic test procedures; ^{3}H-APO binding (I), antagonism of gammabutyrolactone stimulation of DA synthesis (II) and production of stereotypy in rats (III). The activity in I was NPA > APO ≅ TL-99 > PERG ≫ 3-PPP. Preferential interactions with pre- and postsynaptic receptors were assessed in tests II (pre) and III (post). 3-PPP and TL-99 failed to produce stereotypies at nonlethal doses (ED_{50}'s >50 mg/kg i.p.) while NPA, PERG and APO had ED_{50}'s <1.0 mg/kg. All five compounds were active in test II with ED_{50}'s <2 mg/kg i.p., the ED_{50} ratios III:II being; NPA: 1.8; APO: 1.0; 3-PPP: >9.7; TL-99: >21; and PERG: 24. The results support the concept that TL-99 and 3-PPP are selective DA autoreceptor agonists and would also indicate that PERG at low doses preferentially interacts with autoreceptors.

67.15 SELECTIVE DOPAMINERGIC AUTORECEPTOR ACTIVATION: AN IN VIVO TEST SYSTEM. G. E. Martin and R. J. Bendesky*. Merck Institute for Therapeutic Research, West Point, PA 19486.

A pharmacological test will be described which uses the locomotor activity (LMA) of the mouse for assessing dopamine (DA) receptor activation. Apomorphine, in low doses, has been shown to decrease the mouse's LMA presumably due to activation of the DA autoreceptor, whilst greater doses activate the postsynaptic DA receptor causing an increase in LMA. Since apomorphine exerts effects on both DA receptors depending on the dose administered, it generates a U-shaped dose response curve (Carlsson, 1975). Hence, selectivity for the DA autoreceptor would be indicated in this LMA test if a test drug satisfied three criteria. The drug should: 1) produce a dose-related inhibition of LMA with no excitation as the dose is increased; 2) the inhibition should be reversed by sulpiride; and 3) the test drug should inhibit LMA throughout its duration of action.

Using these criteria, 13 reported dopamine agonists were examined in this test. A wide range of doses of each drug was given to 20-30 mice (i.p.) and LMA was recorded automatically in donut-shaped activity chambers and compared with LMA of a control group. The results are outlined in the table. 3-PPP, TL-99, ADTN prodrug and CF 25-397 have a profile of activity consonant with selective DA autoreceptor activity. The first three compounds have been proposed as selective DA autoreceptor agonists by other investigators which suggests that mouse LMA may be a useful measure of DA autoreceptor activation.

Drug	Dose Range(s) (mg/kg i.p.)	(1) Δ LMA	(2) Sulpiride	(3) Duration
3-PPP	0.6-100	↓	+	+
TL-99	0.16-1.28	↓	+	+
ADTN(pro)	8.1-100	↓	+	+
CF25-397	0.019-20	↓	+	+
DPI	0.08-50	↓	-	+
Bromocriptine	5-160	↓	+	-
Lergotrile	0.08-20	↓	+	-
Pergolide	0.01-1.28, 2.56	↓,→		
Apomorphine	0.04-0.64, 1.28	↓,↑		
NPNA	0.004-0.5, 2.5	↓,→		
RU 24926	0.02-2.56, 5.28	↓,→		
RU 24213	0.02-2.56, 5.28	↓,→		
SKF 38393	0.1-48.6,	↔		

+ or - indicate the criterion was or was not met.
Arrows indicate inhibition (↓), excitation (↑) or no effect (↔) on LMA for the corresponding dose ranges.

67.16 INTERACTION OF CATIONS AND ASCORBIC ACID IN [^{3}H]-SPIROPERIDOL BINDING IN RAT BRAIN . L. L. Coughenour* (SPON: J. Abelson) Warner-Lambert/Parke-Davis Pharmaceutical Research Division, Ann Arbor, Michigan 48105.

Numerous studies have been done on the binding of [^{3}H]-dopamine agonists and [^{3}H]-dopamine antagonists to the striatal membranes of rats and other species. In most of these studies ascorbic acid and either a mixture of ions or EDTA was added to the incubation mixture. Recently, it has been reported that in the presence of ascorbic acid, $MnCl_2$ ($1x10^{-4}$ M.) and NaCl ($1x10^{-1}$ M.) increase the binding of [^{3}H]-spiroperidol, a dopamine antagonist, to rat striatal membranes by 192% and 178% of control, respectively (Usdin, et al, 1980). However, others have reported that ascorbic acid inhibits the binding of the dopamine agonist [^{3}H]-ADTN to rat striatal membranes (Kayaalp and Neff 1980) and the binding of the dopamine antagonist [^{3}H]-haloperidol to guinea pig brain membranes (Leslie, et al, 1980). In the present study ascorbic acid (0.001%, 0.01%, 0.1%) was found to inhibit the binding of [^{3}H]-spiroperidol to rat striatal membranes by 50-70% when no ions were present in the incubation mixture. However, when a mixture of ions (120 mM NaCl, 5 mM KCl, 2 mM $CaCL_2$, 1 mM $MgCl_2$) was present in the incubation mixture, ascorbic acid (0.1%) did not inhibit the binding of [^{3}H]-spiroperidol to these membranes. The binding in the presence of both ions and ascorbic acid was not different from the binding in the absence of both ions and ascorbic acid.

When no ascorbic acid was present, NaCl ($1x10^{-1}$ M.) increased the binding of [^{3}H]-spiroperidol by 18% and MnSO4 ($1x10^{-4}$ M.) decreased the binding by 12% while no significant change occurred with EDTA ($1x10^{-4}$ M.) In addition NaCl, MnSO4, and EDTA in the above concentrations could prevent but not reverse the inhibition by ascorbic acid (0.1%). The inhibition by ascorbic acid was time dependent with 50% inhibition occurring after 10 minutes and virtually 100% inhibition occurring after 30 minutes at 25°C. These findings indicate that ascorbic acid may be altering the kinetics of the binding of [^{3}H]-spiroperidol to striatal dopamine receptors. Ascorbic acid also may be modulating or masking the effects of cations on dopamine receptor binding systems. These parameters should be re-evaluated to account for the effects of ascorbic acid in dopamine binding assays.

68.1 EFFECT OF ELECTRICAL STIMULATION OF THE C1 ADRENERGIC CELL GROUP AND THE KOLLIKER-FUSE NUCLEUS ON SYMPATHETIC VASOMOTOR ACTIVITY AND ADRENAL MEDULLARY CATECHOLAMINE SECRETION IN RAT. C.A. Ross, A. Del Bo*, D.J. Reis Lab. of Neurobiology, Cornell Univ. Med. Coll. New York, NY 10021

Segments of the thoracic spinal cord which innervate the adrenal medulla receive afferents from several brainstem presumably autonomic nuclei. In the present study we investigated the effect of electrical stimulation of two of these--the C1 adrenaline containing cell group of the medulla oblongada and the Kolliker-Fuse nucleus (KFN) of the parabrachial complex in the pons (Ross et al. Neurosci. Lett. 1981; Saper and Loewy, 1980). We sought to establish whether neurons in these nuclei excite or inhibit release of adrenal medullary hormones, and if so, whether they act upon the adrenal medulla, or sympathetic vasomotor nerves, or both. Stimulation of both C1 and KFN in chloralose (60mg/ kg) anesthetized, paralyzed, artificially ventilated rats (n=55) with 10 sec trains evoked stimulus-locked pressor responses and tachycardia. Thresholds were 8-15 μa and the responses were graded; at 5x threshold, the elevation of arterial pressure was approximately 80 mm and the increase in heart rate 60 bpm. The optimal stimulus frequencies in the C1 region were 150-300 hz, while those in the KFN were 70-150 hz. Pressor responses and tachycardia from both regions were blocked by spinal cord transections or by phentolamine (5mg/kg, i.v.), but not by acute midbrain transection, nephrectomy, or adrenalectomy. After abolition of vasomotor responses by pretreatment with 6OHDA (100mg/kg) electrical stimulation of both C1 and KF nuclei elicited pressor responses with a delayed onset (15-20 sec) and a prolonged duration (2-5 min), accompanied by vagal bradycardia. Stimulus thresholds and frequency optima were similar to those in untreated rats. The pressor responses were almost completely abolished by phentolamine or bilateral adrenalectomy and therefore resulted from release of adrenal medullary catecholamines. The areas from which maximal responses were elicited corresponded closely with the locations of neurons of C1 and KFN retrogradely labelled after injections of HRP into the lower thoracic spinal cord. The pressor responses from both areas were unaffected by prior ipsilateral midbrain hemitransection, and thus were not dependent on fibers of passage descending from the hypothalamus (Saper et al, 1976). We conclude: (a) excitation of neurons of the C1 adrenergic group and the Kolliker-Fuse nucleus elicits release of adrenal medullary catecholamines and excites sympathetic vasomotor nerves via direct projections from each nucleus to the intermediolateral cell column; (b) within each nucleus, representation of the vasomotor fibers and the adrenal medulla are admixed.

68.2 CNS CONTROL OF THE SYMPATHETIC NERVOUS SYSTEM IN THE DOG: SITE OF ACTION OF BOMBESIN AND A SOMATOSTATIN ANALOG. Marvin Brown, Laurel Fisher* and Victoria Webb*. (SPON: E. Battenberg). Peptide Biology Laboratory, The Salk Institute, La Jolla, CA 92037.

Bombesin, mammalian bombesin (gastrin releasing peptide) and somatostatins (SS), SS-28 and oligo-SS's,given intracisternally or intracerebroventricularly to rats influence epinephrine (E) secretion by the adrenal medulla. Each of these two classes of peptides are distributed in several brain regions thought to participate in regulation of the sympathetic nervous system. Studies to determine the CNS site(s) of action of bombesin and the SS-analog, desAA^{1,2,4,5,12,13}[D-Trp^{8}]-SS (ODT8-SS) to affect plasma levels of E, norepinephrine (NE), glucose (CHO) and glucagon (G) and mean arterial blood pressure (MAP) have been carried out. Adult 10-12 kg male Beagle dogs were used. Multiple, chronic brain ventricular and/or parenchymal cannulae and carotid artery and jugular vein catheters were placed one week prior to experiments. Studies were carried out in unanesthetized dogs placed in a Pavlov sling. Bombesin (5-50 nmoles) placed into the lateral ventricle did not influence plasma levels of E, NE, CHO, G or MAP. Bombesin (5 nmoles) placed into the anterior hypothalamus, dorsal medial hypothalamus (DMH), interpeduncular nucleus, interstitial nucleus of the midbrain and subcumiform nucleus resulted in significant elevations of plasma E, G and CHO and increased MAP. Plasma NE was elevated only in animals with extremely high E levels (> 2 ng/ml). Control injections into these same sites did not result in alteration of E, G, CHO, or MAP. Bombesin placed into the lateral hypothalamus and ventral lateral thalamus resulted in significant elevations of MAP without change of plasma E or CHO. Placement of bombesin into the lateral preoptic area, ventral medial hypothalamus, ventral lateral thalamus, amygdala, hippocampus, several cerebral cortical areas, putamen, bed nucleus of the stria terminalis, posterior hypothalamus, red nucleus, substantia nigra, parabrachial nucleus, and dorsal motor nucleus of the vagus did not result in significant changes of plasma levels of E, NE, or CHO or of MAP. Elevations of plasma E and MAP induced by bombesin placed into the DMH were abolished by simultaneous placement of ODT8-SS into the same site. In contrast, ODT8-SS placed in other brain regions, i.e. hippocampus, amygdala, and red nucleus did not prevent bombesin elevations of plasma E. Conclusions: Bombesin stimulation of specific sites within the dog brain results in elevation of plasma E, G, and CHO and increase in MAP. While overlap between sites at which bombesin elevates E and MAP exist, there are sites where increases in MAP occur without signigicant elevations of plasma E. Likewise, elevation of plasma E is not always associated with increased MAP. ODT8-SS acts in the DMH to prevent bombesin actions.

68.3 BRAIN CONTROL OF GLUCOREGULATION: INVOLVEMENT OF THE RENIN-ANGIOTENSIN SYSTEMS? Laurel Fisher* and Marvin Brown. Peptide Biology Laboratory, The Salk Institute, La Jolla, CA 92037.

Stressful stimuli (e.g. ether, surgery) and several chemical substances placed inside the brain (e.g. bombesin, β-endorphin, carbachol, 2-deoxyglucose) produce hyperglycemia. This effect results from activation of the adrenomedullary sympathetic nervous system. Enhanced sympathetic outflow could elevate circulating Angiotensin II (AII) levels via stimulation of renin secretion. AII is reported to act directly on the adrenals to release catecholamines and thus could in part mediate the response to stress or to centrally-acting hyperglycemic agents. The following studies explored the possible involvement of peripheral and central AII in the neural control of blood glucose levels.

Adult male Sprague-Dawley rats (200-250 g) anesthetized with ether were used in all experiments. Intravenous injection of AII (10 μg) produced hyperglycemia which was blocked by concomitant treatment with the AII receptor antagonist, [Sar^{1},OMeThr^{8}]-AII (20 μg, iv). Insulin levels also were elevated after AII administration, suggesting a hyperglycemic mechanism other than by stimulation of adrenal epinephrine secretion. Consistent with this notion is the fact that adrenalectomy or intracisternal injection of ODT8-SS (1 μg) failed to abolish AII-induced hyperglycemia. Peripheral administration of [Sar^{1},OMeThr^{8}]-AII (20 μg, iv) did not modify the hyperglycemic effects of surgical stress or of intracisternal injections of bombesin (100 ng), β-endorphin (50 μg), carbachol (10 μg), and 2-deoxyglucose (3 mg). Further, intracisternal bombesin (100 ng) produced hyperglycemia in nephrectomized animals and in rats pretreated with propranolol (0.4 and 4 mg, sc). Thus, circulating AII plays a minimal role, if any, in mediating the glucose response to stress or to centrally-acting hyperglycemic substances.

Intracisternal injection of AII (10 μg) also increased blood glucose levels and this effect was blocked by intracisternal injection of [Sar^{1},OMeThr^{8}]-AII (20 μg) or ODT8-SS (1 μg). In contrast, the hyperglycemia observed after intracisternal injections of bombesin, β-endorphin, carbachol, and 2-deoxyglucose (same doses as above) was not antagonized by intracisternal injection of [Sar^{1},OMeThr^{8}]-AII. Hence, these compounds' actions on blood glucose levels probably are not mediated via central AII receptors. However, centrally-administered AII may share the same final pathway as these other substances to produce hyperglycemia in that ODT8-SS is a potent antagonist in all cases. This idea is supported further by preliminary data suggesting that intracranial injection of AII stimulates adrenomedullary epinephrine secretion in unanesthetized rats.

68.4 REGULATION OF ADRENAL MEDULLA BY ALPHA RECEPTORS FOLLOWING CAROTID OCCLUSION IN NORMOTENSIVE RATS. M. Bouvier and J. de Champlain*. Centre de Recherche en Sciences Neurologiques and Dept of Physiology, Université de Montréal, Montréal, Québec, Canada.

The regulation of the sympatho-adrenal tone was studied during bilateral carotid occlusion (CO) in vagotomized normotensive rat. The sympathetic activity was estimated by measuring the plasma concentration of norepinephrine (NE) and epinephrine (E), using a differential radioenzymatic assay. Baseline values under chloralose anesthesia were: NE: 0.111 ± 0.009 ng/ml; E: 0.036 ± 0.010 ng/ml. Following a one-minute carotid occlusion, a rise in mean blood pressure (MBP) simultaneously with a significant ($p < 0.01$) increase in circulating E (+ 0.024 ± 0.006 ng/ml were observed without any change in circulating NE. To eliminate any change in the reuptake during that procedure, the effects of carotid occlusion were studied after an injection of desmethylimipramine (1 mg/kg i.v.). As expected, the baseline values of NE raised significantly to 0.187 ± 0.017 ng/ml but the E values remained unchanged. Following that treatment, it was not possible to detect any elevation of NE following the stimuli, whereas the elevation of E induced by the occlusion remained unchanged. Moreover, the pressor response to occlusion was found to be greatly reduced by acute bilateral adrenalectomy (from 22 ± 3 mmHg to 10 ± 2 mmHg) but the response tended to be potentiated by chemical sympathectomy (6-OHDA, 100 mg/kg i.v. 24 hrs previously) indicating the participation of adrenal CA liberation in this response. The pressure response was partially blocked by treatment with a mixed alpha-1, alpha-2 adrenergic blocker (phenoxybenzamine, 1 mg/kg i.v.) which tended to potentiate the increase of circulating E during that manoeuvre. Furthermore, the injection of a selective alpha-2 adrenergic blocker (Yohimbine, 0.5 mg/kg i.v.) did not change the pressor response but potentiated by more than three folds the increase in circulating E whereas this treatment did not change the catecholamine basal values. These results suggest that an acute stimulation of the carotid reflex arc induces a preferential increase of adrenal medullary activity. Moreover, these results suggest the participation of adrenergic receptors in the regulation of adrenal medulla. The potentiation of E liberation could be mediated by the blockade of alpha-2 adrenergic receptors located on adrenal chromaffin cells. These "auto-receptors" might regulate the liberation of adrenal medullary CA by mediating a negative feedback mechanism as suggested by several in vitro studies.

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68.5 GLUCOCORTICOID RECEPTORS IN RAT ADRENAL MEDULLA: IMMUNOCYTOCHEMICAL LOCALIZATION. P.A. Bernard*, G. Teitelman, D.J Reis and T.H. Joh (SPON: R.A. Ross). Laboratory of Neurobiology, Cornell University Medical College, New York, N.Y. 10021.

The cellular responses to glucocorticoids are mediated by binding to specific cytoplasmic proteins in target cells. The steroid-receptor complexes are then "activated". It has been proposed that these activated receptors are then translocated into the nucleus where they associate with chromatin leading to the production of specific mRNA's. To directly examine the distribution of the activated glucocorticoid receptor (GR) in cells of the adrenal medulla, a target of glucocorticoids, we have raised Abs to the GR and localized them by immunocytochemistry. GR, identified on the basis of triamcinolone acetonide binding and affinity for DNA, was purified from rat liver cytosol. Nonactivated GR, concentrated by protamine sulfate precipitation, was first separated from DNA-binding proteins by DNA-cellulose column chromatography. The receptor preparation was then heat-activated, applied to a second DNA-cellulose column, and washed with low ionic strength (50 mM NaCl) buffer. The DNA-binding form of GR was eluted with high ionic strength (450 mM NaCl) buffer. GR was identified on PAGE on the basis of retention of ^{3}H-steroid in calcium-containing gels. Gel slices containing GR were injected into rabbits. and antibody was harvested. In a radioimmunoassay for GR, antibody reacted with the activated (DNA-binding) but not the non-activated form of GR.

Rat adrenal medullae were immunocytochemically stained for GR by the PAP method in frozen tissue sections. Almost all adrenal medullary cells were specifically stained in two distinct subcellular patterns: In some clusters of cells staining was exclusively nuclear, in others, cytoplasmic. No cells stained in both cytoplasm and nucleus. Administration of low doses of dexamethasone (10^{-7}M) increased (40 min later) only the intensity of cytoplasmic staining, while high doses (10^{-5}M) dramatically increased the number of cells with nuclear staining and markedly reduced the number of cells showing cytoplasmic staining. The present study is the first demonstration of cellular localization of GR and provides direct evidence that the adrenal medulla contains these receptors. The presence of activated GR in the cytoplasm of some cells suggests nuclear translocation is regulated by factors other than high affinity binding and subsequent activation of the receptor. The presence of GR in either nuclei or cytoplasm in untreated rats suggests that in life the GR is in a dynamic state.

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68.6 TRIFLUOPYRAZINE-INDUCED INHIBITION OF CATECHOLAMINE SECRETION BY ISOLATED ADRENAL CHROMAFFIN CELLS. Jack C. Brooks and Suzanne Treml*. Dept. of Basic Sciences, Marquette Univ., Sch. Dent., Milwaukee, WI 53233.

Excitation-secretion coupling in chromaffin cells is known to be regulated by the intracellular calcium concentration and might, therefore, involve the calcium dependent regulator calmodulin. It has been demonstrated that calmodulin can be inactivated in vitro by the drug trifluopyrazine (TFP). We have examined the effect of TFP on catecholamine secretion by isolated chromaffin cells as part of studies designed to determine if calmodulin plays a role in secretion. A dose-response curve was constructed for cells incubated for 5 min. with various concentrations of TFP followed by stimulation with acetylcholine. The drug had no significant effect on secretion at a concentration of 0.01μM, while higher concentrations produced a biphasic effect on secretion. Secretion was reduced to the level of unstimulated control cell suspensions at 1.0μM TFP concentration. Higher TFP concentrations (10 to 100μM) produced an apparent increase in catecholamine release. At the 100μM TFP concentration catecholamine release was five-fold greater than that of cells stimulated in the absence of the drug. However, since dopamine also appeared in the medium, the enhanced release might be due to cell damage. The percentage of norepinephrine, out of the total catecholamine released, was also different for this drug concentration. TFP could not be removed from the cells by repeated washing with drug-free medium. It appears, therefore, that the effect of TFP on secretion is time dependent and either permanent, or the drug can not be removed from the treated cells. Preliminary experiments also indicate that the inhibition of secretion by TFP can not be reversed by increasing the extracellular calcium concentration to 5mM calcium, again indicating a lasting effect of the drug on the secretory system.

As is the case with other secretory cells, these data imply a role for calmodulin in catecholamine secretion by adrenal chromaffin cells.

68.7 RELATIONSHIP BETWEEN Ca^{++} UPTAKE AND CATECHOLAMINE SECRETION IN CULTURES OF ADRENAL MEDULLARY CHROMAFFIN CELLS. R.W. Holz, R.A. Senter*, R.A. Frye*, and T. Blok*. Dept. of Pharmacology, Univ. of Michigan Medical School 48109.

Carbachol or elevated K^+ stimulated $^{45}Ca^{++}$ uptake into chromaffin cells 2-4 fold. The uptake was stimulated by cholinergic drugs with nicotinic activity but not by those with only muscarinic activity. Ca^{++} uptake and catecholamine secretion-induced by the mixed nicotinic-muscarinic agonist carbachol were inhibited by the nicotinic antagonist mecamylamine but not by the muscarinic antagonist atropine. Significant Ca^{++} uptake occurred within 15 sec. of stimulation by carbachol or elevated K^+ at a time before catecholamine secretion was readily detected. At later times the kinetics of secretion induced by carbachol or elevated K^+ paralleled that of Ca^{++} uptake. There was a close correlation between the Ca^{++} uptake from the medium and catecholamine secretion in various concentrations of Ca^{++} which stimulated both processes and Mg^{++} which inhibited both processes. Thus, there is an excellent pharmacological and kinetic correlation between Ca^{++} uptake and catecholamine secretion. This correlation provides strong support for the notion that Ca^{++} entry and a presumed increase in cytosolic Ca^{++} concentration initiates and maintains secretion. Furthermore, these studies also suggest that a number of different stimuli can control the Ca^{++} permeability of these cells.

68.8 BOVINE CHROMAFFIN CELLS IN CULTURE: CHANGES IN PHENOTYPE INDUCED BY ORGAN EXTRACTS, ELEVATED POTASSIUM AND SERUM WITHDRAWAL, BUT NOT BY NERVE GROWTH FACTOR. K. Unsicker and H.-D. Hofmann*. Dept. of Anatomy and Cell Biology, Philipps University, D-3550 Marburg, FRG.

Rat and human adrenal chromaffin cells have the capacity to express a neuronal phenotype by forming axons and growth cones when cultured in the presence of NGF (Tischler et al., Lab. Invest. 43:339, 1980; Unsicker et al., Proc. Natl. Acad. Sci. USA 75:3498, 1978). Recent studies have provided contradictory results regarding effects of NGF on cultured bovine chromaffin cells. The present investigation was undertaken to define the conditions favouring extension of processes of bovine chromaffin cells. The cells were isolated and grown as previously described (Unsicker et al., Neurosci. 5: 1445, 1980).

In the presence of non-chromaffin (fibroblast-like) cells a small proportion of chromaffin cells (approx. 15%) formed processes within 2 weeks that were longer than the 3-fold cell diameter. Elimination of non-chromaffin cells by rigorous differential plating, γ-irradiation or treatment with FdU inhibited the formation of processes. Addition of a cell-free extract from non-chromaffin cells, but not conditioned medium, re-established process formation. Treatment of cultures with extracts of bovine seminal gland, which contains a NGF-like activity (Hofmann and Unsicker, Neurosci. Letters Suppl. 6, 1981), caused extension of processes from about 30% of chromaffin cells within 2 days. Extracts from bovine liver, kidney and brain, mouse submaxillary gland NGF (50ng to 5μg/ml) and the purified NGF-like fraction from bovine seminal gland had no effect.

Process formation was extensively stimulated by elevating K^+ in the medium (11 to 55 mM) and to a lesser extent by serum withdrawal. However, processes rarely resembled the long, varicose axons formed by rat and human chromaffin cells in vitro.

These results suggest that process formation of bovine chromaffin cells, in contrast to rat and human chromaffin cells, is a rather unspecific phenomenon. These processes resemble cytoplasmic extensions rather than neurites. Consequently, bovine chromaffin cells may be considered not to be the most suitable model to study early events of axon formation and the mechanisms underlying neuronal plasticity of chromaffin cells.

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68.9 OPIOID PEPTIDES AND NARCOTIC ANALGESICS MODULATE THE BASAL RELEASE OF CATECHOLAMINES FROM ADRENAL CHROMAFFIN CELLS IN CULTURE.

Deanne M. Dean* and Bruce G. Livett. Division of Neurology, The Montreal General Hospital and McGill University, Montreal, Canada.

We have recently examined the effects of various opioid peptides (enkephalins, enkephalin analogues, β-endorphin, dynorphin) and narcotic analgesics (morphine, levorphanol) on both the basal and nicotine-mediated release of catecholamines (i.e. ^{3}H-NE) from bovine adrenal chromaffin cells in culture. Although these agents inhibit the release of ^{3}H-NE induced by nicotine ($5x10^{-6}$M), the inhibition is non-specific (1).

In contrast, these agents appear to modulate the basal release of catecholamines from adrenal chromaffin cells in culture. At concentrations between $1x10^{-6}$M to $1x10^{-3}$M, these agents enhanced the basal release of ^{3}H-NE. The enhancement of basal release by morphine ($5x10^{-4}$M) was dependent on the presence of Ca^{++} in the medium. The enhancement was stereospecific since levorphanol, but not dextrorphan was active. The enhancement produced by morphine ($1x10^{-4}$M, $5x10^{-4}$M) and levorphanol ($1x10^{-3}$M) was reversed by naloxone and naltrexone. The ability of naloxone to reverse the enhancement of basal release of ^{3}H-NE by dynorphin and β-endorphin was also examined.

Lower concentrations ($1x10^{-8}$M, $1x10^{-7}$M) or many of these agents (e.g. the enkephalins, dynorphin, morphine) inhibited the basal release of ^{3}H-NE from adrenal chromaffin cells in culture. Although the inhibition produced by morphine ($1x10^{-8}$M) was reversed by naloxone ($1x10^{-7}$M, $1x10^{-6}$M), the effect was not stereospecific since dextrorphan was active while levorphanol was not.

These results support earlier studies demonstrating a direct stimulatory effect of morphine on the adrenal gland (2,3) and suggest that endogenous opioid peptides in the adrenal medulla (4) may modulate basal secretion of catecholamines.

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69.1 UNMYELINATED SPLANCHNIC FIBERS OF PREGANGLIONIC AND POSTGANGLIONIC TYPES - AN ELECTRON MICROSCOPIC STUDY. Grace C.H. Yang*, Dwayne S. Yamasaki*, George M. Krauthamer and David C. Kuo. Department of Anatomy, College of Medicine and Dentistry of New Jersey, Rutgers Medical School, Piscataway, New Jersey 08854.

It is commonly believed that most autonomic preganglionic fibers are finely myelinated. In the present investigation, electron microscopic analysis of the unmyelinated fiber component of the left major splanchnic nerve (MSPLN) of the cat indicates that the majority of these fibers are, in fact, preganglionic. The ratio of unmyelinated to myelinated fibers of preganglionic type is approximately two to one. In the normal MSPLN there are 10,000-15,000 unmyelinated fibers. Following a left ventral root rhizotomy (T3-L1)**, unmyelinated fiber counts in the left MSPLN dropped precipitously (see table). A second significant finding in this study is that a large number of unmyelinated fibers in the MSPLN are postganglionic. This is contrary to the generally accepted view that this nerve is primarily sensory and preganglionic. Many undegenerated, unmyelinated fibers were identified in the MSPLN after left spinal nerve cuts (T3-L1) immediately lateral to the dorsal root ganglion (see table). A few neuronal cell bodies were also identified in one MSPLN specimen. Conceivably, some postganglionic fibers may originate from these aberrant cells. In different experiments, the cells of origin of splanchnic postganglionic fibers were studied. Retrograde transport of HRP from the central cut end of the MSPLN labeled a substantial number of paravertebral postganglionic sympathetic neurons, largely located in the region of the sympathetic chain from which the MSPLN has its origin.

Fiber Counts of Normal and Partially Degenerated MSPLN
(UM - unmyelinated; MY - myelinated)

Cat	Normal	Ventral Rhizotomy (T3-L1)	Spinal Nerve Cut (T3-L1)
1	15359(UM) 3801(MY)	-	-
2	9620(UM) 2655(MY)	-	-
3	-	4697(UM) 315(MY)	-
4	-	2609(UM) 284(MY)	-
5	-	-	1643(UM) 13(MY)
6	-	-	7619(UM) 38(MY)

**The spinal levels which contained preganglionic neurons of the MSPLN have been previously defined by HRP method (Kuo et al., Neurosci. Letts., 17(1980) 11-16). (Supported by NS 10922.)

69.2 FINE STRUCTURE OF SUBSTANCE P-LIKE IMMUNOREACTIVE NERVE TERMINALS IN THE CELIAC GANGLION OF GUINEA PIGS. H.Kondo* and R.Yui*. (SPON:T.Chiba). Niigata Univ. Sch. of Med., Niigata, JAPAN

The fine structural localization of substance P-immunoreactive nerve fibers and their synaptic relations to other neuronal elements in the celiac ganglion of guinea pigs were studied by means of the peroxidase-antiperoxidase(PAP) method in electron microscopy. The immunoreactive nerve fibers contained abundant small clear spherical vesicles, 45 nm in diameter, and a few large granular vesicles with a halo, 90 nm in mean diameter. The immunoreactive materials were localized around cytoplasmic components including vesicles and mitochondria and on the inside of the plasma membrane. The immunoreactive nerve fibers directly apposed to unlabelled dendrites of postganglionic principal neurons and also presumably preganglionic axons. Some of the unlabelled axons contained abundant small clear vesicles, 45 nm in means diameter, while others contained abundant large granular vesicles, 90 nm in mean diameter. At sites of apposition, the apposed plasma membranes parallel to each other were separated by a space of about 20 nm. The accumulation of vesicles was observed in the immunoreactive fibers and a cytoplasmic density was slightly observed on the apposed plasma membrane of unlabelled dendrites, but no distinct electron density together with the accumulation of vesicles was seen underneath the apposed membrane of unlabelled axons. No cellular components showing features of SIF(small intensely fluorescent) cells were found in relation to the immunoreactive nerve fibers. The present study shows that substance P-fibers form axodendritic synapses on the principal neuron, which could be the synaptic site where substance P is related to induce the non-cholinergic slow EPSP. This study also suggests the presence of the presynaptic interaction between substance P-fibers and some preganglionic axons in the celiac ganglion.

69.3 INVESTIGATION OF CERTAIN INTRINSIC PROPERTIES OF FELINE CELIAC GANGLION (CG) NEURONS. D. L. Decktor* and W. A. Weems, Dept. Physiol. and Cell Biol., Univ. Texas Med. Sch. at Houston, Houston, TX. 77025

Neural commands that regulate the function of the abdominal viscera are synthesized within the prevertebral ganglia of the solar plexus from both peripheral and central preganglionic inputs. Intrinsic properties of each ganglionic neuron are prime determinents of its ability to integrate these preganglionic inputs and synthesize an output command. The purpose of this study was to investigate some of the intrinsic properties of CG neurons. Solar plexus ganglia were studied in vitro. Transmembrane potentials of neurons were monitored by intrasomatic electrodes. The input resistance and membrane time constant were measured for each neuron. From these values the total input capacitance was calculated. The amplitude and duration of the afterspike hyperpolarization (ASH) was measured and the rhythmic firing mode of the neuron assessed. All of the neurons tested fired in a rhythmic firing mode. Fifty-nine percent were phasic and 41% were tonic. Input resistance ranged from 5 to 58MΩ. This large range of values may be indicative of differences in either the size or the specific membrane resistance of the individual neurons. The mean value for phasic neurons (21MΩ) was significantly less than that calculated for tonic neurons (36MΩ). Time constant ranged from 9 to 21 msec with a mean of 13.8 msec. Input capacitance ranged from 286 to 2750 picofarads. If it is assumed that specific membrane capacitance is relatively constant, then the large range of values for input capacitance would reflect differences in neuron size. ASH durations ranged from 21 to 267 msec and were distributed bimodally with peaks at 60 and 140 msec. It was concluded from these studies that neurons within the celiac ganglion cannot be represented as a single population with similar intrinsic properties. The wide range and non-Gaussian distribution of values reported may reflect functional differences in the physiological role of individual neurons. (Supported by N.I.H. grant HL21351)

69.4 SLOW RHYTHMIC MEMBRANE POTENTIAL CHANGE IN HAMSTER PARASYMPATHETIC NEURONS. T. Suzuki* and K. Kusano. Neurophysiology Lab. Dept. of Biol., Illinois Inst. Technology, Chicago, IL.60616.

Previously we have reported that postganglionic neuron excitability is modulated by hyperpolarizing potentials associated with action potentials and synaptic potentials, and by rhythmic hyperpolarizing potentials (RHP's) activated either spontaneously or by caffeine application. We have concluded that these hyperpolarizing potentials are all due to Ca-activated K-conductance increase. Recently, we have found another cellular activity, namely the slow rhythmic membrane potential change (SRMPC),which is much slower in time scale than the "caffeine-induced" RHP's. The SRMPC is roughly sinusoidal and occurs spontaneously in some neurons. The average membrane potential change from peak to bottom is 9.7±5.7 mV (mean±S.D., range: 4-32 mV) with an average interval of 5 min (range: 2.5-11.5 min). The most negative level of the SRMPC observed is -90 mV in some neurons, but the average is -80±5.6 mV (n=41). The membrane resistance is higher at the most positive E_m level than at the most negative E_m level. The SRMPC can be triggered by applying preganglionic repetitive stimulation. Postganglionic repetitive stimulation, direct stimulation of the neuron by injecting depolarizing current, and bath-applied ACh or Carb are also effective. When the median level of the SRMPC is close to -90 mV the amplitude of SRMPC becomes very small and is hardly seen. The median E_m level of the largest amplitude of the SRMPC is between -60 and -70 mV, and for E_m's lower than this the SRMPC amplitude decreases. The frequency of the SRMPC is not clearly affected by the temporal displacement of the E_m between -50 and -85 mV. The SRMPC is abolished reversibly by 2.5 mM 4-aminopyridine, an agent which reduces membrane K-conductance. In Ca-free saline the amplitude of the SRMPC decreases, and conversely, in the presence of up to about 5 mM Ca the amplitude of the SRMPC increases. In higher than 5 mM Ca the E_m has been found to be more stable near the E_K level. Caffeine of 2-5 mM induces characteristic RHP's on top of SRMPC. When caffeine induces E_m hyperpolarization, the SRMPC decreases gradually. In some neurons, both amplitude and frequency of SRMPC are attenuated by caffeine. Ruthenium red of 10^{-4} M reduces the SRMPC slightly after treatment for 1 hr. Intracellular injection of Ca induces a large transient hyperpolarization when the E_m of the preinjection state had been between -45 and -60 mV. When the E_m had been close to E_K, such transient hyperpolarization is not prominent. Injection of Ca during SRMPC reduces its amplitude and finally the E_m becomes stable at the lower level of the SRMPC. It is concluded that the SRMPC is generated endogenously in these neurons by slow intracellular Ca activity changes which modulate K-conductance. (Supported by NIH Grant, NS12275).

69.5 AN ANALYSIS OF THE INHIBITORY EFFECTS OF LEUCINE ENKEPHALIN ON TRANSMISSION IN VESICAL PARASYMPATHETIC GANGLIA OF THE CAT. August M. Booth*, Nancy Ostrowski*, Sean McLinden*, Irene Lowe* and William C. de Groat. Dept. of Pharmacology, School of Medicine, Univ. of Pittsburgh, Pgh., PA 15261.

Previous studies in situ have shown that transmission in parasympathetic ganglia on the surface of the urinary bladder (UB) is depressed by intra-arterial injections of leucine enkephalin (ENK). In the present investigation the mechanisms underlying this depression were examined with intracellular recording techniques in vitro. Ganglia were removed from the serosal surface of the UB of cats and pinned out in a chamber perfused with Krebs solution at 35-37°C. Activity of ganglion cells was recorded intracellularly with micropipettes. Preganglionic fibers were stimulated with suction or hook electrodes. Stable resting potentials of more than 50mV and action potentials exceeding 70mV were obtained from the majority of cells.

When cell firing was elicited with preganglionic stimulation the application of ENK in concentrations between 12 and 50 μM depressed cell firing by 30-100%. The inhibition was clearly evident within 2-3 min following drug application and lasted 10-20 min following the offset of the drug. More than 80% of those cells tested showed ENK-inhibition. Increasing drug concentrations to 160 μM did not result in demonstrable inhibition in cells that showed no inhibition to 12-50 μM concentrations of ENK.

When cell firing was blocked by hyperpolarizing pulses or when stimulus intensity was reduced to produce epsps that were below the threshold for cell firing ENK reduced the average amplitude of epsps in responsive cells 30-60%, increased the number of epsp "failures" 75-400% and reduced the frequency of spontaneous miniature potentials 40-60%. ENK did not reduce the amplitude of miniature potentials. ENK did not change membrane conductance, resting potential or threshold for cell firing.

To demonstrate the presence of ENK in UB ganglia the FITC indirect immunoflorescence technique was used. ENK immunoreactivity was seen between and surrounding ganglion cell soma. This ENK was presumably contained in terminals of sacral preganglionic neurons which provide the cholinergic, excitatory input to these ganglia and which were shown to contain ENK by Glazer and Basbaum (1980).

In summary, ENK inhibits cholinergic transmission in UB ganglia by a presynaptic mechanism. Since ENK terminals are present in these ganglia, it seems possible that ENK coexists with acetylcholine in the same terminal endings and may modulate ganglionic transmission.

69.6 ORIGIN OF AXONS IN THE BLADDER NERVES. Claire E. Hulsebosch and Richard E. Coggeshall, Depts. of Anatomy and of Physiology and Biophysics and the Marine Biomedical Institute, The University of Texas Medical Branch, Galveston TX 77550.

There are three main nerves which innervate the bladder: the pelvic nerve, the hypogastric nerve and the pudendal nerve. Previous studies using selective surgery determined the number and origin of myelinated (M) axons in these nerves but did not consider the unmyelinated axons (UN) which are more numerous than the myelinated axons. The present ultrastructural study uses selective surgery to determine the number and origin of the unmyelinated as well as the myelinated axons in the bladder nerves of the rat. These data are presented in the following table in which each datum, displayed as a mean value of several rats, represents the number of fibers of ventral root origin (VR), dorsal root origin (DR) or sympathetic origin (SYM):

ORIGIN	PUDENDAL		HYPOGASTRIC		PELVIC	
	UN	MY	UN	MY	UN	MY
VR+SYM	742	53	1353	206	2613	158
DR+SYM	3000	1354	1124	148	1556	444
SYM	165	5	836	141	546	70
VR+DR	2884	1595	644	45	3814	756

In summary, these data suggest the following: 1) 85% of the axons in the pudendal nerve are sensory, 10% are somatic motor and 5% are sympathetic, 2) 55% of the axons in the hypogastric nerve are sympathetic, 30% are somatic motor and 15% are sensory, 3) 50% of the axons in the pelvic nerve are somatic motor, 35% are sensory and 15% are sympathetic, 4) the unmyelinated axons make a substantial contribution in each category. Therefore, it is desirable to characterize the electrophysiological response properties of the unmyelinated fiber population in order to understand the innervation of the bladder. Supported by NIH grants NS 10161, NS 06246, NS 07377 and NS 11255.

69.7 ULTRASTRUCTURE AND SYNAPTIC COVERAGE OF PHYSIOLOGICALLY IDENTIFIED NEURONS OF THE GUINEA PIG SMALL INTESTINE: DOUBLE LABELING BY INTRACELLULAR INJECTION OF HORSERADISH PEROXIDASE AND EM RADIOAUTOGRAPHY WITH ^{3}H-SEROTONIN. S.M. Erde, D.M. Sherman*, and M.D. Gershon, Department of Anatomy, Columbia Univ. P.&S., New York, NY 10032.

Studies of the morphology of neurons of the myenteric plexus of the guinea-pig small intestine, physiologically identified and marked by the intracellular injection of Lucifer Yellow, have previously been reported (Neuroscience Vol. 6:101.1, 1980). We have extended these observations by injecting horseradish peroxidase (HRP; Sigma type VI) into physiologically identified neurons to permit examination of the cells, their processes, and their synaptic inputs by electron microscopy. Electrical activity of myenteric neurons was recorded using intracellular glass micropipettes, filled with HRP [0.2M solution of potassium acetate, 0.1M Tris buffer (pH 8.6), and 5% HRP]. The micropipettes were beveled to a resistance of 150 to 200 megohms. Neurons were categorized as S cells if they fired repetitively on injection of depolarizing current, and as AH cells if they failed to fire repetitively and an after-hyperpolarization, associated with a decreased membrane conductance, followed an action potential. HRP was injected into cells iontophoretically by passing depolarizing rectangular current pulses. Following physiological characterization and injection of HRP, preparations were incubated with tritiated serotonin (0.5uM) for 15-30 minutes in the presence of a 100-fold excess (50uM) of norepinephrine in order to specifically label serotonergic axon terminals. Tissue was fixed with 2.5% glutaraldehyde. HRP was demonstrated with Hanker-Yates reagent and the ^{3}H-serotonin by light and electron microscopic radioautography. Injected ganglia were cut out and HRP-filled cells were sectioned throughout their extent. Morphology was well preserved for electron microscopy despite the long incubation in vitro. Simultaneous identification of HRP-filled neurons and ^{3}H-serotonin proved to be feasible. This double labeling technique, therefore, permits identification of neurons receiving a serotonergic input and the regional localization of that input on those cells. Supported by NIH Grant #NS12969.

69.8 SYMPATHETIC AUTONOMIC NEUROPATHY IN THE HEART IN EXPERIMENTAL DIABETES. S. Y. Felten*, R. G. Peterson, P. A. Shea, and D. L. Felten. Departments of Anatomy and Psychiatry, Indiana University School of Medicine, 1100 W. Michigan, Indianapolis, IN 46223

Diabetes was induced with streptozotocin in male Sprague-Dawley rats at 1, 2, and 4 months of age. Within each of these groups, the animals were allowed to survive with diabetes for periods of 1, 2, and 4 months. Blood glucose levels for diabetes ranged from 550-650 mg% (controls 85-110 mg%) with HbA_1C levels from 4.5-7.5 %Hb (controls 1.9-2.5 %Hb) indicating the presence of diabetes. Motor conduction velocities measured in the sciatic nerve were decreased in all groups of diabetics when compared to controls.

Norepinephrine (NE) levels were measured in the right atrium and the ventricles of the heart using reverse phase, ion-paired HPLC with electrochemical detection. Patterns of sympathetic innervation were studied using glyoxylic acid histofluorescence.

In rats made diabetic at one month of age, atrial and ventricular NE levels were increased over controls at both one and two months of diabetes. At four months of diabetes, both atrial and ventricular NE levels were decreased. In rats made diabetic at two months of age, atrial and ventricular NE levels were increased at one month of diabetes and had returned to or slightly below control levels at two and four months of diabetes. In rats made diabetic at four months of age, atrial NE levels were normal at one month of diabetes, decreased at two months of diabetes, and returned to normal at four months of diabetes. In contrast, ventricular NE levels remained elevated at one, two and four months of diabetes.

Histofluorescence studies of the patterns of distribution of noradrenergic fibers in the heart were done on rats with diabetes of one month duration and have shown overall increases in the density of noradrenergic innervation with no change in the actual patterns of distribution. These findings are in agreement with the HPLC measurements showing an early increase of NE tissue levels in the diabetic rat heart.

This preliminary evidence demonstrates a two phase change in noradrenergic innervation of the heart in diabetes, agreeing with previous findings from this laboratory in diabetic rat penile corpora. The present findings suggest that during the early phases of diabetes the noradrenergic nerves are still intact and may be susceptible to pharmacologic manipulations. The later fall of NE back to or below control levels may indicate actual neuronal damage, suggesting that early intervention may be necessary to protect these nerves from degeneration.

This work was funded by the Juvenile Diabetes Foundation and the American Diabetes Association - Indiana Affiliate.

69.9 ABNORMAL BIOPHYSICAL PROPERTIES OF AUTONOMIC NEURONS IN SPONTANEOUSLY HYPERTENSIVE RATS. P. Yarowsky, D. Weinreich, and R. Snow. Dept. Pharmacol. & Exp. Therap., Univ. of MD, Sch. Med., Baltimore, MD, 21201.

Previous physiological and pharmacological investigations have shown that there is increased sympathetic nerve activity in Spontaneously Hypertensive rats (SHR) (Judy et al., Circ. Res. 38 (supp. II):21, 1976; Juskevich et al., Europ. J. Pharmacol. 51:429, 1978). It is not known whether the altered sympathetic nerve activity is due to changes in the biophysical properties of the postganglionic sympathetic neurons. We have recorded from the in vitro superior cervical ganglion (SCG) from the adult (7-10 wks of age) SHR with standard intracellular techniques. A remarkable feature of these neurons was that they showed repetitive action potentials (n = 36/40) during a depolarizing pulse or following anodal break; a behavior rarely observed in normotensive Sprague-Dawley or Wistar rats. In addition, some cells displayed spontaneous action potentials for prolonged periods of time. Furthermore, addition to Locke's solution of tetrodotoxin (TTX;1 μM) and TEA (5mM) did not abolish the repetitive action potential discharge. Multiple action potentials could be blocked, however, by the addition of any of the following Ca^{++} antagonists to Locke's solution: Co^{++} (5mM), Cd^{++} (3mM), or Ni^{++} (1mM). The remaining single action potential could be eliminated by adding TTX to the Locke's solution containing the Ca^{++} antagonists. These results indicate that the first spike is mediated by Na^{+} current, while the subsequent multiple action potentials are produced by regenerative Ca^{++} currents.

Examination of the membrane properties of SCG neurons in the SHR consistently revealed a lower resting membrane potential (-50mV vs. -60mV), yet they displayed normal input conductance, spike amplitude, spike threshold, and after hyperpolarization (C & K currents). The contribution of M-currents (Brown and Adams, Nature 283:673, 1980) to the electrogenesis of the observed repetitive spiking in SHR neurons was tested by using Ba^{++} (4 mM), a blocker of M-currents (Constanti et al., Brain Res. 206:244, 1981). Ba^{++} treatment of SHR ganglion cells yielded a slight decrease in the resting slope conductance (-15%) at or around resting potential and accentuated repetitive spiking. The % change in slope conductance produced by Ba^{++} was much smaller than is expected, based upon the values reported by Brown and Constanti (Br. J. Pharmac. 70:593, 1980). These data suggest that the postganglionic neurons of the SHR possess fewer or less efficacious M-channels than are present in cells from normotensive animals. (Supported by The Homer & Martha Gudelsky Foundation, Inc.)

69.10 PARASYMPATHETIC PREGANGLIONIC NEURONS AND PRIMARY AFFERENT PROJECTIONS IN THE SPINAL CORD OF THE RHESUS MONKEY. I. Nadelhaft, J. Roppolo, W. de Groat, C. Morgan, and S. Ames*. VA Medical Cent. & Depts. of Neurosurgery and Pharmacology, Univ. of Pgh. Medical School, Pittsburgh, Pa.

Horseradish peroxidase (HRP) applied to the central stump of the transected pelvic nerve of anesthetized Rhesus monkeys labeled afferent and efferent neurons innervating the pelvic viscera. Preganglionic neurons forming the sacral parasympathetic nucleus (SPN) were located in a narrow band 150-200 μm wide on the ipsilateral border of the intermediate gray matter. The SPN contained an average of 800 neurons (range: 330-1300) and characteristically extended over two or three sacral segments with the majority of cells in S2. The average length of the SPN was 13.2 mm (range: 9.1-18.2). The SPN was composed of medium sized elongated somata (17.5x32.0 μm) with their major axes in the transverse plane and oriented parallel to the lateral border of the gray matter. Dendrites extended deep into the lateral funiculus, horizontally into the medial posterior commissure, and dorsolaterally along the lateral edge of the dorsal horn. Preganglionic axons followed the lateral border of the ventral horn to the ventral rootlets.

Pelvic nerve afferent axons carried HRP to the dorsal root ganglion (DRG) neurons and by transganglionic transport to the spinal cord. The average number of labelled DRG cells was 3066 (range: 2224-4363) with the largest fraction (80%) in S2. The segmental frequency of DRG cells correlated with the frequency of SPN cells in corresponding spinal cord segments. Within the spinal cord labelled primary afferent fibers were prominent in the sacral segments and were also observed in decreasing numbers within two to three segments beyond the rostral and caudal limits of the SPN. In transverse sections, primary afferents were found throughout Lissauer's tract (LT) and in the lateral half of the dorsal columns. A prominent collateral fiber bundle from LT was located along the lateral edge of the dorsal horn. This bundle extended into the SPN and a few collaterals continued medially into the posterior commissure and the contralateral side of the cord. A much less prominent collateral fiber group from LT passed medially over the apex of the dorsal horn and descended along the midline into the posterior commissure. Horizontal sections revealed that these two collateral pathways were organized into periodically spaced bundles of fibers about 250 μm apart. HRP reaction product in terminal fields related to these afferent collateral projections were found in the lateral marginal zone, along the dorsal medial septum, and bilaterally in the medial region of the posterior commissure. In summary, the most striking observation of this investigation is prominent projection of visceral afferent fibers directly into the sacral parasympathetic nucleus.

69.11 GUANETHIDINE-INDUCED DESTRUCTION OF PERIPHERAL SYMPATHETIC NEURONS: AN AUTOIMMUNE PHENOMENON. P. T. Manning, C. W. Powers* and E. M. Johnson, Jr. Wash. Univ. School of Medicine, Dept. of Pharmacology, St. Louis, MO 63110.

Guanethidine (GUAN), a guanidinium adrenergic neuron blocking agent which, when chronically administered at high doses to newborn or adult rats (other species are not affected), causes destruction of peripheral sympathetic neurons. Neuronal destruction is preceded by small cell infiltration of the sympathetic ganglia, and is suggestive of an immunologically-mediated mechanism. We have demonstrated that administration of immunosuppressive agents prevents the sympathectomy produced by GUAN administration. Adoptive transfer experiments using spleen and bone marrow cells obtained from naive donors transferred to lethally irradiated recipients were thus carried out to demonstrate that GUAN-induced destruction occurs by an immunologically-mediated mechanism. To determine the dose of irradiation necessary to protect against neuronal cell death induced by GUAN, 3-week-old Lewis rats were treated with either 600, 750, or 900 rads of γ-irradiation six hours prior to the initiation of GUAN treatment. Rats received 50 mg/kg of GUAN sulfate for 5 days, were killed 2 days later, and the superior cervical ganglia (SCGs) dissected for assay of tyrosine hydroxylase (TOH) activity and for histological evaluation. Irradiation protected against GUAN-induced destruction in a dose-related manner with virtually complete protection afforded by doses of 900 rads. Similar results were obtained using neonatal Sprague-Dawley rats. Adoptive transfer recipients (3-week-old Lewis rats) were irradiated with 850 rads immediately prior to cell transfer. Adoptive transfer experiments involved 4 groups of animals: Group A (GUAN only); Group B (irradiation only), Group C (irradiation + GUAN); and Group D (irradiation + $5x10^6$ spleen and bone marrow cells + GUAN). Spleen and bone marrow cells were obtained from naive adult Lewis rats of the same sex. Sympathetic ganglia from animals in Groups B and C were normal, whereas ganglia from animals in group A showed the usual massive small cell infiltration and cell destruction. Animals in Group D, in contrast to Group C, showed clear small cell infiltration of the ganglia and neuronal destruction. These results indicate that small cell infiltration and neuronal destruction due to GUAN treatment require immune competency. The specific antigen that induces this autoimmune response as well as the specific immune mechanism responsible for the neuronal destruction remain to be elucidated. Supported by NIH grant HL-20604 and NIH Cardiovascular Training Grant 5-T32-HL-07275. EMJ is an established investigator of the American Heart Association.

69.12 LEVELS OF PLASMA NOREPINEPHRINE AND EPINEPHRINE FOLLOWING STANDARD HYPOTHALAMIC STIMULATION IN THE AWAKE CAT. S.L. Stoddard-Apter, A. Siegel, and B.E. Levin. Dept. of Neurosciences, N.J. Medical School, Newark, N.J. 07103, and Dept. of Neurology, V.A. Medical Center, East Orange, N.J. 07019.

This study was designed to map areas in the hypothalamus which were capable of activating the peripheral adrenergic fiber (plasma norepinephrine) and adrenal medullary (plasma epinephrine) components of the sympathetic nervous system. Hypothalamic sites were stimulated in 13 adult, ovariectomized, female cats, while the unanesthetized animal was restrained in a headholder; the standard stimulus was a 5-sec train of biphasic, square-wave pulses (0.4 mA; 60 Hz). Blood samples were withdrawn through an indwelling venous cannula prior to stimulation, and at 0.25, 0.5, 1, 2, 3, 4, and 5 min following stimulus onset. Plasma levels of norepinephrine (NE) and epinephrine (Epi) were determined by radioenzymatic assay. Cardiovascular (CV) parameters of heart rate (HR) and mean arterial blood pressure (MAP) were monitored continuously through an indwelling arterial cannula. The pooled data from 120 diencephalic sites were mapped from AP: 14.5-7.5 and L: 0.0-4.0. Since no correlations were found between baseline levels of plasma catecholamines (CAs) and the absolute levels reached following hypothalamic stimulation, sympathoadrenal (SA) responses were classified on the basis of the absolute level of NE or Epi within the first 75 sec after stimulation. Maximal responses were defined as levels of NE > 7.0 ng/ml and Epi > 10.0 ng/ml. The results of this study were as follows: (1) Sites which maximally activated the SA system were located throughout the rostro-caudal extent of the hypothalamus, with the most effective areas in the dorsomedial preoptic area and the medial tuberal hypothalamus. (2) Several sites were found which differentially increased one component of the SA system (NE or Epi). (3) Significant increases in plasma CAs were elicited from hypothalamic sites, primarily in the preoptic area, without coincident increases in HR and MAP. (4) Conversely, stimulation of numerous lateral hypothalamic sites elicited maximal CV activation (increases in MAP > 50 mm Hg and HR > 65 beats/min) without concomitant increases in plasma CAs. These results suggest that the hypothalamic systems which control or modulate the sympathetic nervous system exhibit a degree of divergence in the control of the CV, sympathetic nervous, and adrenal medullary components of the peripheral nervous system.

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70.1 TANYCYTES IN ESTROGEN-TREATED LONG-TERM OVARIECTOMIZED EWES DISPLAY ULTRASTRUCTURAL EVIDENCE SUGGESTING HEIGHTENED CELLULAR ACTIVITY. Penelope W. Coates and Steven L. Davis*. Dept. Anatomy, Texas Tech Univ. Health Sciences Center, Lubbock, TX 79430 and Dept. Animal Science, Univ. Idaho, Moscow, ID 83843.

Scanning electron microscopy (SEM) and transmission electron microscopy (TEM) was used to analyze fine structure changes in tanycytes lining the floor of the third ventricle of 17β-Estradiol ($E_2β$)-treated long-term ovariectomized (OVX) female sheep (ewes) compared to tanycytes from OVX ewes alone. Eight mature ewes were OVX, maintained for 8½ months, at which time four ewes received daily injections of 10 µg $E_2β$/kg over a two week period while the remaining control ewes (OVX) received daily injections of vegetable oil. At the end of this time, blood was drawn from each animal for radioimmunoassay (RIA) of luteinizing hormone (LH), and tissues were prepared for SEM and TEM by procedures previously employed (Coates and Davis, Biol. Reprod. 17: 567-573, '77). LH concentration in the OVX group was 45.1 ± 28.7 ng/ml, compared to 2.2 ± 1.3 ng/ml for the $E_2β$-OVX group. SEM revealed that most tanycytes from OVX ewes displayed a characteristic lack of surface features, especially microvilli compared to the $E_2β$ - OVX group. Those structures that were present appeared prominent by virtue of the absence of surrounding features. This contrasted with the appearance of tanycytes from the $E_2β$-OVX ewes where long, slender branching microvilli were consistently seen, giving an overall fuzzy appearance to the floor of the third ventricle. Miniblebs were also evident. TEM of the OVX group showed that tanycytes presented a more heterogeneous appearance than suggested by SEM alone, possibly due to limited sampling. Tanycytes which lacked microvilli did not exhibit the rich development of internal organelles characteristic for tanycytes with microvilli from the $E_2β$-OVX group: i.e., numerous mitochondria, Golgi complexes and ribosomes, profiles of r.e.r. filled with an electron dense substance, microfilaments and microtubules, and prominent nucleoli. RIA showed that LH in OVX ewes was 20 fold greater than LH in the $E_2β$-OVX group, indicating the latter was strongly under the negative feedback influence of $E_2β$. These data are highly reminiscent of the appearance of tanycytes in wethers (castrate rams) and testosterone propionate treated wethers reported earlier (Coates & Davis, SEM/1979/III:497-504). Taken as a whole, the ultrastructural evidence suggests a positive correlation between tanycytes displaying morphological characteristics suggesting increased synthetic, secretory and/or absorptive activity and the presence of gonadal steroid hormones.

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70.2 IN SITU NUCLEAR RETENTION OF ^{3}H-ESTROGEN BY NEURONS IN HYPOTHALAMUS AND AMYGDALA: TEMPORAL ASPECTS. Donald A. Keefer, Department of Anatomy, University of Virginia Medical School, Charlottesville, Va. 22908.

Certain neurons in the phylogenetically old regions of the brain contain cytoplasmic estrogen receptors. Upon entering these neurons estrogen binds to the receptor and translocates to the nucleus where the estrogen-receptor complex becomes associated with chromatin. In this study the quantitative dry autoradiographic-immunocytochemical method was used to analyze and compare the temporal parameters of nuclear retention of radioactivity in several different neural regions after ^{3}H-estradiol administration. Sixteen adult female rats, ovariectomized for four days were given i.v. injections of 5.4 µg ^{3}H-estradiol/kg. body weight. The brain and other tissues were removed 15m, 1h, 3h or 7h after injection and were frozen in toto in liquid freon at -150° C. Six micron-thick sections were cut in a cryostat at -30° C and thaw mounted onto desiccated emulsion coated slides (Kodak NTB-2). After 40 days exposure at -20°C the autoradiograms were photographically processed (Keefer, D.A., J. Histochem. Cytochem. 29: 167, 1981). Silver grains were counted over nuclei of neurons in the medial preoptic (MPO) arcuate (ARC), ventrolateral ventromedial (VLVM) and medial amygdaloid (MA) nuclei. Classification of neurons as "labeled" and "unlabeled" was determined by the Poisson distribution (Arnold, A.P., J. Histochem. Cytochem. 29: 207, 1981). Silver grain counts were maximal in neurons of all four regions at 15m after injection. Counts in the ARC and VLVM were very similar at all time intervals, decreasing almost linearly from 15m to 7h. Uptake by POA neurons remained constant from 15m to 1h then decreased from 1h to 7h. MA neuron uptake dropped rapidly between 15m and 1h, and slowly between 1h and 7h. The labeling indices of POA and VLVM neurons remained at .80 to .90 over all time intervals. Indices for MA and ARC neurons dropped from .75 to .85 at the early times to .50 at 7h. These data indicate that the temporal aspects of nuclear retention of radioactivity after ^{3}H-estradiol injection may vary for target neurons in different regions of the brain.

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70.3 MODIFICATIONS IN THE OLFACTORY BULD ANTIDROMIC FIELD POTENTIALS BY 17 β- OESTRADIOL. M. de la Rosa-Viejo*, H.U. Aguilar-Baturoni, and R.Guevara-Aguilar. Depto. de Fisiología, Div. de Investigación, Fac. de Medicina, Univ. Nal. Autónoma de México, México 20, D.F.

In previous papers we reported modifications in the electroencephalographyc activity of the olfactory structures by stimulation of the posterior hypothalamus after subcutaneus administration of 17 β- oestradiol in castrated famale cats. Furtheremore the components of the olfactory pathway potentials evoked by posterior hypothalamus stimulation increase following topical application of 200 µg of 17 β-oestradiol. The purpose, of this study was to elucidate if there results could be explained by changes in the exitability of the olfactory bulb. We recorded in rats anaesthetized with chloral hydrate (450mg/Kg) the antidromic field potentials in the olfactory bulb produced by stimulation of the lateral olfactory tract. The rats had been oophorectomized two weeks before. The I.V. administration of 50 µg of 17 β- oestradiol produced an increase of both components of the antidromic potentials. The fast components (originated in the Mitral cells) increase in a 158%, while the slow components (fromthe granulose cells) in a 495% with respect to the control. This results suggest that the sexual hormones exert modulatory influences upon the exitability of the olfactory bulb.

70.4 MEDIAL PREOPTIC-SEPTAL AREA (mPO-S) AND MIDBRAIN CENTRAL GRAY (MCG) UNIT RESPONSES TO ESTROGEN IONTOPHORESIS AND GENITAL STIMULATION. J.T. Haskins and R.L. Moss. Dept. of Physiology, University of Texas Health Science Center, Dallas, TX 75235.

The mPO-S and MCG concentrate radiolabeled estrogen and are important in regulating the release of luteinizing hormone (LH). The present study investigates neuronal responses in the mPO-S and MCG to the iontophoresis of 17B-estradiol (17B-E_2S) and to vaginocervical stimulation. Female Sprague-Dawley rats (200-400g) in either late diestrus (LD) or late proestrus (LP) were anesthetized with urethane (1.2g/kg) and prepared for extracellular, single unit recording. Genital stimulation was accomplished by means of light-pressure applied to the uterine cervix with a cotton swab soaked in mineral oil.

The spontaneous activity of 87 neurons in the mPO-S and 79 neurons in the MCG was recorded. The activity of mPO-S neurons was significantly lower ($p<.05$) on LP (3.08±0.41 spikes/second; n=51) than on LD (4.27±0.42; n=36), while spontaneous activity of MCG neurons was significantly lower ($p<.05$) on LD (2.26±0.27; n=29) than on LP (4.14±0.57; n=50). The iontophoretic deposition of 17B-E_2S onto mPO-S and MCG neurons evoked both excitatory and inhibitory responses. However, most neurons in both areas failed to respond to the iontophoresed 17B-E_2S. No significant differences in the numbers or types (excitations or inhibitions) of estrogen responsive neurons were found in either the mPO-S or MCG.

Genital stimulation also had marked excitatory and inhibitory effects on the unit activity of neurons in both the mPO-S and MCG. These same neurons did not respond to toe or tail pinch or to stroking of the back fur. No significant differences were found in the number or types of neuronal responses to genital probing in either the mPO-S or MCG. However, unlike iontophoresed 17B-E_2S, some responses were slow in onset and outlasted the probing stimulus for seconds or, in some cases, minutes. Latencies and durations of inhibitory responses recorded from mPO-S neurons in LD were significantly longer than those recorded in LP ($p<.025$). We believe prior exposure to high endogenous estrogen levels during early proestrus may account for the differences in spontaneous neuronal activity in both areas, i.e. faster activity in the mPO-S during LD and slower activity in the MCG during LD. Similarly, endogenous estrogens may alter the integrative properties of mPO-S or MCG neurons prior to and during the period of sexual heat such that mating, if necessary, could insure the release of sufficient LH to cause ovulation. In view of these results it seems reasonable that the mPO-S and MCG are central nervous system centers important in integrating sexual stimuli and that this integration is modulated throughout the estrous cycle by fluctuating estrogen levels.

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70.5 THE VENTROMEDIAL MIDBRAIN AND LORDOSIS BEHAVIOR IN THE GOLDEN HAMSTER. J. D. Rose and M. D. Havens*. Dept. of Psychology, Univ. of Wyoming, Laramie, WY 82071.

In sexually-receptive female golden hamsters, lordosis can be elicited and maintained by continuous application of lumbosacral tactile stimuli. Previous single-unit recording work in hamsters (Rose, J. D., Exp. Neurol., 60: 499, 1978) identified neurons responding to lordosis-triggering forms of stimuli in various midbrain locations, especially deep tectum. The present investigation reexamined the midbrain to locate neurons with the strongest and most sustained responses to lumbosacral tactile stimuli. Single neurons were recorded from the midbrain in urethane-anesthetized, ovariectomized hamsters under four different hormonal conditions: pretreatment with estradiol benzoate (EB) and progesterone (P), at 44 and 4 hrs before recording, respectively; EB alone; P alone and oil vehicle alone. Neurons were sought which displayed changes in firing of more than two times their spontaneous rates throughout a 20 sec application of lumbosacral tactile stimulation. Such cells were found most often in the deep superior colliculus, the central gray, in scattered locations in the reticular formation and in a ventromedial region including the interpeduncular nucleus, median raphé nucleus and adjacent tegmentum. The ventromedial cluster of strongly-responsive cells was of particular interest since it lay in a region having a high progesterone binding capacity (Wade, G. N., et al., Brain Res., 61:357, 1973). To evaluate the potential significance of the ventromedial region in lordosis, ovariectomized hamsters were prepared with electrolytic lesions placed there. Each hamster received a series of three pre-lesion and three post-lesion behavioral tests, following appropriate injection of EB and P, to assess lordosis in response to a male and to manual lumbosacral stimuli. The lesions were highly effective, producing either a complete abolition of lordosis or a reduction in the lordosis-eliciting capacity of lumbosacral tactile stimuli. In the latter case, lordosis was elicited by males but not by manual stimulation alone. These lesion effects may have been due to destruction of neurons responsive to lumbosacral tactile stimuli and/or to damage of the midbrain neuronal substrate for P uptake. In addition, some of the more dorsal lesions may have interrupted descending tectal efferents, thereby impairing the contribution of the superior colliculus to the control of lordosis (Muntz, J. A., et al., Brain Res. Bull., 5: 359, 1980). It is unlikely that the most ventral of the lesions, such as those confined to the interpeduncular nucleus, produced their effects by interrupting tectal efferents.

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70.6 CHRONIC HYPERPROLACTINEMIA DOES NOT INCREASE STRIATAL DOPAMINE RECEPTOR BINDING. P. C. Doherty*, S. Lane*, K. Pfeil*, W. W. Morgan, A. Bartke*, and M. S. Smith* (SPON: L. F. Mercer). Depts. of Anat. and Ob.-Gyn., Univ. of Tex. Hlth. Sci. Ctr., San Antonio, TX 78284, and Dept. of Physiol., Univ. of Pittsburgh, Sch. of Med., Pittsburgh, PA 15261.

Hyperprolactinemia (hyperPRL) induced by grafting 4 ectopic pituitary grafts under the kidney capsules suppresses copulatory behavior in male rats. Recent reports have indicated that short-term hyperPRL induced by 2 pituitary grafts or prolactin administration can induce increases in copulatory behavior (Drago et al., Physiol. Behav. 26:277, 1981) and increases in stereotyped behavior and in the number of dopamine (DA) receptors in the striatum (Hruska et al., Abstr., Soc. Neurosci. 6:441, 1980). Thus experiments were performed to investigate the short-term effects of 4 grafts on copulatory behavior and the chronic effects of 4 grafts on DA receptor binding in the striatum. Adult sexually-experienced male rats were observed for copulatory behavior at 4 day intervals after induction of hyperPRL. No changes were observed until day 8, when the post-ejaculatory interval (PEI) was found to be significantly increased in the grafted animals (5.34±0.35 vs 4.21±0.20 min.; $\bar{x}\pm SE$; $p<0.02$). By day 16, when the grafted animals showed the complete pattern of behavioral suppression, the PEI was no longer increased, in agreement with previous findings from this laboratory. Similar results were obtained in a second experiment with two additional groups of animals. To determine if chronic hyperPRL results in changes in DA receptor binding in the corpus striatum, the binding of ^{3}H-spiroperidol to homogenates of the caudate-putamen (Fields et al., Brain Res. 136:578, 1977) was assessed in animals which had been grafted for 11 months. No differences in binding were observed between the grafted and sham-operated groups either in K_d for the receptor site (23.4±1.9 vs 25.4±4.0 pM) or in the maximum number of binding sites (39.2±2.2 vs 39.8±3.4 pmoles/g-tissue). At autopsy, the grafted animals had significantly larger adrenal glands and accessory reproductive organs, indicative of their hyperprolactinemic state. Previous reports of increases in behavior and DA receptor binding were not confirmed under the conditions of these experiments. These results do not exclude the possibility of early increases in behavior or receptor sensitivity, but rather indicate that at a time when the suppression of copulatory behavior occurs in hyperprolactinemic animals, striatal dopamine receptor binding is not increased or has returned to pre-treatment levels. The early increase and then return to normal in the PEI may be indicative of such a change. Supported by NIH grants HD-12671 and NS-14855.

70.7 PROLACTIN INCREASES THE DENSITY OF STRIATAL DOPAMINE RECEPTORS IN NORMAL AND HYPOPHYSECTOMIZED MALE RATS. K.T. Pitman*, R.E. Hruska, L.M. Ludmer*, and E.K. Silbergeld (SPON: P.D. Thut). Neurotoxicology Section, NINCDS, NIH, Bethesda, MD 20205.

We previously reported (Neuropharm. 19: 923, 1980) that estrogen administered to adult male rats significantly increases the density of the striatal dopamine (DA) receptors. In hypophysectomized (Hypox) rats this effect is blocked. We now report that administration of ovine prolactin (oPRL) to normal male or Hypox male rats increases the density of the striatal DA receptors. oPRL was administered to male rats by two methods. First, oPRL was dissolved in sesame oil and injected s.c. daily at doses from 8 to 1000 µg/d for 4 to 6 days. Second, oPRL was dissolved in saline and placed in Alzet osmotic mini-pumps. The concentration was adjusted so that either 120 or 6000 ng/h of oPRL was continuously injected s.c. for 7 days. Hypox rats were obtained from Taconic Farms and maintained on a drinking solution of dextrose and balanced salts. The rats were killed by decapitation. Hypox rats were inspected for complete absence of the pituitary, and in appropriate rats the pumps were removed and inspected. Striatal DA receptors were measured in our standard ^{3}H-spiroperidol binding assay. The daily administration of oPRL by s.c. injection to normal male rats did not alter the density of the striatal DA receptors at doses of 8 to 100 µg/d. While a dose of 250 µg/d had a tendency to increase the density, a significant increase was obtained only with the 1000 µg/d dose. In Hypox rats administration of 1000 µg/d of oPRL did not alter the density of the striatal DA receptors. The lack of effect in Hypox rats may result from the need for larger amounts of exogenous oPRL because the endogenous supply is gone. The continuous infusion of oPRL via mini-pumps also increased the density of the striatal DA receptors in normal male rats, but at a much lower dose of oPRL (120 ng/h for 7 days). This dose was not effective in Hypox male rats. Increasing the dose 50-fold to 6000 ng/h did increase the striatal DA receptor density in Hypox rats. Evidently, continuous s.c. administration of oPRL allows a lower dose to be used than is necessary by single large daily s.c. injections. In addition rat PRL (from the Rat Pituitary Hormone Distribution Program, NIADDKD) was active in normal male rats to increase the density of the striatal DA receptors. The affinity of the receptors was not changed in any experiment by prolactin administration. These results suggest that PRL alone can increase the density of the striatal DA receptors and that PRL could be the common mediator of the increase in striatal DA receptor density produced by estrogen, haloperidol, and other chemicals or drugs.

70.8 ESTROGEN POTENTIATES HALOPERIDOL-INDUCED CATALEPSY/AKINESIA IN MALE RATS. M. De Ryck*, R.E. Hruska and E.K. Silbergeld. (SPON: W. Himwich) Neurotoxicology Section, NIH, NINCDS, Bethesda, MD.

Estrogen may modulate behaviors controlled by the basal ganglia. In ovariectomized rats estrogen modifies behaviors induced by drugs acting, at least in part, on the nigrostriatal dopamine (DA) system. In male rats, a single injection of estrogen produces 6 days later a 20 percent increase in the number of striatal DA receptors and enhances DA agonist-induced stereotyped behaviors (Hruska and Silbergeld, Eur. J. Pharm., 1980, 61:397). Similar in vitro and in vivo changes are typical of DA receptor supersensitivity following chronic DA receptor blockade by haloperidol (HAL). Chronic HAL also causes tolerance to the catalepsy/akinesia induced by its acute administration. In contrast, as we show below, estrogen, rather than attenuate, actually potentiates HAL-induced catalepsy/akinesia.

Adult male rats were injected s.c. with 125 µg 17 Beta-estradiol valerate (EDV) in sesame oil (n = 40) or, as controls (CON), with sesame oil (n = 38). Six or 7 days later, catalepsy was rated by means of multiple measures (see De Ryck et al., Brain Res., 1980, 201:143): (1) Akinesia: crouched posture; freezing/shivering reaction; and latency till movement; (2) Bracing reactions; (3) Ptosis; (4) Blepharospasm; (5) Leaning; (6) Clinging; (7) Tonic grasp; (8) Righting; and (9) Tremor. These measures were separately analyzed and converted into a catalepsy index (CI; range = 0-100). EDV and CON groups were compared once before (saline) and repeatedly up to 7 hrs after a suprathreshold dose of .25 mg/kg HAL. Other EDV and CON groups were similarly tested up to 12 hrs after a threshold dose of .10 mg/kg HAL. Rats were also used as their own controls by running EDV and CON animals once before and for 5 hrs after saline, followed by 3 hrs after the threshold dose of .10 mg/kg HAL. Without HAL, repeated tests induced a neuroleptic-like catalepsy in 30 percent of drug-free controls. Chronic EDV reduced such "learned" catalepsy (acute EDV had no effects). In contrast, after HAL treatment, EDV rats became significantly more cataleptic than CON rats. After .25 mg/kg HAL, the CI of EDV rats was enhanced by 25 percent over control. Between- and within-animal comparisons showed that EDV lowered the catalepsy threshold after .10 mg/kg HAL. These results may not fit a simple model of DA receptor function after chronic estrogen. It is unclear whether decreased drug metabolism is responsible for these results. Alternatively, estrogen, known to enhance somatosensory inputs, could increase the gain of reflexive mechanisms underlying DA-mediated catalepsy and stereotypy.

70.9 COMPARISON OF THE BIOCHEMICAL AND BEHAVIORAL EFFECTS OF ESTROGEN IN MALE AND FEMALE RATS. R.E. Hruska, K.T. Pitman*, L.M. Ludmer*, M. De Ryck*, and E.K. Silbergeld. Neurotoxicology Section, NINCDS, NIH, Bethesda, MD 20205.

In striatal tissue from male or female rats, the addition of estrogen, in vitro, does not alter the binding of ^{3}H-spiroperidol to DA receptors. Similarly, in striatal tissue from male or female rats the inhibition of binding by d-butaclamol (an antagonist) or DA (an agonist) is the same and not altered by the addition of estrogen.

In vivo, estrogen administration (125 µg/rat of 17ß-estradiol valerate in sesame oil, injected 6 days before s.c.) increases the density of the striatal DA rceptors in adult male rats. This treatment does not affect the density of striatal DA receptors in adult female rats. Ovariectomy (ovex) also does not alter the density of the striatal DA receptors. However, estrogen administration to ovex rats significantly increases the density of striatal DA receptors as well as the dissociation constant (Kd). Therefore, rats without estrogen present (i.e. male or ovex rats) respond similarly to the administration of estrogen and have an increase in the density of the striatal DA receptors.

Behavioral stereotypy results are measured in grouped rats and are more complex. Normal female rats display more stereotyped behavior after apomorphine administration than normal male rats. The amount of stereotyped behavior in the female rat does not vary with the stage of the estrous cycle. Male rats administered estrogen show increased amounts of stereotyped behavior after apomorphine treatment (Eur. J. Pharm. 61: 397, 1980). If female rats have more stereotyped behavior than male rats, and male rats administered estrogen have more stereotyped behavior than normal male rats, it may be expected that ovex rats would have less stereotyped behavior than female rats. Paradoxically, ovex rats have more stereotyped behavior than normal female rats, and this is decreased to levels of normal female rats by administration of estrogen. Stereotypy regulation is probably complex in both male and female rats. In male rats there is a correlation between increases in stereotyped behavior and increases in density of striatal DA receptors. In female rats there is a dissociation. Possibly estrogen acts at other sites which regulate stereotyped behavior, such as at acetylcholine or GABA neurons. It might be expected that male and female rats exhibit different responses after hormone treatment, especially since the sexual differentiation and hormonal environment of the brains are dissimilar. Indeed, it may be serendipitous to find a correlation in male rats, while in female rats other effects are more important.

70.10 SEX DIFFERENCES IN RESPONSE TO CONFLICT: BEHAVIORAL AND PITUITARY-ADRENAL EFFECTS. J. Weinberg, M.R. Gunnar, L.P. Brett, C.A. Gonzales and S. Levine. Dep't. of Psychiatry and Behavioral Sciences, Stanford Univ. Sch. of Med., Stanford, CA 94305

Behavioral and pituitary-adrenal responses of male and female rats were compared in an approach-avoidance conflict situation. Animals were exposed to a novel (milk) solution, and a conditioned taste aversion was produced by pairing drinking with LiCl injection. Reexposure to milk then occurred following 72 hr of ad lib food and water or 72 hr of food and water deprivation. Thus avoidance tendencies were produced by aversive conditioning and approach tendencies were produced by deprivation prior to reexposure. No consistent sex differences were observed during the pretoxicosis phase of testing. At the end of the deprivation period females exhibited greater plasma corticoid elevations than males. On the first reexposure day males maintained elevated corticosterone levels while females showed a significant suppression of corticoids below presession levels. However, males and females showed similar suppressions in drinking on this day. If ad lib access to food and water was then reestablished and daily reexposures to milk were continued, sex differences in behavior appeared. Males showed a greater initial suppression of intake than females and females recovered to pretoxicosis intake levels faster than males. Gonadectomy eliminated these sex differences. Presession corticosterone levels were lower in ovariectomized females and higher in castrated males than they were in intact animals. When reexposed to milk castrated males exhibited a response pattern similar to that of the females: i.e. a corticoid suppression on the first reexposure day and an accelerated recovery to pretoxicosis intake levels. These data indicate that intact males and females respond differently to conflict. That females showed a greater corticoid response to deprivation suggests that the deprivation regimen might be more aversive for females than males. An increased response to deprivation should increase motivation to approach the milk, and if approach tendencies become stronger than avoidance tendencies, conflict and hence arousal, should decrease. The finding that females exhibited a corticoid suppression upon reexposure, as well as less suppressed intake and faster recovery to pretoxicosis drinking levels suggests that females experienced less conflict than males. Furthermore, in this situation testosterone has primarily an activational effect. The sexual dimorphism in both behavioral and pituitary-adrenal responsiveness appeared to depend upon the presence of testosterone in the adult male.

70.11 EFFECTS OF COPULATION ON FOOD INTAKE IN MALE RATS. A. A. Nunez, L. I. Siegel* and G. N. Wade. Department of Psychology, Michigan State University, East Lansing, Michigan 48824 and Univ. Massachusetts, Amherst, MA 01003.

In adult male rats, repeated mating tests or cohabitation with intact females reduces body weight gain and carcass fat (Neuroscience Abstracts, 4: 182, 1978). Two lines of evidence suggest that copulation-induced changes in body weight and adiposity may be mediated by testicular testosterone. First, it has been reported that male rats increase their secretion of testosterone when exposed to receptive females. Second, prolonged treatment of castrated male rats with high doses of testosterone propionate (TP) reduces weight gain and body fat (Neuroscience Abstracts, 6: 45, 1980). This decrement seems to be mediated by a reduction in adipose tissue lipoprotein lipase (LPL) activity. In addition to changes in body weight and adiposity, high doses of TP reduce caloric intake. Dietary self-selection experiments have shown that this reduction in total calories is the result of a specific reduction in carbohydrate intake.

The present experiment aimed to further investigate the effects of copulation on behavioral and physiological endpoints that are sensitive to testosterone. Sexually active and sexually deprived male rats were given access to a pair of isocaloric diets that were equal in fat, but that contained differing amounts of carbohydrate and protein. Body weight and food intake were measured twice weekly for six weeks. Copulation (2 mating tests/ week) significantly reduced body weight. However, sexual activity failed to reduce caloric intake and did not affect the intake of protein or carbohydrate. Using the same self-selection paradigm, daily injections of 2 mg of TP produced a selective increase in protein intake, and reduced carbohydrate and caloric intake. Adipose tissue LPL activity is reduced by doses of TP that reduce food intake, body weight and carcass fat content in castrated rats. We found no evidence for changes in LPL activity in sexually active males that were gaining significantly less weight than controls.

In summary, although copulation and TP treatment produce similar effects on body weight and adiposity, these effects do not seem to share a common mechanism. (Supported by Grants NS 10873, NS 05854-01 and NS 00090 from NINCDS, by Grant AM 20785 from NIAMDD and by Training Grant MH 11823 from NIMH).

70.12 THE EFFECTS OF o,p'-DDT ON NEUROENDOCRINE PARAMETERS IN FEMALE RATS. Anne M. Etgen. Dept. Biology, Livingston College, Rutgers University, New Brunswick, NJ 08903.

Previous research has shown than 1-(o-chlorophenyl)-1-(p-chlorophenyl)-2,2,2-trichloroethane (o,p'-DDT), a contaminant in technical DDT, possesses potent estrogenic properties. It stimulates uterine growth, induces the synthesis of specific estrogen-inducible uterine protein, and has a low affinity for uterine estrogen receptors. Since chlorinated insecticides have been reported to produce reproductive disorders in both humans and animals, it seemed possible that o,p'-DDT might also have estrogenic activity at the level of the central nervous system and that disruption of neuroendocrine interactions might contribute to the observed reproductive dysfunction. As a preliminary test of this possibility, the effects of o,p'-DDT on sexual behavior, gonadotropin release, and body weight, three estrogen sensitive neuroendocrine responses, were tested.

For sexual behavior tests, ovariectomized adult female Sprague Dawley rats received sc injections of 50, 200, 350 or 500 mg/kg of o,p'-DDT followed 48 hr later by 500 ug of progesterone (P). Tests for lordosis behavior began 4 hr after P treatment. On successive weeks, animals received a given dose of DDT either alone or in combination with an optimal (2 ug) or suboptimal (1 ug) dose of estradiol benzoate (EB), or they received 1 or 2 ug of EB alone. The order of treatments was randomized, and no consistent treatment order effects were noted. DDT failed to facilitate lordosis when given alone or combined with 1 ug of EB, regardless of the dose. When given with 2 ug of EB, no dose of DDT significantly antagonized EB-induced sexual behavior.

Another group of female rats with equivalent body weights was unilaterally ovariectomized (left ovary) and given daily sc injections of vehicle, 0.1 ug of estradiol (E_2), 200 mg/kg of DDT, or 0.1 ug of E_2 plus 200 mg/kg of DDT. After 15 days, the body weights of the animals were recorded and the remaining (right) ovary was removed. The weight of the right ovary was compared to that of the left to determine if any of the treatments had attenuated gonadotropin secretion. Animals receiving DDT alone or in combination with E_2 had significantly lighter right ovaries than did animals receiving vehicle or E_2 alone; the latter two groups did not differ. In addition, both groups which received DDT had significantly lower body weights than the control or E_2-treated animals. DDT thus appears to mimic estrogen depression of body weight and gonadotropin release but to neither facilitate nor inhibit estrogen-induced lordosis behavior. The interaction of DDT with pituitary and hypothalamic estrogen receptors will be compared with its effects on these neuroendocrine responses.

70.13 CHANGES IN BRAIN IRON DURING THE ESTROUS CYCLE. Joanna M. Hill* (SPON: P.D. MacLean). Laboratory of Brain Evolution and Behavior, National Institute of Mental Health, Bethesda, MD 20205.

Iron is unevenly distributed in the brain with the highest levels occurring in the ventral pallidum (VP), globus pallidus (GP), and substantia nigra (SN). With the use of the sensitive diaminobenzidine intensification of the Perl's histochemical method for iron and measurement of tissue ferritin iron concentration by spectrophotometry, a sex difference in brain iron, with females having the greater iron reserves, recently has been demonstrated (Hill, Neurosci. Abstr. 6, 131, 1980). A sex difference brought about by sex steroids occurs in many aspects of iron metabolism. This study was undertaken to determine if brain iron changes with the naturally occurring fluctuations of ovarian hormones during the estrous cycle. Using the sensitive methods outlined above, this study demonstrates that: 1) iron is localized histochemically not only in the high-iron areas of the VP, GP, and SN but also in the organum vasculosum of the lamina terminalis (OVLT), the tanycytes, and in granules in the arcuate nucleus and median eminence (sites known to contain luteinizing hormone releasing hormone, LHRH); and 2) total non-heme iron concentration in the VP, GP, and SN, measured by spectrophotometry, fluctuates during the estrous cycle with levels at proestrus significantly higher than at other stages of the cycle.

	μg Fe/g wet weight ± S.E.M.
Proestrus	43.48±5.55*
Estrus	25.44±5.11
Diestrus	23.02±4.73
Metestrus	15.40±2.49

*F=6.24, df=3,27 p<0.005

The increase of endogenous iron in the VP and GP during proestrus could be: 1)directly influencing the activity of the adjacent medial preoptic area and thus causing ovulation (iron salts or or electrochemical stimulation with steel electrodes of the medial preoptic area causes luteinizing hormone (LH) release and ovulation), 2) reflecting the endogenous iron changes occurring in the high-iron areas of the hypothalamus, thus influencing LHRH directly through the association of iron with LHRH in the OVLT, tanycytes, the arcuate, and median eminence or 3) indirectly affecting gonadotropin release through the effects of iron on monoamines (iron is required for the synthetic and degradative enzymes as well as, perhaps, the storage and receptor mechanism of brain monoamines which, in turn, are involved in gonadotropin release and sexual behavior).

70.14 EXERCISE REINSTATES ESTROUS CYCLES IN HAMSTERS MAINTAINED IN SHORT PHOTOPERIOD. K.T. Borer, C.S. Campbell, L. Gordon*, K. Jorgenson* & J. Tabor*. Dept. Phys. Educ., Univ. Mich., Ann Arbor, MI 48109 & Dept. Biol. Sci., Northwestern Univ., Evanston, IL 60201.

Exposure to short photoperiod renders female hamsters anestrous (Seegal, R.F. & Goldman, B.D., Biol. Reprod. 12:223-231, 1975) and reduces their volume of daily running activity (Widmaier, E.P. & Campbell, C.S., Physiol. Behav. 24:923-930, 1980), while exposure to running reinstates rapid growth in adult hamsters (Borer,K.T. & Kelch,R.P., Amer. J. Physiol. 234:E611-E616, 1978). To determine whether the photoperiod would affect exercise-induced growth and whether exercise would affect photoperiodically-induced anestrus, we subjected four groups of 7 hamsters to: short day and exercise (10L:14D-EX); long day and exercise (16L:8D-EX); short day and sedentary (10L:14D-SED); long day and sedentary existence (16L:8D-SED). Five consecutive estrus cycles were monitored before exposure to different photoperiods, and seven cycles were monitored after ten weeks of exposure to a given photoperiod. After ten weeks in a given photoperiod, EX groups were given a 4-week exposure to running activity in rotating wheels. Hamster length was determined at the start and at the end of the experiment. In addition, on the last day of the experiment, blood was collected by decaptiation between the second and third hour after the onset of light. Serum was used for measurements of growth hormone (GH) and prolactin (PRL) with homologous radioimmunoassays. Percentage of body fat was determined by petroleum ether extraction of freeze-dried homogenates of hamster carcasses.

Our findings are: (1) Photoperiod had no effect on exercise-induced growth or serum GH concentration, but short photoperiod abolished or reduced (to 29 and 71%) estrous cycles and was associated with significantly lower serum PRL concentration ($p < 0.05$); (2) Exercise resulted in significant elongation of the body (41% $p < 0.001$), in complete or partial (14-86%) reinstatement of estrus cycles in short-day hamsters ($p < 0.001$), and in significantly higher levels in serum GH concentration ($p < 0.05$); (3) Exercise was associated with significant reduction in the percentage of body fat in animals of both photoperiods ($p < 0.005$).

We conclude that the neuroendocrine changes associated with the exposure to voluntary running activity interfere with the neuroendocrine consequences of exposure to the short photoperiod. Photoperiodically-induced neuroendocrine changes, however, have no impact on the exercise-induced facilitation of growth.

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70.15 DIFFERENCES BETWEEN ANDROGEN-RESISTANT RAT AND MOUSE MUTANTS. K. L. Olsen* and T. O. Fox. Long Island Research Institute, State Univ. of New York, Stony Brook, NY 11794 and Dept. Neuropathology, Harvard Med. Sch. and Dept. Neuroscience, Mental Retardation Research Center, Children's Hosp. Med. Cntr., Boston, MA 02115.

To assess the possible involvement of androgen receptors in behavior, androgen-resistant mutants that are deficient in androgen receptors have been analyzed. We have compared some aspects of the phenotypes of rats (tfm) and mice (Tfm) which have the syndrome of testicular feminization. Both rat and mouse mutants exhibit profound deficiency in responsiveness to androgen. Correlated with this deficit, each exhibits only 10 - 20% of the level of the putative androgen receptors of the respective wild-types. We report that these two mutants are distinguishable from their wild-type controls and from one another with regard to spontaneous male sexual behavior and to DNA-cellulose elution patterns of putative androgen receptors from brain.

Both wild-type rats and mice spontaneously display the full copulatory sequence of behaviors. The spontaneous behavior of the mutants is reduced relative to that of the wild-type males. Androgen-resistant rats mounted receptive females and 20% even exhibited the ejaculatory pattern. In contrast, none of the mutant mice displayed any spontaneous male sexual behavior.

The residual androgen receptors from both the rat and mouse mutants were chromatographed on DNA-cellulose to compare them qualitatively and thus obtain a biochemical characterization of the mutational effects. Receptors from mutant rat brain eluted with the same pattern, but at a uniformly lower level, compared with wild-type rats. In contrast, receptors from mutant mice eluted with an altered pattern as compared with wild-type mice. This revealed a virtual lack of a detectable major receptor form from the mouse, with the residual receptor being either a minor or mutant form. Thus, the receptor deficiencies in the two mutants appear to be non-identical. The levels and elution patterns of estrogen receptors were the same in mutant and wild-type mice and rats.

These experiments indicate that these two mutations differentially affect behavior and androgen receptors, and this must be taken into account when using, or comparing, these mutants in genetic experiments.

70.16 ANDROGEN AND ESTROGEN RECEPTORS IN HAMSTER BRAIN. C. C. Vito, J. F. DeBold and T. O. Fox, Dept. of Neuropathology, Harvard Med. Sch., and Dept. of Neuroscience, Mental Retardation Res. Ctr., Children's Hosp. Med. Ctr., Boston, MA 02115; and Dept. of Psychology, Tufts Univ., Medford, MA 02155.

Among hamsters, mice and rats, there are both species and sex differences in the behavioral responsiveness of adults to androgens and estrogens. We are investigating the possible relevance of sex hormone receptor mechanisms to such differences.

Using ^{3}H-dihydrotestosterone (DHT), ^{3}H-estradiol and DNA-cellulose affinity chromatography, we have demonstrated that putative receptors for both androgens and estrogens are present in cytosol extracts of hypothalamus-preoptic area (HPOA) and remaining brain (whole brain minus HPOA = RB) from gonadectomized adult male and female hamsters. By all qualitative and quantitative criteria tested to date, both the androgen and estrogen receptors in cytosols of male HPOA and RB are indistinguishable from those of female HPOA and RB. Moreover, the androgen and estrogen cytosol receptors in hamster (male and female) HPOA and RB are qualitatively similar to those in both mouse and rat brain as evidenced by their ability to adhere to DNA, characteristic behaviors during elution from DNA-cellulose with a linear gradient of [NaCl], affinity for their respective hormones, specificity of hormone-binding, and tissue specificity. Cytosols of HPOA and RB contain DHT-binding activity which adheres to DNA-cellulose and elutes below 150 mM NaCl, saturates between 5 and 10 nM DHT, and binds testosterone; the concentration of putative androgen receptor in HPOA is about 5-fold greater than that in RB. Cytosols of HPOA and RB also contain estradiol-binding activity which adheres to DNA-cellulose and elutes above 150 mM NaCl, saturates between 5 and 10 nM estradiol, and binds the synthetic estrogen diethylstilbestrol; the concentration of putative estrogen receptor in HPOA is about 10-fold greater than that in RB.

Our data suggest that there is a quantitative species difference in both the androgen and estrogen receptor systems in HPOA. The ratio of androgen receptor to estrogen receptor is higher in adult hamster HPOA (male and female) than that observed in either adult mouse or adult rat HPOA; in adult male and female hamsters, this ratio is higher by virtue of the fact that androgen receptor concentrations are about 4-fold higher and estrogen receptor concentrations are about 2-fold lower than in either mouse or rat HPOA. These observations correlate with a generally lowered estrogen sensitivity of female hamsters and heightened DHT sensitivity of male hamsters relative to mice and rats.

70.17 INHIBITION OF ANDROGEN BINDING BY ATD AND FLUTAMIDE. J. F. DeBold, T. O. Fox and K. L. Olsen* Dept. Psychol., Tufts Univ., Medford, MA 02155; Dept. Neuropathol., Harvard Med. Sch. and Dept. Neurosci., Mental Retardation Res. Ctr., Boston, MA 02115; Long Island Res. Inst., S.U.N.Y., Stony Brook, NY 11794.

The extent to which androgen action in the brain is mediated by androgen receptors or by estrogen receptors after aromatization of androgen to estrogen is currently uncertain. ATD (1,4,6-androstatriene-3,17-dione), an inhibitor of aromatization of androgen to estrogen, and flutamide (4'-nitro-3'-trifluoromethylisobutyrylanilide), an antiandrogen, are often used to assess pharmacologically the mode of androgen action. Thus, if ATD is found to block androgen action in a particular system it is generally concluded that aromatization is important; however, if flutamide blocks androgen action it is thought to reflect direct action on androgen receptors. We have tested biochemically some of the assumptions behind these conclusions.

Cytosol extracts from the hypothalamus-preoptic area (HPOA) and kidney of castrated male rats were incubated (4° C) for 1 hr with 5 nM ^{3}H-DHT alone or with 100 nM or 1 uM ATD, flutamide or DHT. Bound ^{3}H-DHT was measured after filtration with Sephadex G-25. Although less effective than unlabelled DHT, ATD and flutamide both reduced the apparent amount of specifically bound ^{3}H-DHT.

	Inhibition by ATD		Inhibition by Flutamide	
	100 nM	1 uM	100 nM	1 uM
HPOA	16.3%	32.9%	17.0%	29.4%
Kidney	32.0%	59.6%	31.0%	48.3%

This effect was greater in kidney cytosols than HPOA but was concentration-dependent in both. In a preliminary study we found similar effects of ATD in mouse kidney.

Possible competition by flutamide and ATD for androgen binding was also assessed with DNA-cellulose affinity chromatography. Kidney and HPOA extracts were incubated (4° C) for 1 hr with 10 nM ^{3}H-DHT alone or with 1 uM ATD, flutamide or DHT. The labelled extracts were layered onto DNA-cellulose columns and further incubated at 22° C for 1 hr. ATD reduced androgen *binding (eluted at 100 - 150 mM NaCl) by 12% in HPOA and 34% in* kidney, while flutamide had little effect in HPOA (2%) and inhibited kidney by 12%. The difference in degree of inhibition with Sephadex vs DNA-cellulose chromatography may reflect differences in kinetic vs equilibrium conditions.

Clearly, in vitro ATD can interfere with androgen binding; in fact better than flutamide, an antiandrogen. Although conditions in vivo are different, some caution must be used in interpreting ATD effects as purely inhibition of aromatization.

71.1 SYMPATHETIC INNERVATION OF THE RABBIT APPENDIX: FURTHER EVIDENCE FOR NEURAL INPUT TO LYMPHOID TISSUE. J.M. Overhage*, S. Y. Felten *, J.F. Schmedtje*, and D.L. Felten. Department of Anatomy, Indiana University School of Medicine, 1100 W. Michigan St., Indianapolis, IN 46223

Recent morphological and functional studies (J.M. Williams et al., Brain Res. Bull., 6:83-92, 1981) have demonstrated sympathetic noradrenergic innervation of parenchymal lymphoid tissue in murine thymus and spleen, and an inhibitory control by these fibers over response to sheep red blood cell antigen. The well documented existence of lymphoid tissues in the wall of the appendix of the rabbit prompted us to search for sympathetic innervation. The vermiform appendix from 17 New Zealand white rabbits (five 21 day old, five 42 day old, seven adults) was prepared for sucrose-phosphate-glyoxylic acid (SPG) histofluorescence. In the appendix (J. Schmedtje, J. Morph., 166:179-195, 1980), domes with a central core of B-lymphocytes and a surface epithelial zone of migrating T-lymphocytes, and intradomal regions with a subepithelial layer of immunoglobulin-secreting plasma cells are found. The moats surrounding the domes separate elements of the cellular immune system on one side from the humoral immune system on the other. A dense sympathetic innervation was found in specific regions of the appendix in rabbits of all three ages. Fluorescent plexuses were found in the outer muscular layer, mainly in association with blood vessels. From the muscular layer, radially oriented clusters of fluorescent fibers coursed inward between the domes. Upon reaching the floor of the moats surrounding the inner portion of the domes, these sympathetic fibers arborized into an extensive plexus in the intradomal regions. A dense component of these intradomal plexuses traveled parallel to the furface of the epithelium in the subepithelial plasma cell zone. Viewed from the luminal surface, these fibers surrounded the domes, separated from them by the moats. Associated with the intradomal adrenergic plexuses were yellow fluorescent cells which did not demonstrate metachromatic staining. We suggest that the sympathetic innervation of the intradomal plasma cell zone and the proximity of fibers across the moats from the surface epithelium of the domes may represent further evidence for neural input to lymphoid tissue. In the rabbit, the appendix represents an important lymphoid structure. In humans, the appendix is not as highly organized or important a lymphoid structure as in the rabbit, but still may play a role in immune responsiveness. We suggest that the rabbit appendix is a well organized and readily accessible model for sympathetic-lymphoid tissue interactions in the gastrointestinal tract, and may represent another important functional link between the nervous and immune systems. Supported by an Alfred P. Sloan Fdn. Fellowship (D.L.F.)

71.2 MODULATION OF LYMPHOPROLIFERATIVE T-CELL RESPONSES BY THE CEREBRAL CORTEX. K. Bizière, P. Bardos*, D. Degenne*, G. Renoux*. C.R.C.M., rue du Pr. Blayac, 34082 Montpellier Cedex, France. Lab. d'Immunologie, Fac. Med., 37032 Tours Cedex, France.

Surgical lesions of the left cerebral cortex have been shown to impair immunological responses by depressing T-cell mediated responses and spleen NK activity (Renoux, G., Bizière, K., Renoux, M., Guillaumin, J.M. C.R. Acad. Sci., 290 D : 719 ; Bardos, P., Degenne, D., Lebranchu, Y., Bizière, K., Renoux, G. Scand. J. Immunol., 1981 in press). In the present report we describe the effets of surgical lesions of cerebral cortex on lymphoproliferative T and B-cell responses.

Similar lesions were performed on either the left or the right cerebral cortex of 6-7 week old female C_3H/He mice. Animals were anaesthetized by i.p. administration of 0.085 ml/g of a 5 % solution of chloral hydrate ; the skull overlying the cortex was removed and the cortex was ablated by shallow knife cuts ; the ablated area was gently packed with sterile gelfoam and the scalp was apposed with sutures. The lesion involved the dorsal and lateral aspects of the frontal, parietal and occipital cortex, without penetrating the corpus callosum. Animals with a left cortical lesion served as controls for the surgical trauma induced by lesioning the right cortex, and vice versa. Sham operated mice in which the cortex was not lesioned during surgery, were also used as controls. Eight weeks after surgery phytohemagglutinin, (PHA), concanavaline A, (CON A) and pockweed, (PWM) induced responses were measured by $|^3H|$- Thymidine incorporation. The allogenic antigen response was measured in a one way mixed lymphocyte culture (MLC) using allogenic spleen cells.

A surgical lesion of the left cortex resulted in a significant 30 % decrease of $|^3H|$- Thymidine incorporation in T- Lymphocytes in response to PHA and Con A, but did not affect the response to PWM (a B cell response in mice). In contrast a surgical lesion of the right cortex caused a significant 30 % increase of $|^3H|$- Thymidine in response to PHA, but did not affect the reponse either to Con A or to PWM. The kinetics of the MLC response were deeply modified after either left or right cortical lesions, the maximum response occurred at seven days as compared to five days in controls and was significantly greater than that observed in controls.

These results bring new evidence that the cerebral cortex may be involved in the elaboration of immune responses by modulating T-cell mediated events.

71.3 SEXUAL DIMORPHIC AND STRAIN DIVERSITY IN ANTIGEN SPECIFIC HUMORAL IMMUNITY: NORMAL ENDOCRINE MODULATION OF IMMUNITY. P. Trefts*, K. Bulloch[1] and R.N. Hamburger*,(Spon: T. Melnechuk), Pediatric Immunology and Allergy Div., Dept. of Pediatrics, Univ. of Ca., School of Med., LaJolla, Ca. 92093. [1]Div. of Neuroimmunology, Dept. of Neurology, SUNY at Stony Brook, N.Y. 11794

Many human diseases show definite patterns of susceptibility based on sex, age and the major histocompatibility complex. In order to determine if certain aspects of normal immunity express a hierarchy of responses based on these same parameters, a study was undertaken to examine antigen and class specific humoral responses of 5 strains of mice. Aged matched wildtype staggerer (C3H and B57Bl/6J mixed background), inbred BIOS as well as BALB/c, C57Bl/6J, A/J and their Fl crosses were used in these experiments. The mice were injected i.p. either with DNP-OVA, DNP-KLH or DNP - ascaris in 0.5 ml. of normal saline with 2 mg. aluminum hydroxide $Al(OH)_3$ as the adjuvant. Retro-orbital sinus bleeds were collected from individual animals on day 10 and day 23 and the serum fraction prepared. All injections and serum collections were carried out at the same time in every experiment. Dilutions of individual serum were then tested in a solid phase radioimmune assay for the class and antigen specific response to the hapten DNP. Standards for IgG_1 & IgA were tested in each assay to quantify the class specific responses to DNP. The results of these experiments show that in all strains and Fl crosses regardless of the carrier used, female mice elicited higher antigen specific IgA and IgG_1 responses on day 10 and day 23 than males, regardless of age. The magnitude of the sexual dimorphic responses ranged from 6-9 fold differences for the BlOS and staggerer wildtype, to 2-4 fold differences for the A/J BALB/c and C57Bl/6J. Fl showed intermediate responses. These data demonstrate a consistent difference between male and female class specific response to a given antigen (hapten-carrier complex) that is related to the strain. Although age did not appear a factor in these experiments, others (Murphy, W.H.,Prog. in Neurol. Res. 175, 1979) have shown that age dependent loss of immune function is a viable factor in susceptibility to some viruses in certain strains independent of sex. Testosterone and progesterone treatment of the male and female wildtype staggerer (Bulloch, K., Trefts, P. and Hamburger, R.N., man. in prep.) show there is not a qualitative difference between male and female immune cells since the sexual dimorphism can be abrogated with the appropriate hormone. It is clear from the experiments "normal immunity" is under endocrine modulation. The data presented here provides a model system to evaluate the exact nature of these mechanisms and a basis for interpreting the aberrant diseased state.

71.4 EVIDENCE FOR A CNS-THYMUS AXIS INVOLVING THYMOSIN PEPTIDES. N. R. Hall, E.W. Palaszynski*, T. W. Moody and A. L. Goldstein*. Dept of Biochemistry, George Washington Univ. Sch. of Med., Washington, D. C. 20037.

Peptide hormones produced by the thymus gland play a central role in T-cell dependent immunity. The possibility that these thymosin peptides may also play a regulatory role as part of a CNS-thymus gland axis is suggested by two types of experimental evidence: 1) The presence in rat hypothalamus and pituitary gland of the peptide, thymosin α_1, and 2) differential effects of intracerebral thymosin administration upon immunologic and endocrine parameters.

Discrete rat brain regions were extracted and assayed for thymosin-like peptides using a RIA capable of detecting as little as 50 pg of thymosin α_1. The density of endogenous thymosin-like peptides was 20-fold greater in high regions such as the hypothalamus and pituitary than in low regions such as the cortex and spinal cord. Intermediate peptide densities were present in the striatum, midbrain, thalamus and hippocampus. In all brain regions assayed, one major peak of immunoreactivity was present which had a molecular weight similar to that of thymosin α_1 and a minor peak which may represent a low molecular weight metabolite which is derived from the major peak.

The biological effects of thymosin α_1, thymosin α_7 and the partially purified thymosin fraction 5 were evaluated following intracerebral injection into unanesthetized adult mice through stereoxatically positioned guide cannulas. Antibody titers to SRBC were found to be significantly depressed in animals that received thymosin fraction 5 or thymosin α_7 when compared with saline injected control animals. In contrast, mice that received intracerebral thymosin α_1 had elevated antibody titers. No significant differences were observed in the mixed lymphocyte response or spleen cell response to LPS. However, spleen cell responsiveness to PHA and Con-A were significantly depressed in animals that received thymosin fraction 5. The possibility that some of these immunologic changes might have been due to an altered neuroendocrine axis was suggested by the observation that the pituitary glands and ovaries were slightly enlarged in the thymosin fraction 5 treated animals, but not in those that received thymosin α_1 or thymosin α_7.

Consequently, it is proposed that the endocrine thymus is part of a neuroendocrine axis that may play a prominent role in the mechanism by which the brain might influence the course of disease.

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CNS DEVELOPMENT IN INVERTEBRATES. J.G. Hildebrand (Chairman, Columbia Univ.), C.S. Goodman (Stanford), J.W. Truman (Univ. Washington), and D.A. Weisblat (U.C. Berkeley).

Much interest in developmental neurobiology focuses on the genesis of populations of neurons and the mechanisms that direct their interactions and sculpture them to form the CNS. This symposium samples recent advances resulting from efforts to probe cellular events in the development of the CNS in experimentally advantageous invertebrate preparations. Studying the leech CNS, D. Weisblat and coworkers have determined the embryonic origins of cells by injecting cell-lineage tracers into blastomeres early in development. Normally each of 4 bilateral pairs of ectodermal precursor blastomeres contributes distinct, stereotyped subpopulations of cells to the 32 segmental ganglia of the ventral nerve cord. Departure from the normal pattern follows ablation of specific blastomeres and can also result from fundamental ambiguities in the identity of certain blastomeres. To study the sequential choices made by growth cones of developing neurons, C. Goodman and associates examine living grasshopper embryos with Nomarski optics to observe early neuronal growth cones as they establish the first axonal pathways and subsequent growth cones as they choose between different axonal pathways. Experiments under way include manipulation of identified growth cones and the individual guide fibers they follow as well as use of monoclonal antibodies to explore cell surfaces of the neurons that lay down the early axonal pathways. J. Truman and coworkers explore the role and control of programmed neuronal death in shaping the developing adult CNS. The metamorphosis of moths is followed by a stereotyped program of death among mature neurons within the CNS. Both steroid and peptide factors influence the time of onset of this program, although neuronal interactions can selectively alter the time-course of the degeneration of certain cells. Sexual dimorphism in the CNS suggests plasticity in the development of ensembles of neurons. J. Hildebrand and coworkers find that sexually dimorphic neurons and neuropil structures in the olfactory pathway of the male moth brain develop as a consequence of direct interactions with olfactory afferents from male antennae. Surgical gynandromorphs reveal that male afferents can "masculinize" the female antennal lobe while a male lobe receiving innervation from a transplanted female antenna fails to show the characteristic, sexually dimorphic male elements.

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MECHANISM OF ACTION OF ANTIDEPRESSANTS. E. Costa (Chairman, NIMH); S.Z. Langer* (Cochairman, L.E.R.S.); F. Goodwin (NIMH); F. Sulser (Vanderbilt Univ.); K. Fuxe* (Karolinska Inst.); and S.H. Snyder (Johns Hopkins Univ. Sch. of Med.)

F. Goodwin will introduce current neurobiological theories on the etiology and pathogenesis of endogenous depression. Symptoms of depression will be analyzed in the light of these theories.

F. Sulser will discuss the basis for the belief that chronic treatment with typical or atypical antidepressants will decrease density and reduce responsiveness of β-adrenergic receptors located in brain. He will show that changes in density or function of α-adrenergic receptors are inconsistent responses to chronic antidepressants, that the reduced function of β-adrenergic receptors involves uncoupling from adenylate cyclase and actual loss of receptors, and that iprindole fails to produce subsensitivity in absence of signal input due to lesion of locus coeruleus. He will discuss the implications on psychobiology and pharmacotherapy of affective disorders.

K. Fuxe will show that subchronic treatment with zimelidine, desipramine, imipramine, alaproclate and mianserin leads to adaptive changes in 5HT receptor mechanisms. The major finding seems to be the induction of large numbers of low affinity binding sites for 5HT-1 receptors leading to a possible stabilization of 5HT neurotransmission at the 5HT-1 receptors, while a reduction at 5HT-2 receptors is induced. Long-term treatment with zimelidine, desipramine, and imipramine also reduces 5HT dependent behavior in mice and rats. Thus, it is speculated that 5HT neurotransmission may be reduced and mainly switched over to 5HT-1 receptors following subchronic antidepressant treatment.

S. Langer will report the progress of the study of high affinity labeling of specific receptors with various antidepressants. The relationship between imipramine binding and 5HT uptake indicates an involvement of presynaptic 5HT mechanisms in the action of antidepressants.

E. Costa will show that the high affinity binding sites for desipramine are not located exclusively on 5HT presynaptic mechanisms. Moreover, mianserin, an atypical antidepressant, lacks presynaptic binding on 5HT neurons. Based on the simultaneous binding of antidepressants to various synaptic mechanisms, he suggests that the action of synaptic modulator(s) acting as receptor regulators in a number of synapses may be modified by antidepressants.

S. Snyder will stress that the antidepressants because of their clinical similarities may act on a final pathway though the specific mechanisms to attain the pathway may vary. Common themes seem to be down-regulation of NE dependent cyclase, the number of β-adrenergic receptors, the number of 5HT-2 receptors, and 5HT uptake mechanisms.

75.1 SPINALLY PROJECTING, SEROTONIN STAINED NEURONS CO-EXIST WITH ONE OR MORE PEPTIDES. R.M. Bowker*, K.N. Westlund, and J.D. Coulter. Depts. Psychiat. and Physiol. and Marine Biomedical Institute, Univ. of Texas Medical Branch, Galveston, TX. 77550.

The origins of spinally projecting neurons that stain for serotonin, substance P, leucine- and methionine-enkephalin and somatostatin immunoreactivity were localized in rats using a combined method of the retrograde transport of HRP and the immunocytochemistry. Injections of HRP were made into different spinal cord levels of anesthetized rats. The spinally projecting neurons were identified by incubating the tissue sections in $CoCl_2$ and diaminobenzidine prior to the immunocytochemical staining for the respective antisera using the unlabeled antibody, peroxidase-antiperoxidase (PAP) method of Sternberger (Brain Res. 211, 1981). Serotonin stained neurons which projected to all spinal cord levels were distributed throughout the medullary raphe nuclear complex in the nucleus raphe obscurus, raphe pallidus, raphe magnus and the ventral parts of the nucleus gigantocellularis. The locations of these descending serotonin stained neurons correspond to cell groups B_1-B_3 of Dahlström and Fuxe (Acta Physiol. Scand., 1965). The quantitative data to date indicate that over 85% of the retrogradely labeled cells in the raphe nuclei and ventral reticular formation were found to be positively stained for serotonin. In colchicine treated animals large numbers of neurons stained positively for substance P, enkephalin and somatostatin immunoreactivity were distributed in these same midline raphe and ventral reticular nuclear groups. Other medullary cell groups, such as spinal nucleus of the trigeminal complex and nucleus of the solitary tract, were also found to contain positive peptidergic neurons. Of the spinally projecting neurons in the raphe nuclei and ventral nucleus gigantocellularis over 60% and nearly 50% were found to be stained for substance P, and enkephalin-like immunoreactivity, respectively. Fewer spinally projecting neurons contained positive immunoreactivity for somatostatin. These findings suggest that a large fraction of the spinally projecting neurons in the raphe nuclear complex and ventral reticular formation have serotonin co-existing with one or more neuropeptides.

(Supported by NIH Grants NS 12481 and NS 11255).

75.2 DISTRIBUTION OF ENKEPHALIN, SUBSTANCE P AND SEROTONIN IMMUNOREACTIVITY IN THE VISUAL SYSTEM OF THE LOBSTER, CRAYFISH AND HORSESHOE CRAB. Jorge Mancillas*, Jacqueline McGinty+ and Allen Selverston++ (SPON: W. Kristan). Depts. of Neuroscience* and Biology++, Univ. Calif., San Diego and Salk Institute+, La Jolla, CA

Neuroactive peptides first characterized in mammalian nervous systems have been recently detected in invertebrates. We report that immunoreactivity for enkephalin, substance P and the indoleamine serotonin can be localized to specific neural elements in the visual system of the spiny lobster, crayfish and limulus.

The indirect immunocytochemical technique was used. Frozen sections of lobster and crayfish eyestalks and the lateral eyes of limulus were incubated in leu-enkephalin (at 1:1000), substance P (1:100) and 5-HT (1:1000) antisera, which was then localized with an FITC labelled IgG. Specificity of the immunoreactive staining was tested by incubation with antisera previously absorbed with their respective synthetic antigen (at 0.1, 1, 10 and 100 micromolar concentrations) and by substituting the primary antibody with non-immune serum or PBS.

The patterns of immunoreactive staining were almost identical in the crayfish and lobster. Enkephalin-like immunoreactivity (lir) was observed in the primary photoreceptors (retinular cells) and their projections to the lamina ganglionaris, as well as in the medulla interna and terminalis. Additional enkephalin-lir terminals and cell bodies were observed in the medulla externa of the crayfish. Substance P-lir was present in perikarya, fibers and terminals of identifiable cell types in the lamina, medulla interna, externa and terminalis. Substance P-lir was also observed in cell bodies of the medulla terminalis X organ and in neurosecretory terminals in the sinus gland. 5-HT-lir was present in cell bodies, terminals and fibers in the medulla terminalis, interna and externa. All three substances were also present in the optic nerve. Interestingly, enkephalin and substance P-lir was observed in synaptically related cells, the retinular cells and the "neurosecretory" cells of the lamina, both of which terminate on the ganglion cells. The role of these substances as possible neurotransmitters (especially enkephalin in the photoreceptors) and their possible interactions are currently under study.

In the limulus lateral eye, only substance P-lir was observed in centrifugal fibers travelling through the optic nerve, the lateral plexus and ending around the edges of the ommatidia and the cornea. Preliminary evidence suggests that substance P can induce morphological changes in the ommatidia that may be associated with centrally controlled circadian changes in sensitivity or environmentally induced light-dark adaptation. These possibilities are being tested neurophysiologically.

75.3 COEXISTENCE OF GLUTAMATE DECARBOXYLASE IMMUNOREACTIVITY AND SOMATOSTATIN-LIKE IMMUNOREACTIVITY IN NEURONS OF NUCLEUS RETICULARIS THALAMI OF THE CAT. W.Oertel$^{\Phi}$,A.M.Graybiel+,E.Mugnaini$^{\Omega}$,R.Elde+,D. Schmechel*@ and I.Kopin$^{\Phi}$.NIMH$^{\Phi}$,MIT+,U.Conn$^{\Omega}$,U.Minn.+,Duke Univ.@

Several instances of coexistence of neuropeptides and biogenic amines have been reported in peripheral and central neurons (Hökfelt et al,Nature 284,1980;285,1980).To date, however,no peptide has been localized in neurons that release the inhibitory neurotransmitter γ-aminobutyric acid(GABA). In the present study we investigated by immunocytochemical methods the possibility that glutamate decarboxylase (the biosynthetic enzyme for GABA) immunoreactivity(GAD) and somatostatin-like immunoreactivity(SOM) coexist in neurons of the feline nucleus reticularis thalami. Nine young cats were perfused with buffered(pH7.4) 4% paraformaldehyde with or without 0.1% glutaraldehyde. Vibratome or frozen sections (25μm) were processed either according to the preembedding unlabeled antibody enzyme method of Sternberger or the immunofluorescence technique with specific antisera against somatostatin(Elde and Parsons,Am.J.Anat.144,1975) and GAD(Oertel et al,Neuroscience,in press). On light microscopic examination of adjacent sections most of the neurons appeared to react for GAD (Houser et al,Brain Res.200,1980) and SOM(Graybiel et al,Anat.Rec 199,1981).In the electronmicroscope strong SOM was localized exclusively in the Golgi-apparatus; weak GAD was found in the cytoplasm and proximal dendrites,medium GAD in the Golgi-apparatus and very strong GAD in boutons. To demonstrate coexistence of these two antigens which have a different intracellular distribution,a sequential double staining method was employed:Sections were incubated in rabbit anti-somatostatin,followed by fluorescein coupled swine anti-rabbit. After fluorescence photography the sections were incubated in sheep anti-GAD,donkey anti-goat, goat peroxidase anti-peroxidase complex and 3',3-diaminobenzidine (DAB).Many neurons were stained for SOM(cytoplasmic skeins,green) and GAD (rather homogeneous reaction product,brown). In addition a double immunoperoxidase method was used to simultaneously visualize SOM with 4-chloro-1-naphthol(blue) and GAD with DAB (brown) with bright field microscopy. Despite the weak cross-reactivity of the two linking antibodies (control experiments), the differential distribution of SOM and GAD allowed the use of these sequential protocols without antibody elution.

This study strongly suggests that in the nucleus reticularis thalami of the cat,most neurons are GABAergic and also contain a compound immunohistochemically indistinguishable from the inhibitory neuropeptide somatostatin.

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75.4 NEUROTENSIN IMMUNOREACTIVE SYSTEMS IN THE CENTRAL NERVOUS SYSTEM OF RAT. L. Jennes*, W.E. Stumpf, W.C. Beckman*, S.K. Burgess, D. Luttinger, C.B. Nemeroff and A.J. Prange, Jr. Dept. Anatomy and Biological Science Research Center, Univ. North Carolina, Chapel Hill NC 27514.

The distribution of neurotensin (NT)-containing structures in the central nervous system has been studied with immunohistochemical techniques. Male and female adult Holtzman rats received an intraventricular injection of colchicine (50 μg/100g body weight) 48 h before sacrifice by intracardiac perfusion with 4% paraformaldehyde. Immunohistochemistry was performed on vibratome and thick frozen sections using Mason's single bridge technique. The primary antiserum was shown to react with the C-terminal portion of the NT molecule by radioimmunoassay analysis of the binding of NT analogues with single D-amino acid substitutions. The antiserum was purified on a Protein-A column or on a DE 52 ion exchange column and exhibited no cross-reactivity in the radioimmunoassay with GnRH, β-endorphin and bombesin. Highest accumulations of NT immunoreactive cell bodies are found in the lateral portion of the lateral septum, the bed nucleus of the stria terminalis, the diagonal band, the nucleus (n.) paraventricularis pars parvocellularis, n. arcuatus, n. periventricularis thalami and the medial and central amygdala. Dispersed NT-containing neurons exist in the lamina fibrosa of the olfactory bulb, septo-preoptic region including the n. triangularis septi, medial forebrain bundle, zona incerta, n. ventralis thalami and central gray. In the lower brainstem, NT-containing neurons are seen in the n. raphe dorsalis and centralis, locus ceruleus, n. tractus solitarii, n. tractus mesencephali and formatio reticularis. A few neurons are located in the substantia gelatinosa and in the area surrounding the central canal. Neurotensin positive nerve fibers are seen in all regions which contain immunoreactive neurons. In addition NT fibers are present in the olfactory bulb in the n. olfactorius anterior, in different layers of the cortex, claustrum, caudate-putamen, globus pallidus, n. accumbens, amygdala with highest concentrations in the n. centralis and medialis, certain thalamic structures and in sensory projections of the trigeminus and the substantia gelatinosa, as well as other layers in the dorsal and central spinal cord. In addition to neurons, certain cells in the pituitary and in the subrachoroid space react positively with the antibodies. The results demonstrate that the neurotensinergic system is an extensive neuropeptide system, probably involved in the regulation of neuroendocrine, autonomic, sensory, and motor functions. Supported by PHS NS09914, NIMH MH-32316, 34121, NICHHD HD-03110 and Deutsche Forschungsgemeinschaft Je 105/2.

75.5 PRO ACTH/ENDORPHIN ANTIGENICITIES IN MEDULLARY NEURONS OF THE RAT[1]. D.G. Schwartzberg* and P.K. Nakane* (SPON: R. Lasher). Department of Pathology, University of Colorado Health Sciences Center, Denver, CO 80262.

Immunohistochemically, neurons containing pro ACTH/endorphin antigenicities (PACTH-Ag) have been reported to be confined to the basal hypothalamus (Krieger, D.T., New England J. Med. 304: 876, 1981). Antigenicities found outside of the diencephalon (Watson, S.J. et al., Nature 275:226, 1978) are thought to be derived from hypothalamic neurons. In the course of the investigation of the morphogenesis of neurons containing PACTH-Ag, a group of neurons in the medulla also containing PACTH-Ag was demonstrated immunohistochemically.

For this study the indirect peroxidase-labeled antibody method was used. Affinity purified rabbit antisera against (1-9) β-endorphin, N-terminal ACTH, and rabbit anti-16K fragment, all donated by Drs. Mains and Eipper, and rabbit anti-tyrosine hydroxylase (TH), donated by Dr. J.K. Stephens, were used as the first antibody; affinity purified peroxidase-labeled Fab' fragment of sheep anti-rabbit IgG was used as the second antibody.

Perikarya immunoreactive for all 3 major determinants of PACTH-Ag exist within the medulla adjacent to TH-containing neurons of the A2 system, and contribute axons to superficial basolateral tracts. The medulla is not a known site of action for ACTH-related peptides. However, noradrenergic neurons of the A2 cell group have been implicated in the regulation of sexual function (Sar, M., Nature 289:500, 1981), and β-endorphin exerts central effects on luteinizing hormone secretion (Parvizi, N., Nature 286:812, 1980). The anatomic association of medullary neurons containing PACTH-Ag, with A2 neurons suggests that these two systems may interact to control sexual function.

[1]Supported in part by NIH grants (AI 09109, CA 15823, and AM 06569) and a gift from R.J. Reynolds, Inc.

75.6 THE PRESENCE OF SUBSTANCE P-LIKE AND SOMATOSTATIN-LIKE IMMUNOREACTIVITY IN THE ABSENCE OF UNMYELINATED PRIMARY AFFERENT INPUT IN THE SPINAL CORD OF THE STINGRAY. Teresa C. Ritchie and R.B. Leonard, Marine Biomed. Inst. and Dept. of Physiology and Biophysics, the Univ. of Texas Med. Branch, Galveston TX 77550.

Previous studies in mammals have indicated the presence of substance P or substance P-like immunoreactivity in various regions of the spinal gray horns, and emphasis has been placed on its association with the primary afferent input to the spinal cord. Specifically, substance P (SP) and somatostatin (SS) have been localized in small neurons of the DRG's and fibers in Lissauer's tract. Terminal labeling is dense in the superficial laminae of the dorsal horn, the targets of fine myelinated and unmyelinated fibers. This study was undertaken to examine the distribution of SP and SS in the spinal cord of the Atlantic stingray, Dasyatis sabina, an animal in which unmyelinated axons comprise only 0.4% of the total population of primary afferent fibers in spinal nerves and roots. However, despite the virtual absence of unmyelinated primary afferents, histological examination of the spinal cord reveals a large structure of the superficial dorsal horn resembling the substantia gelatinosa of mammals.

In this study, the distribution of SP-like and SS-like immunoreactivity was studied with antibodies to these peptides, using the unlabelled antibody, PAP technique of Sternberger. Controls consisted of preabsorption of the antisera with excesses of their respective antigens.

Sp-like immunoreactivity was found in a band occupying approximately the outer one-half of the substantia gelatinosa. The labeling was most dense in the lateral third of this structure, around the root entry zone. Scattered label also occurs in deeper regions of the dorsal horn and in the ventral horn. Preliminary results indicate a reduction in labeling in the substantia gelatinosa following multiple dorsal rhizotomies.

SS-like immunoreactivity also occurs in the stingray spinal cord. Fascicles of labeled fibers are seen running through the nucleus proprious and substantia gelatinosa, and a narrow band of presumptive terminals is seen along the superficial margin of the substantia gelatinosa. These fibers may originate from either the dorsal root or from a prominant tract in the dorsolateral funiculus. In addition, some fibers turn ventrally to distribute in the ventral horn.

These findings demonstrate the presence of both SP-like and SS-like immunoreactivity in the spinal cord of an elasmobranch which is characterized by a virtual absence of unmyelinated primary afferents. Additionally, preliminary results indicate that a portion of the labeling is associated with dorsal root input. Supported by grants NS 16093 and NS 11255.

76.1 ELECTROPHYSIOLOGY OF CULTURED HYPOTHALAMIC NEURONS: CA-DEPENDENT PLATEAU POTENTIALS. P. Legendre *, I. Cooke and J.D. Vincent. (SPON: B. Bioulac). INSERM U. 176, 33077 BORDEAUX-CEDEX, France.

All-or-none plateau depolarizing responses with durations of mins are recorded intracellularly (KCl electrodes) from the largest neurons (type (a), Theodosis et al., this vol.) of antibiotic-free primary cultures of dispersed fetal mouse hypothalamus 40 days or more old. During recording, the culture is continuously perfused with Hank's medium supplemented with Ca (final conc., 4 mM), glucose (50 mM), and HEPES (10 mM, pH 7.1), 32° C. The neurons exhibit a constant barrage of depolarizing synaptic activity. From this, or in response to depolarizing current arise three types of regenerative response: 1) overshooting action potentials (APs) of 2-5 msec (at 1/2 ampl.). 2) APs having a short plateau on their falling phase. This has variable duration, up to 2 sec, and reaches a depolarized level of -30mV. 3) Plateau responses of long duration. These arise from the falling phase of an AP or the short plateau potential, and take 50 msec to depolarize to the plateau, during which up to 8 impulses of declining amplitude may occur. The plateau level, -20mV, is constant from neuron to neuron and independent of cell holding potential (V_h), -50 to -60mV. Duration of the response varies from 1/2-5 min. During the plateau, cell input resistance (80-150 MΩ) is decreased 85%. The plateau gradually declines to -25mV, then a rapid (5-10 sec) repolarization to within 10mV of V_h follows with a parallel increase in resistance. The final repolarization requires 1/2-2 min. During slow repolarization, synaptic activity or current evokes type 2 responses; the amplitude and duration of their short plateau become progressively larger until a long plateau is again evoked. In some neurons having strong synaptic input, long plateaus are driven to recur regularly. The period (1/2-7 min) is correlated with the duration of plateau. Some neurons show plateaus only in response to brief (10 msec) depolarizing current. TTX reversibly blocks synaptic input and impulses, but does not change the amplitude or time course of plateaus. Presence of Co or Cd reversibly blocks plateaus. Ejected during a plateau, they provoke repolarization. If applied briefly and early during the plateau the plateau may redevelop. TEA doubles the duration of plateaus. Thus plateaus are endogenous, regenerative depolarizing responses involving a voltage-dependent Ca-conductance increase. Their regular recurrence and duration is remeniscent of patterned activity of vasopressin (VP) neurons in vivo (Poulain et al., Proc. R. Soc. B 196: 367, 1977). Long plateaus have only been observed in neurons of the morphological type (a) which sometimes react with VP antisera (Theodosis et al., this vol.).

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76.2 DENDRITIC AND SOMATIC ACTION POTENTIALS IN SINGLE PURKINJE CELLS CHANGE $[K^+]_o$ and $[Ca^{2+}]_o$. J. Hounsgaard* and C. Nicholson (SPON: R.P. Kraig). Dept. of Physiol. and Biophys., New York Univ. Med. Ctr., 550 First Avenue, New York, N.Y. 10016.

While syncronous activation of large aggregates of neurons substantially changes $[K^+]_o$ and $[Ca^{2+}]_o$ (Nicholson, 1980; Neurosci. Res. Prog. Bull 18: 177-322) little is known about the ion changes around single neurons under normal levels of impulse activity. We have recorded the $[K^+]_o$ and $[Ca^{2+}]_o$ in the vicinity of visually identified Purkinje cell bodies in guinea pig cerebellar slices (Llinás and Sugimori, 1980; J. Physiol. 305: 171-195) using ion selective microelectrodes.

Intermittantly active cells were selected for the experiments. Spikes of somatic and dendritic origin were clearly distinguishable when monitored with the reference barrel of an ion-selective electrode positioned close to the cell body. Na^+-mediated somatic spikes with frequencies of 30-40 Hz in the early phase of an active period were associated with an increase of $[K^+]_o$ to a steady level approaching 1mM above base line (6mM). The onset of Ca^{2+}-mediated dendritic spikes in the terminal phase of an active period produced a dramatic increase in $[K^+]_o$ in the soma region reaching levels 3-4mM above baseline. The K^+ activity returned to the 6mM level of the superfusing medium within the first 10s of a silent period.

In addition to spike frequency, the amplitude of the K^+ signal was clearly related to the distance between the tip of the electrode and the cell membrane since it changed in parallel with the amplitude of the action potentials when the electrode was moved. The duration of the action potentials also seemed to be an important determinant of the K^+ signal. Single somatic spikes lasting 0.5 msec gave no measurable increase in $[K^+]_o$ while single dendritic spikes lasting 10 ms were associated with a distinct transient increase in $[K^+]_o$. These long duration spikes and associated K^+ signal were insensitive to tetrodotoxin. Spikes lasting several seconds appeard when 1mM Ba was substituted for 1mM Ca in the bathing solution. These Ba^{2+}-mediated spikes evoked an increase in $[K^+]_o$ of several mM.

Spike activity also changed the $[Ca^{2+}]_o$ level around Purkinje cell bodies, but the changes were small (≤ 0.2mM) and in the opposite direction to the K^+ changes.

The possible functional significance of the results and the capability of ion selective electrodes to determine the location of ion conductances will be discussed.

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76.3 MODIFICATION OF GABA RESPONSE BY REPETITIVE ACTIVATION IN THE HIPPOCAMPUS. D.J. Watkins and R.K.S. Wong. Dept. of Physiol. LSU Med. Ctr. Shreveport, LA 71130.

There is now a considerable amount of neurochemical and pharmacological data suggesting that GABA may function as an inhibitory neurotransmitter in the hippocampus. Our recent studies showed that GABAergic inhibition activated during orthodromic stimulation suppressed the elicitation of endogenous burst firing in the soma-dendritic regions of the hippocampal pyramidal cell. Recent studies also suggest a decrease in the efficacy of the inhibitory process may occur upon repetitive stimulation of the efferent pathways at frequencies of 10/sec or higher. The present study is designed to examine whether modification of the inhibitory process induced by repetitive activation could in part be accounted for by alterations in the postsynaptic response to GABA. The in vitro hippocampal slice preparation was used in the study. GABA was applied by pressure pulses onto the slice. Typically 3 msec pulses of 30 psi amplitude were used to eject GABA (10^{-3} M) from a 30 MΩ pipette. The amount of GABA ejected in this fashion was calculated to be about 10^{-15} M. Intracellular recordings from CA1 pyramidal cells showed that a pulse of GABA applied at the somatic region typically produced an increase in membrane conductance with an associated hyperpolarization and a suppression of spontaneous firing at the resting potential. Hyperpolarizing the cell by current injection reduced the amplitude of the GABA induced hyperpolarizing (H) response and revealed an additional depolarizing (D) response. These responses exhibit interesting use-dependent changes. When paired pulses of GABA was applied at short intervals (up to 12 sec), the H response to the second application decreased in amplitude and the D response appeared to facilitate. These changes in the second GABA response could be observed for intervals up to 12 seconds following the initial GABA response. Additional experiments showed that similar changes could also be induced in the CA1 pyramidal cell by tetanic stimulation of the Schaffer Collateral pathway. The D response could increase 3-4 folds while the H response attenuated following tetenic shock. This alteration of the GABA response could last up to a minute following the tetanic stimulation. Our data demonstrate that a phasic application of GABA produces sustained changes in the response of the hippocampal pyramidal cell to GABA such that the inhibitory action of GABA to a subsequent application is attenuated. These changes in GABA response could also be induced by tetanic stimulation suggesting that they may play a physiological role in regulating the efficacy of the inhibitory transmission in the hippocampus. (Supported by NIH Grant NS16606. R.K.S.W. is a Klingenstein Fellow in Neuroscience.)

76.4 GENERATION OF SYNCHRONIZED BURSTING IN POPULATIONS OF HIPPOCAMPAL NEURONS. R.K.S. Wong and R.D. Traub. Dept. of Physiol. LSU Med. Ctr. Shreveport, LA 71130 and IBM Watson Res. Ctr. Yorktown Hts. NY 10598.

When the GABAergic inhibitory process is blocked by pharmacological agents in the hippocampus, spontaneous and evoked synchronized oscillations are often recorded in populations of neurons. The present study represents an initial attempt to define some of the cellular processes underlying these synchronized oscillations. The in vitro hippocampal slice preparation was used. Extracellular recordings in the CA1 region showed that upon disinhibition, stimulation of the Schaffer Collateral pathway often elicited two phases of synchronized bursting. A first phase followed the stimulus at short, fixed latencies (5-10 msecs) and a second phase occurred at longer, more variable latencies (50-150 msec). These two phases of synchronized bursting could be further differentiated by the fact that while the first phase activity could follow the stimulus input in a 1:1 fashion up to 5Hz, the second phase activity had much longer refractory periods and could follow the stimulus frequency only up to about 0.3Hz. Intracellular recordings showed that burst firing was elicited in CA1 pyramidal cells during both phases of synchronization. The data also suggest that both phases of bursting were triggered by underlying excitatory postsynaptic potentials (EPSPs). The EPSP triggering the first phase bursting was elicited directly by the electrical stimulation of the Schaffer Collaterals. The EPSP underlying the second phase bursting followed the occurrence of synchronized bursting in the CA2-CA3 region of the hippocampus. Additional data showed that synchronized bursting could be elicited by orthodromic activation of neurons in a surgically isolated CA2 region of the hippocampal slice. This synchronized bursts had the following characteristics: (a) They could be elicited nonspecifically by the stimulation of any excitatory afferent input or by antidromic activation. (b) Their latency of occurrence was prolonged (50-150 msec). Our results suggest that upon disinhibition orthodromic stimulation of the CA1 cells directly elicit a phase of short latency synchronized bursting of these cells. In addition, the excitatory volley also triggers long latency, intrinsically generated synchronized bursting of the cells in the CA2-CA3 region. The excitatory volley arising as a result of the synchronized activity in CA2-CA3 region in turn projects onto the CA1 neurons, producing a second phase synchronization in the CA1 population. The cellular mechanisms for the generation of synchronization in the CA2 cell group is discussed in a companion abstract (Traub and Wong, this volume). (Supported by NIH Grant NS16606, R.K.S.W. is a Klingenstein Fellow in Neuroscience.)

76.5 MULTIPLE BINDING SITES FOR OUABAIN IN THE CENTRAL NERVOUS SYSTEM. R. Hauger*, H.M. Do*, F.K. Goodwin, and S.M. Paul*. (SPON: F. Bruns), Clinical Psychobiology Branch, NIMH, Bethesda, MD 20205.

[^{3}H]Ouabain has been previously shown to bind specifically to multiple brain areas in the cat with equilibrium dissociation constants (K_D) ranging from 510 to 2000 nM (Eur. J. Pharmacol. 56:297-304, 1979). Since [^{3}H]ouabain binding sites in the human erythrocyte exhibit a much higher binding affinity (K_D = 11.3 nM) (Clin. Pharmacol. Ther. 28:346-349, 1980), we examined whether "high affinity" binding sites for ouabain exist in rat brain as well. Specific binding of [^{3}H]ouabain was readily demonstrated in homogenates of rat brain incubated with varying concentrations of [^{3}H]ouabain (1 to 1000 nM) and 5 mM ATP for 30 min. at 37°C. Scatchard analysis revealed the presence of at least two distinct binding sites in all areas of the brain examined. In the rat striatum a "high affinity" site characterized by an apparent K_D of 17.4 nM and a binding capacity (B_{max}) of 12.0 pmoles/mg protein and a "low affinity" site with an apparent K_D of 400 nM and B_{max} of 36.0 pmol/mg were present. In contrast, the "high affinity" site was absent from heart (left ventricle) and kidney. Ouabain and strophanthidin competitively inhibited ouabain binding in the brain with potencies similar to their inhibition of Na^+ - K^+ ATPase. Following lesions of the rat striatum with kainic acid, a marked decrease (40-60%) of both the "high affinity" and "low affinity" ouabain binding sites was observed. These results suggest that both binding sites are localized to neurons. This hypothesis was further supported by studies on postmortem brain samples from patients with Huntington's disease; where a marked decrease in [^{3}H]ouabain binding was observed in the caudate nucleus when compared to matched controls. [^{3}H]Ouabain binding appears to be a useful biochemical probe of neuronal Na^+-K^+ ATPase activity.

76.6 ASSESSMENT OF INJURY IN INTRACELLULARLY PENETRATED NEURONS OF THE CAT MOTOR CORTEX. C.D. Woody, E. Gruen*, and K.McCarley*Depts. of Anatomy & Psychiatry, UCLA Medical Center, Los Angeles,CA 90024

Many investigators have employed intracellular recording to assess unit activity in conjunction with local membrane properties,yet few studies have been directed toward assessment of possible injury arising from cell penetrations. The present studies compared the response of penetrated neurons to repeated click stimuli with that of unpenetrated (extracellularly recorded) units of the same cortical region. Recordings were made in awake cats using methods described elsewhere (Woody and Black-Cleworth, J.Neurophysiol.,1973). Penetrated neurons were pressure-injected with HRP at the conclusion of each study. Of 92 HRP-injected neurons, 30 showed only dendritic processes on examination of serially sectioned core biopsies. All but one of these cells were characterized by action potentials (AP) of amplitude smaller than the recorded resting potentials (RP), i.e. spike undershoot. These recordings were thought to represent dendritic penetrations with electrotonic spread of the spike through passive cables. Analysis of the HRP-injected cells showed that the penetrations with dendritic recoveries had higher input resistances than those with recoveries of both somas and dendrites (24±11 MΩ, s.d., and 18±12 MΩ, respectively, $P < .01$,Student t). Increases in spike height at the time of pressure injection were greater in spike undershoot than in spike overshoot cells (13±7 mV versus 11±5 mV). Increases in spike height were also greater in recordings with only dendrites recovered (16±8 mV) than in recordings with somas and dendrites recovered (11±7 mV). The latter differences were significant at $P < .01$. Responses to click were obtained from 83 penetrated neurons. The data from these neurons were separated into four groups according to the size of the recorded action potential. The magnitude of the response to click was much the same in three of the groups (AP 50-60 mV, AP 40-50 mV, and AP 30-40 mV) and slightly greater in the group with AP 20-30 mV as was the level of spontaneous activity. The response profiles (PSTH to click) were comparable to those of extracellularly recorded units (Woody et al, J.Neurophysiol., 1970,72). Studies using K^+ ion-sensitive microelectrodes intracellularly found comparable values of E_{K^+} with either spike undershoot or spike overshoot penetrations. We conclude that whatever injury arose from the penetrations of cortical neurons performed herein was not sufficient to impair the ability of the cells to respond with spike activation to natural stimuli such as weak click. Many of the penetrations appeared to be of passive dendritic cables rather than of cell somata. (Supported by AGO - 1754 and BNS 78-24146)

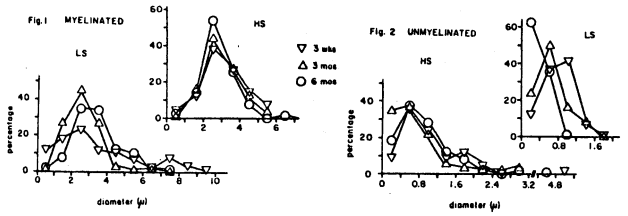
77.1 CORRELATION OF NEUROMA ULTRASTRUCTURE WITH AUTOTOMY IN RATS. M.M. Bosma* and W.K. Dong. Dept. of Anesthesiology, School of Medicine, University of Washington, Seattle, WA 98195.

Experimental amputation neuromas, as a model of anesthesia dolorosa, are known to be accompanied by autotomy of the denervated portion of the foot. Changes in neural structure may contribute to the abnormal firing patterns underlying the putative pain-producing mechanisms.

Sciatic nerve neuromas were produced and the severity of autotomy scored as described by Wall et al. (Pain, 7:103, 1979). Rats paired for high (7-10) and low (1-4) scores were sacrificed at 3 wks, 3 mos, and 6 mos. The most distal portion of the neuroma was prepared for EM. Each sample was scanned at low magnification and a representative area chosen for morphometry. Pictures were systematically taken at 3290X and 6680X. The 3290X series was used to measure myelinated (M) axon size, and to compare total numbers of M and unmyelinated (U) axons. The 6680X series was used to measure U axon size. Equal areas were sampled in each rat. Only areas of the nerve perpendicular to the section were sampled, but axons shaped or oriented abnormally were not measured. About 150-300 axons were sampled at each magnification.

Rats with high autotomy scores (HS) had U:M ratios of about 6:1; those with low score (LS) had ratios of about 1:1. Comparing M axons in the two groups showed that LS rats have a wider range of axon diameters (Fig. 1). However, the M diameters in both groups were smaller than in normal sciatic nerves. The HS rats had a wider range of U axon diameters than the LS rats (Fig. 2). LS rats showed a reduction in U axon diameter with longer survival times. All HS rats had a group of unusually large U axons. These large axons were neither watery, nor swollen with organelles or fibrils; they were grouped with other U axons wrapped in Schwann cell cytoplasm.

The correlation of this unusual ultrastructure with autotomy over long survival times may elucidate some of the anatomical substrates involved in painful sensation or paresthesia from amputation neuromas. (Supported by USPHS Grant NS 16329)



77.2 AFFERENT FIBERS IN MERIDIAN POINTS AND THEIR ROLE IN ACUPUNCTURE ANALGESIA. G.W. Lu* (SPON: M. HOFFERT). Dept. of Neurophysiology, Beijing Second Medical College, Beijing, China.

We studied the relation of peripheral afferent fibers to meridian points and acupuncture analgesia on 308 rabbits, 15 cats, 71 normals and 845 patients. We found: a) compared to non-meridian points, points zusanli possess a special axon diameter spectrum characterized by more myelinated (myelinated/unmyelinated = 2.7), more large-sized ($A\alpha\beta\gamma/A\delta$ = 2.8), and more $A\beta\gamma$ ($A\beta\gamma/A\alpha\beta\gamma\delta$ = 52) fibers. b) The velocity spectrum of afferent fibers in points zusanli also showed a special pattern. The frequency of $A\alpha$, $A\beta\gamma$, and $A\delta$ was 7.9%, 67.8% and 24.3%, respectively. The ratio of $A\alpha\beta\gamma$ to $A\delta$ was 3.1. c) The analgesic effect induced by needling points zusanli was markedly reduced by blocking the activity of $A\beta\gamma$ and some $A\delta$ fibers. The same analgesic effect could be produced by direct stimulation of $A\beta\gamma$ fibers. d) The disappearance of the sensation produced by needling acupoints occurred between the loss of touch and pain sensations during limb compression, extradural and spinal anesthesia. Adequate needling sensation was accompanied by $A\beta\gamma$ and some $A\delta$ fiber activity. e) In n.VPL of the thalamus the unitary activity activated by peripheral C fiber stimulation was markedly inhibited by the conditioning activity of $A\beta\gamma$ fibers, decreasing the frequency and duration of its late discharges. Activity induced by peripheral $A\beta\gamma$ fiber stimulation, however, could not be modulated by the conditioning activity of both $A\beta\gamma$ and C fibers. f) The conditioning activity of $A\beta\gamma$ and some $A\delta$ fibers could significantly prolong the refractory period of cortical neuron populations and decrease the amplitude of the primary component of the evoked potential: P2>N1>P1. g) Analgesia was significantly increased in experimental and clinical surgery when meridian points were stimulated and electro-stimulation was applied to the surgical field through the surgical instruments. Using two tourniquets alternately on two places in the same extremity markedly reduced the tourniquet reaction and increased acupuncture analgesia. h) By injection of dilute anesthetic solution into acupoints the skin pain threshold and the efficacy of acupuncture analgesia were significantly raised. We believe that (1) the predominance of large afferent fibers (mainly $A\beta\gamma$ fibers) is one of the structural and functional characteristics of the meridian points; (2) needling impulses arising from meridian points are transmitted along the $A\beta\gamma$ and some $A\delta$ afferent fibers; (3) the inhibitory action exerted by large fiber activity on small fiber activity in the CNS is one of the mechanisms of acupuncture analgesia; (4) increased activation of large fibers and/or decreased activity of small fibers is one means of promoting acupuncture efficiency.

77.3 UNMYELINATED AXONS IN THE DORSAL WHITE COLUMNS OF HUMANS & RATS. Lauren A. Langford and Richard E. Coggeshall. Depts. of Anatomy & of Physiology and Biophysics & the Marine Biomedical Institute. The University of Texas Medical Branch, Galveston TX 77550

The dorsal white column of the mammalian spinal cord is said to consist of myelinated primary afferent fibers. Recently, however, unmyelinated fibers were seen to be more numerous in rats. Because of the role of this pathway in somatic sensation and because stimulation of this pathway is reported to influence pain, it is important to determine how many unmyelinated fibers are present in humans. The present study reports the results from two humans indicating that the unmyelinated fibers are predominant in this pathway in humans. If these preliminary data are confirmed, the presence of these fibers must be considered when stimulation of the dorsal white columns is done and a functional understanding of these fibers is of considerable interest.

To determine the function of the unmyelinated axons in the dorsal white columns, it is necessary to use experimental animals. Our first step is to determine where the fibers originate in rats. Dorsal rhizotomies remove primary afferent fibers, spinal transections remove ascending or descending fibers, and isolation of a spinal segment removes all fibers but propriospinal axons. Our preliminary results are shown in the following table:

	DORSAL COLUMN PROPER		CORTICOSPINAL TRACT		TOTAL	
	MY	UN	MY	UN	MY	UN
UNILATERAL DORSAL RHIZOTOMY L5, L6 and S1						
L6 NOR	14,442	15,238	12,752	14,721	27,194	29,959
OP	5,687	7,370	5,107	7,450	10,794	14,820
S2 NOR	4,229	5,787	2,882	3,536	7,111	9,323
OP	3,352	4,182	4,065	4,596	7,417	8,778
TRANSECTION C4						
S2	6,662	3,556	1,503	1,179	8,165	4,735

Note that there is a decrease in the unmyelinated fibers after each of the above surgical procedures. The greatest decrease occurs with the cervical transection. Our preliminary conclusions are that there are a significant number of primary afferent, long descending, and propriospinal unmyelinated axons in rat dorsal white columns. The largest group seems to be descending axons which originate above the mid-cervical level, but the presence of significant numbers of unmyelinated sensory fibers in the dorsal white columns is also of interest. Supported by grants NS 10161, NS 07377 and NS 11255 from NIH.

77.4 SYMPATHETIC ACTIVITY SHOWN TO HAVE NO SHORT-TERM EFFECT ON POLYMODAL NOCICEPTORS IN CATS. William J. Roberts and Amy U. Lindsay*, Neurololgical Sciences Inst., Good Samaritan Hospital and Med. Ctr., Portland, OR 97209.

The pain in causalgia is burning in character and is therefore likely to be mediated by C-polymodal nociceptors, the receptors thought to subserve burning, aching pain. Causalgic pain is triggered or exacerbated by sympathetic arousal and is relieved by sympathetic block. Such findings suggest that sympathetic efferents may have an excitatory effect on C-fiber terminals in the skin and, in this way, contribute to causalgic pain. The present study was designed to test for sympathetic effect on the responses of polymodal nociceptors to noxious heat.

The study was done in chloralose anesthetized cats in which both carotid arteries were ligated; a stimulating electrode was placed on one lumbar sympathetic trunk. Central arterial pressure was recorded from one carotid artery. The pressor response to noxious thermal stimulation of the skin on a hindleg site was used as a measure of activity in a population in polymodal efferents.

It was found that the pressor response was graded with stimulus temperature (50-60°C). The amplitude of the pressor response was found to be relatively stable during repeated, short-duration stimuli (30 sec. duration, 5 min. intervals).

Supramaximal sympathetic stimulation produced no significant change in the pressor response to noxious heat during either brief (3 min.) or prolonged (1 hr.) periods of sympathetic stimulation. The results therefore failed to support the hypothesis that sympathetic activity contributes to causalgic pain by acting upon polymodal nociceptors in the skin.

77.5 PAIN AND ITCH FROM C FIBER STIMULATION. H.E. Torebjörk* and J.L. Ochoa (SPON: J. Metzler). Dept. of Clinical Neurophysiology, University Hospital, Uppsala, 75014 Sweden and Dept. of Neurology, Dartmouth Medical School, Hanover, NH. 03755.

Introduction. Delayed pain and itch have some common neuroanatomical and neurophysiological substrates, and the question has repeatedly been asked as to whether itch can be induced by low frequency discharges in nociceptive fibers which mediate pain at higher frequencies.

Methods. Tungsten electrodes were used for microneurographic recordings of impulse activity in single afferent A and C units in the median or radial nerves in alert humans. Such electrodes were also used for intraneural microstimulation (INMS) of afferent fibers.

Results. INMS in cutaneous nerves at liminal intensity for sensation sometimes elicited itch, referred to a focal area in the skin, or pain, referred diffusely to a larger area. Such sensations were evoked only from certain points in the nerve fascicles, and could be induced even after selective A fiber block. Recordings from such points consistently revealed polymodal nociceptive units with C fibers close to the electrode tip and with receptive fields in the precise area of referral of pain or itch in the skin. No qualitative difference was recorded between receptive properties of C nociceptors identified by referral of itch sensation as compared to those identified by referral of pain. The C polymodal nociceptors could be activated by mechanical, thermal and chemical skin stimuli which elicited itch if the stimuli were focal, and pain if the stimuli were widespread or strong.

Pain and itch could be perceived simultaneously at the same frequency of INMS. Changes in frequency did not transform itch into pain or vice versa. Itch often persisted for many seconds after cessation of INMS, in contrast to pain. Scratching suppressed itch at a stage when only minor fatigue of C nociceptors was observed.

Conclusions: Delayed pain and itch are subserved by C afferent fibers. Itch cannot be explained by low frequency activation of nociceptive units. So far we have failed to demonstrate a specific itch receptor. If delayed pain and itch are induced by activity in a common receptor type, the C polymodal nociceptor, then spatial interactions as well as different central processing of the signals may be involved in the differentiation of pain from itch. Scratching seems to suppress itch by central inhibition rather than just by peripheral receptor fatigue.

77.6 EVIDENCE FOR TWO DISTINCT CLASSES OF UNMYELINATED NOCICEPTIVE AFFERENTS RESPONSIVE TO MECHANICAL AND THERMAL STIMULI IN THE MONKEY. J.N. Campbell[1], R.A. Meyer[1,2], and S.R. Jaffe[1,*], Department of Neurosurgery[1] and Applied Physics Laboratory[2], The Johns Hopkins University, Baltimore, Maryland 21205 USA

We determined that C-fiber nociceptive afferents responsive to both intense mechanical and heat stimuli (CMHs) may be subdivided into two classes based on their temporal response to heat stimuli. Other properties of the two groups of units were found also to differ. The thermal response properties of 40 CMHs innervating the hairy skin of monkey were studied using standard single fiber recording techniques. A laser thermal stimulator under radiometer feedback control provided step increases in skin temperature with rise times of less than 150ms. In response to 41°-49°C stimuli (3s duration), the CMHs displayed either a quickly adapting or a slowly adapting response. The quickly adapting units (QCs) displayed an initial burst of neural activity (spike frequencies as high as 115 impulses/sec) which decreased within 1s to a low steady discharge rate (< 8 impulses/s). The slowly adapting units (SCs) did not display this initial burst of activity but instead responded at a relatively uniform rate throughout the stimulus. Of the 40 CMHs, 20 were QCs and 20 were SCs. The mean Von Frey mechanical threshold, initial thermal threshold, and response to a 53°C, 30s burn of the QCs were significantly lower than that of the SCs, whereas the mean receptive field areas and conduction velocities were not significantly different. Another 31 CMHs innervating glabrous (26) and transitional (5) skin were also subdivided into QCs (14) and SCs (11). Six glabrous CMHs had characteristics of each class: a slowly adapting response to stimuli below 49°C but a quickly adapting response to 53°C stimuli. Could the QC receptor correspond to the elusive "first pain" receptor in monkey? (Supported by USPHS grants NS-14447 and NS-00519)

77.7 INHIBITION OF PRIMATE SPINOTHALAMIC TRACT NEURONS BY STIMULATION WITHIN IPSILATERAL OR CONTRALATERAL VENTROPOSTEROLATERAL (VPL) THALAMIC NUCLEI. K.D. Gerhart, R.P. Yezierski and W.D. Willis. Marine Biomedical Institute and Departments of Physiology & Biophysics and of Anatomy, University of Texas Medical Branch, Galveston, Texas 77550

Electrical stimulation within several medial brainstem areas, including the nucleus raphe magnus (NRM) and periaqueductal gray matter (PAG), has been shown to produce behavioral analgesia in rats, cats and monkeys, and also to inhibit responses of primate spinothalamic tract (STT) neurons to noxious and innocuous stimuli. The spinothalamic tract is thought to be the main somatosensory pathway in primates, including man, to carry information concerning painful stimulation of the body. Stimulation of ventrobasal thalamic areas, including the ventral posterior lateral (VPL) nucleus, has been shown to be clinically effective in reducing chronic pain in man. In order to examine further descending influences on primate STT cell responses, this project studies the effects of stimulation in the VPL nuclei of both ipsilateral and contralateral thalamus on STT cell background activity and on evoked responses.

Monkeys (M. fascicularis) were anesthetized with α-chloralose and pentobarbital, paralyzed, and artificially ventilated. Spinothalamic tract cells were antidromically activated from the contralateral VPL nucleus. A second thalamic stimulating electrode was positioned in the ipsilateral VPL nucleus. STT cells were classified as wide dynamic range or high threshold, and their excitatory and inhibitory cutaneous receptive fields were mapped. The effects of thalamic stimulation were examined using trains of 0.2-2 second duration at 333 Hz with currents of 25-400 μA (but not exceeding the antidromic activation threshold from contralateral thalamus).

Inhibition of spontaneous background STT cell activity, as well as of responses to innocuous or noxious cutaneous stimulation, was caused by stimulating the ipsilateral or contralateral VPL nuclei. Inhibition of responses to A- or C-fiber volleys in the ipsilateral sural nerve was also observed; the inhibition of responses to C fibers was more potent than inhibition of responses to A fibers.

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77.8 A FURTHER EXAMINATION OF THE EFFECTS OF CORTICAL STIMULATION ON PRIMATE SPINOTHALAMIC TRACT CELLS. R.P. Yezierski, K.D. Gerhart and W.D. Willis. Marine Biomedical Institute and Department of Physiology and of Anatomy, University of Texas Medical Branch, Galveston, Texas 77550

Stimulation in the sensorimotor cortex with short trains of electrical pulses (four 0.2 millisecond pulses at 333 Hz) has been shown by our laboratory to inhibit activity of primate spinothalamic tract (STT) cells evoked by innocuous cutaneous stimulation. The failure of cortical stimulation to inhibit activity evoked by noxious stimulation is in contrast with our findings following stimulation in the nucleus raphe magnus and periaqueductal gray. Long trains of electrical pulses delivered to these brainstem sites has been shown to inhibit both noxious and non-noxious responses evoked in primate STT cells. The present study was therefore designed to examine the effects of cortical stimulation on STT cells using stimulus parameters comparable to those used for brainstem stimulation.

Monkeys (M. fascicularis) were anesthetized with α-chloralose and pentobarbital, paralyzed and artifically ventilated. Recordings were made from identified STT cells in the lumbosacral spinal cord using glass or stainless steel microelectrodes. The ipsilateral sural nerve was dissected free from connective tissue and prepared for electrical stimulation. A monopolar stimulating electrode was positioned in the sensorimotor cortex. Inhibition of STT cell activity was obtained using 0.2-2 second trains (333 Hz) delivered once every 2 or 10 seconds, respectively, at stimulus intensities of 200-300 μA.

The results of the present study confirm previous observations using short train stimulation of the sensorimotor cortex. In addition, we have now shown that activity of STT cells evoked by noxious and non-noxious natural stimuli or by C-fibers in the sural nerve can be inhibited by stimulation in sensorimotor cortex using long stimulus trains.

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77.9 CHARACTERISTICS OF NEURONAL RESPONSES TO NOXIOUS STIMULI IN RAT SI SOMATIC SENSORY CORTEX. Y. Lamour, J.C. Willer* and G. Guilbaud* (SPON : M. Abdelmoumène). Unité de Recherches de Neurophysiologie Pharmacologique de l'INSERM, U. 161, 2 rue d'Alésia 75014 Paris, France.

Little is known about the physiological properties and laminar distribution of cerebral cortical neurons receiving noxious input. The present experiment was designed to study such neurons in rat's first somatic sensory cortex (SI) and to determine their laminar distribution. Male Sprague Dawley rats (250-350 gr) were anaesthetized with fluothane (0.5 % in a mixture of 66 % N_2O, 33 % O_2) paralyzed with gallamine triethiodide and artificially ventilated. Single unit activity was recorded in the different parts of SI corresponding to forelimb, hindlimb or tail representation, during penetrations perpendicular to the cortical surface, using glass microelectrodes filled with 2 % pontamine blue in 1 M NaCl. Each electrode penetration was reconstructed on a camera lucida drawing of 100 µm frozen sections using the dye deposit made at the last recording site. The receptive fields of the units were characterized using a variety of innocuous and noxious stimuli. Innocuous stimuli consisted of hair movement, light touch,pressure and joint movements ; noxious stimuli included intense mechanical stimulation and noxious heat (radiant heat or hot water bath from 48° to 60°C). To date 319 neurons have been recorded, and a peripheral receptive field characterized for 129 of them. 24 (16 excited, 8 inhibited) received convergent noxious and non-noxious (deep or superficial) inputs and 28 pure noxious inputs (25 excited, 3 inhibited). About 63 % of the neurons discharged tonically and 37 % phasically in both groups. Most of the "tonic type" neurons were observed to have large peripheral fields. 22 out of 27 neurons receiving mechanical noxious inputs were also responsive to noxious heat. Neurons responsive to noxious inputs increased their discharge rate with stimuli of increasing strength. The threshold of the responses to noxious heat was always above 46°C but usually below 50°C. Both types of neurons were rarely found in the superficial layers (I to IV). In a given penetration neurons receiving noxious inputs were intermingled with other neurons receiving pure cutaneous or joint inputs from the same area. The different types of neurons could be recorded successively in typical "columnar" penetrations.

Supported by DGRST (DN/80.7.0256).

77.10 NUCLEUS RAPHE MAGNUS AFFERENTS IN THE RAT: A RETROGRADE STUDY USING HORSERADISH PEROXIDASE GEL IMPLANTS AND TETRAMETHYLBENZIDINE NEUROHISTOCHEMISTRY. S.M. Carlton*, E.G. Young*, G.R. Leichnetz, D.J. Mayer (SPON: J.H. Johnson). Departments of Anatomy and Physiology, Medical College of Virginia, Virginia Commonwealth University, Richmond, Va 23298.

The nucleus raphe magnus (NRM) is known to play an important role in a descending antinociceptive system through its projections to the spinal cord dorsal horn and trigeminal subnucleus caudalis. Thus the study of its afferents is essential in determining other CNS cell groups which could influence analgesic mechanisms by virtue of their connections with NRM.

Accordingly, tiny fragments of HRP gel were stereotaxically implanted within NRM and in adjacent areas in 16 adult Sprague-Dawley rats. The gel was introduced through previously implanted stainless steel cannulae to prevent contamination of the tract, a factor which has compromised previous studies of this area. After survival periods of 48 hours, the brains were processed according to the TMB-HRP protocol of Mesulam (1978).

Retrogradely-labelled cells were consistently found in the thalamic parafascicular nucleus, zona incerta, dorsal periaqueductal gray, lateral periaqueductal gray, deep layers of the superior colliculus, nucleus cuneiformis and a cell group in the ventralmost periaqueductal gray which was ventral to, and continuous with, the nucleus of Darkschewitsch. Even though the technique accomplished extremely small placements, the presence of retrogradely-labelled cells in the deep superior colliculus made us consider that the inferior olivary nucleus could have been involved. However, since we never observed labelled cells in the red nucleus, which also projects to the inferior olive, this seems unlikely. Control HRP gel implants in the spinal cord retrogradely filled cells in the zona incerta, deep superior colliculus and nucleus of Darkschewitsch, and thus the possible involvement of axons of passage originating from these nuclei and passing through NRM must be considered. However, the heavy and consistent retrograde labelling of the parafascicular, dorsal periaqueductal gray, lateral periaqueductal gray, and nucleus cuneiformis indicates that these nuclei are important constituents of the NRM afferent system.

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77.11 BRAINSTEM AREAS PROJECTING TO LAMINAE I AND II OF THE SPINAL CORD IN CAT. AN AUTORADIOGRAPHICAL STUDY. Gert Holstege, Department of Anatomy, Erasmus University Rotterdam, P.O.Box 1738, 3000 DR Rotterdam, The Netherlands.

Physiological studies indicated that neurons in Rexed's laminae I and II of the spinal cord are especially involved in painperception. Such studies also suggested an inhibitory influence on these neurons from supraspinal levels. In the framework of a study of brainstem-spinal pathways in cat three different brainstem areas were found that project to spinal laminae I and II.

^{3}H-leucine injections (± 0,5 µl containing 50 µCi) were made in mesencephalon, pons and medulla. The cats were allowed to survive the operations for six weeks after which they were sacrificed and perfused with saline followed by 10% formalin. Brainstem, all cervical and lumbosacral segments and 7 thoracic segments were cut in transverse frozen sections 25 µm thick. They were prepared for autoradiography with Ilford G5 emulsion, exposed for three months and then counterstained with cresyl violet.

Three brainstem areas were found to project to spinal laminae I and II:

1: The medial reticular formation at rostral medullary and caudal pontine levels, including the nucleus raphe magnus. Remarkably the nuclei raphe obscurus and pallidus did not project to spinal laminae I and II.
2: The lateral pontine reticular formation at levels rostral to the motor V nucleus. This area distributed labeled fibers to spinal laminae I and II and the lateral part of lamina V especially contralaterally.
3: The area of the nucleus subcoeruleus. This area distributed labeled fibers to all spinal laminae among which laminae I and II.

The first area, which partly contains serotonergic neurons, could be involved in a "pain level setting system". The second area receives many afferents from especially the spino-thalamic tract which is strongly involved in painperception. The neurons which axons constitute this spino-thalamic tract are especially located in the contralateral laminae I and II. The lateral pontine area, which contains virtually no serotonergic and no noradrenergic neurons, distributed labeled fibers to laminae I and II especially contralaterally. This suggests that this area could serve as a feedback system for the spino-thalamic tract and thus could modulate painperception. The third area, nucleus subcoeruleus, contains many noradrenergic neurons and distributes fibers to large parts of the central nervous system. For this reason the nucleus subcoeruleus does not seem to be a typical pain modulating area.

77.12 THE PROJECTION OF THE CINGULATE CORTEX TO THE PERIAQUEDUCTAL GRAY IN THE RAT. J. M. Wyss, Department of Anatomy, Univ. of Alabama in Birmingham, Birmingham, AL 35294.

The mesencephalic periaqueductal gray matter (PAG) has attracted considerable interest because of its apparent role in the modulation of pain input. Behavioral evidence has suggested that several cortical areas including the cingulate gyrus may also be involved in the modulation of pain and therefore are in some fashion functionally connected into the same pathways. In a series of ongoing studies of the connections of the cingulate gyrus, the present paper reports that as Domesick (1969) (Brain Research 12, 296-320) suggested on the basis of degeneration techniques, the anterior portion of this cortex does project to the lateral dorsal portion of the PAG. In thirty-five rats, iontophoretic injections of a mixture of ^{3}H proline, ^{3}H leucine and ^{3}H lysine were placed into the various division of the cingulate cortex and adjacent cortex. Following injections into the anterior ventral cingulate cortex (area infraradiata [IR] a and b α) silver grains were consistently transported to the lateral portion of the dorsal PAG. Injections into slightly more dorsal areas (IR c α and the precentral agranular cortex) result in only slight labeling in this same area of the PAG. The cortex immediately caudal to IR a and b α, i.e. IR a and b β, also projects to the same region of PAG; however, these projections are somewhat more restricted toward the lateral margin. Since in both cases a limited projection passes on to the deeper layers of the superior colliculus, it is unclear whether the overlap that exists between the two projections is a true overlap of the terminal fields or simply fibers of passage from the anterior injections overlapping the terminal field of the IR β cortex. No projections to either the PAG or the superior colliculus arise from the more caudal portions of the IR β cortex or from any sector of the retrosplenial cortex. These results demonstrate that the ventral anterior portion of the cingulate cortex is directly connected to the area of PAG known from previous anatomical and behavioral studies to be involved in the modulation of pain.

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78.1 |^{14}C|-2-DEOXYGLUCOSE MAPPING OF THE DORSAL COCHLEAR NUCLEUS OF THE KITTEN. R. J. Nudo* and R. B. Masterton. Dept. of Psychology, Florida State Univ., Tallahassee, FL 32306.

The distribution of metabolic activity in the dorsal cochlear nucleus (DCN) of the kitten during free-field auditory stimulation was examined by use of the |^{14}C|-2-DG method. The autoradiographs show that a continuous pure-tone stimulus results in an approximately horizontal band of labeled tissue through the DCN cortex. Increases in stimulus intensity widen the labeled band. Increases in stimulus frequency shift the band dorsally; decreases in frequency shift the band ventrally. A section through the active band anywhere along its length shows a compact column of labeled tissue normal to the nuclear surface, extending from the most superficial into the deepest layer, and demonstrating a columnar organization of DCN cortex. However, throughout the most superficial, or molecular layer of DCN, high metabolic activity is present regardless of stimulus parameters. This spontaneous activity is not lessened by ipsilateral nor bilateral destruction of the cochlea.
(Supported by NIH Grant NS 07726)

78.2 MEDIAL GENICULATE PROJECTIONS TO AUDITORY CORTICAL FIELDS A, AI AND P IN THE CAT. T.J. Imig and R.A. Reale*, Dept. of Physiology, K.U. Medical Center, Kansas City, KS 66103 and Dept. of Neurophysiology, University of Wisconsin School of Medicine, Madison, WI 53711.

Cat auditory cortex has been divided into a number of fields on the basis of tonotopic mapping experiments. We have studied the locations of medial geniculate neurons projecting to the anterior (A), primary (AI) and posterior (P) auditory fields using retrograde transport of horseradish peroxidase (HRP) and tritiated bovine serum albumin. Tonotopic maps were obtained in each experiment and used as guides for placement of injections. One tracer was injected into an identified portion of the tonotopic representation of one field, the other tracer was injected into the same portion of the frequency representation of another field. By combining HRP histochemistry and autoradiography in the same tissue section, it was possible to visualize distributions of neurons projecting to the same portions of the frequency representations in two different fields.

The caudal pole of the medial geniculate was devoid of neurons projecting to fields A, AI or P. Following injections of tracer into AI, a dense accumulation of labeled cells formed a band coursing through pars lateralis and pars ovoidea of the ventral divison of the medial geniculate (terminology of Morest). Injections of tracer into field P labeled neurons aggregating around the dorsal, ventral, caudal and rostral perimeter of the band of neurons labeled by AI injections. Few neurons projecting to field P were found within the band of AI projection neurons. On the other hand, neurons projecting to field A were scattered throughout the band of AI projection neurons in the ventral division, although neurons projecting to field A were fewer in number than neurons projecting to field AI in this region.

All three fields received projections from the medial and dorsal divisions of the medial geniculate. Field A received a much more dense projection from the lateral division of the posterior group than did fields AI or P. Although projections overlap in many areas within the geniculate, there is a clear spatial segregation of neurons projecting to these three fields.

In each of the geniculate subdivisions, neurons were found which were labeled with both tracers suggesting that they projected via axon collaterals to at least two cortical auditory fields. (Supported by BRSG SO7 RR05373, Biomedical Research Support Grant Program, NIH; NSF Grant BNS76-19893, NIH Program Project Grant NS12732, NIH Core Support Grant HD03352, and NIH Grant NS 17220).

78.3 AUDITORY THALAMOCORTICAL PROJECTIONS IN CATS. A. Jayaraman. Department of Neurology. LSU School of Medicine, New Orleans, La. 70112.

Several auditory cortical regions in the cat have been shown to have tonotopic organization. To evaluate the presence of a precise topographical projection pattern between the auditory cortical regions and the different subdivisions of the medial geniculate nucleus, small quantities of horseradish peroxidase (HRP) were injected into the auditory cortex of 42 adult cats. After a survival of 24 to 36 hours, the cats were sacrificed and the brains processed according to the method of Mesulam. Injections covering the entire rostrocaudal extent of area AI resulted in the transport of the label to neurons located within the ventral and magnocellular nuclei. The labelled cells within the ventral nucleus were organized mediolaterally and rostrocaudally. Injections in tonotopically different regions of the anterior auditory field resulted in retrograde labelling of neurons of the lateral division of the posterior thalamic nucleus, deep dorsal nucleus and the nucleus ovoidea. Several injections in the cochleotopically organized dorsal and ventral divisions of the posterior ectosylvian gyrus led to transport of HRP to the suprageniculate nucleus, the Poi division, the ventral and ventromedial regions of the ventrolateral nucleus and the magnocellular nucleus. HRP was transported from area AII regions to neurons of the deep dorsal nucleus, magnocellular and ventrolateral nucleii. After injections of the insular cortex the neurons within the suprageniculate nucleus and the ventrolateral subdivisions were labelled. In three cats HRP injections were made in the suprasylvian fringe region and this resulted in transport of the enzyme to neurons within the suprageniculate nucleus, but more towards the lateral parts bordering the intermediate division of the LP nucleus. Temporal injections led to labelling of cells within the caudal subdivisions of MGB primarily.

The present study confirms the previous observations from anatomical and physiological studies that the projection pattern between the ventral nucleus and area AI are tonotopically organized, but fails to confirm similar anatomical substrate for a tonotopical projection pattern between the other subdivisions of the medial geniculate nucleus and auditory cortical regions.

Supported by The Deafness Research Foundation.

78.4 THALAMIC CONNECTIONS TO AND FROM BINAURAL INTERACTION BANDS IN AI OF THE CAT. John C. Middlebrooks and John M. Zook. Coleman Laboratory, HSE 863, UCSF, San Francisco, CA 94143.

The primary auditory cortex (AI) of the cat contains "binaural interaction bands" oriented orthogonal to the lines of constant characteristic frequency. Bands of neurons that receive excitatory inputs from both ears (EE neurons) alternate with bands of neurons that are excited by contralateral stimulation and inhibited by ipsilateral stimulation (EI neurons). In previous anatomical tracing studies, loci in AI larger than the mediolateral width of a single binaural interaction band have been shown to interconnect reciprocally with continuous sheets of cells within the ventral nucleus of the medial geniculate body. With smaller tracer injections, discontinuous banded arrays of label were seen within the ventral nucleus. This discontinuous labelling might reflect a segregation within the ventral nucleus of neurons interconnected with different cortical binaural interaction bands. To test that hypothesis, the thalamocortical and corticothalamic connections of single binaural interaction bands were demonstrated using neuroanatomical tracing methods in conjunction with microelectrode cortical mapping. AI was mapped in fine grain using conventional multiunit recording methods and diotic sound stimulation. Small ($\ll$50nl) injections of retrograde or anterograde tracers then were introduced into given binaural bands. Different tracers, separately or together, were injected into binaural interaction bands of the same or of different classes. Following a short survival period, cats were perfused and the tissue was processed histochemically and autoradiographically. Injections of anterograde or retrograde tracer that apparently were restricted to a single binaural interaction band resulted in a banded pattern of label within at least part of the ventral nucleus. These patterns of label appeared in transverse section as interrupted contours, and could be reconstructed in three dimensions to reveal multiple rostro-caudally oriented slabs of label extending for a significant distance along that axis of the nucleus. These data enable a comparison of the anatomical connections of single EE and EI bands as well as a description of the relationship between thalamocortical and corticothalamic connections. These results support a model of the central auditory system in which: 1) input to EE and EI bands is distinguishable and anatomically distinct throughout several levels from the binaural nuclei of the pons to AI; 2) there are convergent and divergent interconnections among anatomical subunits within a single binaural interaction class; and 3) the ventral thalamus, like AI, apparently contains repeating layers or bands corresponding to a simple functional dichotomy.

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78.5 RESPONSE PROPERTIES AND TOPOGRAPHIC MAPS OF CAT AI AND AII AUDITORY CORTEX. C. Schreiner* and M. Cynader. Coleman Laboratory, U.C.S.F., San Francisco, CA 94143.

A finegrain analysis of parts of cortical areas AI and AII was made by taking up to 200 penetrations within a 4x4 mm area near the AI/AII border.

Neurons within the middle layers of AI were characterized by sharp frequency tuning, low thresholds, predominantly phasic responses, short latencies and binaural interactions of the excitatory-excitatory (EE) or excitatory-inhibitory type (EI). In AII, we observed broader frequency tuning, higher thresholds and both EE and EI binaural interaction classes. In parts of AII, responses were more sustained and latencies could be much longer than those recorded in AI.

Area AI displayed clear isofrequency contours usually oriented across the medial to lateral dimension of the field. EE and EI cells clustered into aggregates which had a tendency to run orthogonal to the isofrequency contours. A prominent EI patch marked the border between ventralmost AI and dorsalmost AII. Whereas the response thresholds of AI cells tended to decrease as the border was approached, the AI/AII border itself was a zone of great variability in threshold. Here, cells with thresholds differing by more than 40 dB could be found in nearly contiguous locations.

Isofrequency contours established in AI sometimes continued uninterrupted into AII, with cells in AII displaying the same center tuning. In other cases, we noted abrupt shifts of best frequency preferences as the border was crossed. Nonetheless, the dorsal part of AII displayed a best frequency map similar to that of AI, with low frequency responses represented posterior to high frequency responding neurons. In more ventral parts of AII, frequency tuning broadened still more, with neurons responding well over more than one octave. The broad tuning blurred the topographic map in the ventral part of AII.

Aggregates of EE and EI cells were found in AII. In contrast with AI, the patches of EE and EI cells tended to be elongated in the dorsal to ventral rather than the anterior to posterior dimension. The properties of AII cells are consistent with a possible role for AII in a binaural localization process based on comparison of broadband spectral characteristics of inputs from the two ears.

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78.6 AUDITORY RECEPTIVE FIELDS: THEIR ORIGIN IN THE BRAINSTEM NUCLEI. Andrew Moiseff and Masakazu Konishi, Division of Biology 216-76, California Institute of Technology, Pasadena, CA 91125

Auditory neurons having small, discrete spatial receptive fields have been described in the midbrain auditory nucleus (MLD) of the barn owl (Tyto alba). We have been recording from single auditory neurons in the owl's brainstem auditory nuclei to determine at which stage in the auditory system neurons with spatial receptive fields emerge.

Anatomical studies have shown that with the exception of the primary cochlear nuclei (nucleus angularis and nucleus magnocelularis) and one of the lemniscal nuclei (lemniscus lateralis, pons ventralis) the brainstem auditory nuclei receive binaural input. We recorded from single auditory neurons within these binaural nuclei (nucleus laminaris, nucleus olivaris superior, and nuclei of lemniscus lateralis) with glass-insulated Platinum-Iridium microelectrodes and glass microelectrodes. Electrolytic lesions or injection of HRP enabled us to positively identify the recording site and, in some experiments, confirm the input and output pathways of the auditory nuclei.

Our preliminary results indicate that auditory neurons within the owl's lateral lemniscus are selective for specific binaural on-going time disparities. Selectivity to dichotically presented on-going time disparities corresponds to selectivity to specific azimuthal positions under free-field stimulus conditions. The auditory responses exhibited by these lemniscal neurons are indicative of complex neuronal interactions. When either ear is stimulated alone the neurons are excited; when both ears are stimulated simultaneously, under dichotic conditions, the magnitude of the excitation is dependent on the on-going time disparity presented. Unlike MLD neurons with small receptive fields, which are narrowly tuned to both on-going time disparities and interaural intensity differences, neurons in lateral lemniscus are sensitive to on-going time disparity over a broad range of intensity differences. In as much as interaural intensity differences provide the owl with a cue for determining the elevation of a sound source, these neurons would not have spatial receptive fields with well-defined elevational borders.

Our data suggest that neurons in the auditory brainstem nuclei respond to the binaural cues responsible for sound localization and can, through additional interactions, give rise to the small receptive field auditory neurons present in MLD. (Supported by a Helen Hay Whitney postdoctoral fellowship to A. M., and an NIH grant to M. K.)

78.7 TONOTOPIC ORGANIZATION AND BEST FREQUENCY DISTRIBUTION OF SINGLE UNITS IN THE INFERIOR COLLICULUS OF THE MUSTACHE BAT. R.D. Bodenhamer* and G.D. Pollak. Dept. of Zoology, Univ. of Texas, Austin, TX 78712.

Echolocating bats identify objects around them by emitting ultrasonic biosonar signals and listening to the returning echoes. The biosonar signals of the mustache bat, Pteronotus parnellii, are characterized by a relatively long duration, constant frequency (CF) component having a fundamental frequency of 30 kHz with second and third harmonics. The dominant harmonic is the second, which lies between 60-63 kHz depending upon the individual animal. The mustache bat is sensitive to Doppler shifts of this "60 kHz" CF component, and if the echo CF returns at a frequency greater than the emitted CF, it lowers the frequency of subsequent signals. In this way, the bat stabilizes the echoes within a very narrow frequency band.

In this study, we monitored the activity of neurons in the mustache bat's inferior colliculus (IC), directing special attention toward the distribution of best frequencies (BFs) and the manner in which different BFs are organized within the nucleus. By sampling large areas of the IC, we encountered neurons tuned to frequencies ranging from 10-100 kHz. However, the population of cells we sampled was not uniformly distributed over this frequency range. On the contrary, each bat devoted approximately 50% of its neurons to a band of frequencies only 300-500 Hz wide. This overrepresented frequency band corresponds to the frequency of the echo CF and ranged from 61-63 kHz depending, again, upon the individual animal. Neurons having BFs in this frequency band were much more sharply tuned than neurons outside the overrepresented band, having an average Q10-dB value of over 120.

The different BFs were systematically arranged within the IC, and different frequency regions could be repeatedly located in a reliable fashion. The most striking feature of the tonotopic organization was that the population of sharply tuned neurons constituting the overrepresented frequency band was located in a specific, clearly demarcated region of the IC. This essentially isofrequency region occupies a significantly disproportionate percentage of IC volume. In short, the mustache bat devotes an extremely large amount of neural tissue to processing information contained in the stabilized echo CF component.

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78.8 RESPONSE PROPERTIES OF THE FM-FM COMBINATION-SENSITIVE NEURONS IN THE AUDITORY CORTEX OF THE MUSTACHED BAT. I. Taniguchi,* H. Niwa* and N. Suga. Dept. of Biology, Washington University, St. Louis, MO. 63130.

The FM-FM area of the auditory cortex of the mustached bat processes target-range information, analyzing time interval between the emitted pulse and its echo (O'Neill and Suga, Science, 203: 69, 1979). Neurons in this area exhibit remarkable facilitation when they are stimulated by the first harmonic component (FM_1) of a pulse followed by one of the higher harmonic FM's (FM_n, n=2,3,4) of an echo. For facilitation, about 40% of the FM-FM neurons studied require the downward sweeping FM_n as seen in the natural pulse and echo. About 60% of the neurons, however, show good facilitation regardless of sweep direction. For the maximum facilitation of these neurons, an FM_n should be delivered with particular time delays (0.4-18 msec) from an FM_1 (Suga and O'Neill, Science, 206: 315, 1979). The mechanism for the facilitation however, has not yet been explored. Measurements of the latencies of responses to FM_1 and FM_n demonstrate that the response to FM_1 arrives at the FM-FM area with a latency longer than that of FM_n. Therefore, the integration of these inputs for maximal facilitation requires an FM_n delay from FM_1 which is comparable to the difference in latency between the FM_1 and FM_n responses. What kind of neural mechanism is responsible for the long latency of the FM_1 response? Two working hypotheses, the multiple-delay hypothesis and the inhibition hypothesis, are conceivable. The former predicts that the long latency of the FM_1 response is due to multiple synaptic delays en route to the FM-FM area from the periphery. The latter proposes that the long latency is due to inhibition evoked by the initial portion of FM_1. Our data show that in many FM-FM neurons, inhibition is evoked by the initial portion of FM_1 preceding its essential component for facilitation, but the removal of this initial portion, i.e., inhibition does not cause any significant change in the latency of FM_1 response and facilitation. Therefore the multiple-delay hypothesis is more likely than the inhibition hypothesis. The functional role of inhibition may be in increasing the contrast of temporal pattern of neural activity. (Work supported by NSF and the Bendix Corp.)

78.9 AUDITORY RESPONSES IN THE ZEBRA FINCH'S MOTOR SYSTEM FOR SONG. L. C. Katz* and M. E. Gurney (SPON: M. Konishi) Division of Biology 216-76, California Institute of Technology, Pasadena, CA 91125

Vocal learning of the motor program for song by oscine birds depends upon the bird using auditory feedback; thus, auditory information must be accessible to the vocal control system. This system includes at least 3 telencephalic nuclei: the hyperstriatum ventrale, pars caudale (HVc), the nucleus robustus archistriatalis (RA), and Area X. Lesion of either HVc or RA produces a deficit in song (Nottebohm et al., J. Comp. Neurol. **165**:457, 1976). We have discovered neurons within HVc of the zebra finch (Poephilia guttata) which respond to auditory stimuli, as demonstrated by using intracellular recording and horseradish peroxidase (HRP) staining of single neurons.

HRP filled glass microelecrodes (resistance 150-200 MΩ) were used for recording and staining. Successfully penetrated cells were presented with noise or tone bursts through a closed sound delivery system. Cells were filled with HRP by passing depolarizing current pulses.

In 22 adult males, we tested 59 HVc neurons for auditory responses. 36 (61%) responded to noise bursts; 23 neurons (39%) did not. Most auditory neurons were excited or inhibited by the onset and/or the offset of the noise burst. Excitatory response latencies to stimulus onset were 25-40 msec; response latencies to stimulus offset were 35-50 msec.

HVc projects rostrally to Area X, and caudally to RA. Our results indicate that HVc auditory neurons project to Area X, and that RA-projecting HVc cells do not show auditory responses. Area X-projecting HVc neurons (nine filled) had dendritic arms heavily endowed with spines, somatal diameters of 10-15 μm and dendritic field diameters of 150-200 μm. Two classes of non-responding HVc neurons were seen: RA-projecting and Area X-projecting. The four RA projecting neurons had somatal diameters of 6-8 μm and a sparse dendritic arborization 80-120 μm in diameter. The morphologies of the non-responding Area X-projecting neurons (3 filled) closely resembled those of the auditory Area X-projecting cells. Two additional auditory neurons filled were within the neostriatum caudale (NC) immediately beneath HVc, which receives a projection from Field L in canaries (Kelley, D. B. and Nottebohm, F., J. Comp. Neurol. **183**:455, 1979). Axonal collaterals from these two cells arborized dorsally into HVc as well as ventrally into Field L. Their response patterns resembled that of many HVc neurons.

Interfaces between auditory and motor pathways for song define levels in the brain at which the motor output can be modified. The existence of auditory units in HVc presents the possibility that such an interface may exist in this nucleus. (Supported by NSF Graduate Fellowship to L.C.K. and a NRSA (No. 3 F 32 HD05940-01) to M.E.G.)

78.10 SINGLE NEURON RESPONSES TO A NATURAL SEQUENCE OF SPECIES SPECIFIC VOCALIZATIONS IN THE AUDITORY CORTEX OF AWAKE SQUIRREL MONKEYS. I. Glass* and Z. Wollberg. Dept. of Zoology, Tel-Aviv Univ., Tel-Aviv, Israel.

The activity of 82 auditory cortex (AC) neurons was extracellularly recorded in the awake, unmedicated squirrel monkey. A sequence of 70 species-specific vocalizations (total duration = 82 sec) emitted by a colony of squirrel monkeys during feeding was presented consecutively 15 times to the experimental monkey, at 75 ± 10 dB SPL. The responses of each unit to 21 representative calls of the 70 comprising the sequence were analyzed in detail. The 21 calls consist mainly of two call types, a twitter (10 calls) and a long-peep (6 calls), which differ in their temporal and spectral structures. Eighty-five percent of the cells responded to at least one of the 21 calls with an average of 7.9 effective calls per neuron. This responsiveness was significantly lower than the responsiveness of AC neurons to isolated species-specific vocalizations (Glass, I. and Wollberg, Z., Exp. Brain Res., 34:489, 1979). The most common response pattern was a simple excitatory response. A particular response to a given stimulus was not necessarily correlated with a response to other stimuli. A significant difference was found in the number of neurons responding to each of the 21 stimuli. However, no apparent correlation was observed between the number of responding neurons and the order of the calls in the sequence, their intensity, or their spectral content. The potency of the 10 twitter calls to evoke responses differed significantly. That was also true for the 6 long-peep calls. The average number of responding units per twitter call was not significantly different from the corresponding value for a long-peep call. It appears that the responsiveness to natural calls of a certain type is variable even if these calls are produced in the same behavioral context, and thus presumably have the same communicative content. Moreover, the presentation of species-specific vocalizations in a consecutive sequence appears to result in a suppression or adaptation of the AC neurons in comparison to individual vocalizations presented in an isolated manner.

78.11 METABOLIC RESPONSE OF THE HUMAN BRAIN TO VERBAL AND NON-VERBAL AUDITORY STIMULATION. J.C. Mazziotta*, M.E. Phelps, R. Carson* (SPON: D.E. Kuhl). Dept. of Neurology and Div. of Biophysics, UCLA School of Medicine, Los Angeles, CA 90024.

Normal right-handed male volunteers receiving auditory stimulation were studied using fluorodeoxyglucose and positron computed tomography. Paired studies were always performed. Control states consisted of plugging the ears and covering them with sound-proof headsets with eyes either open or closed. The following day a manaural (equal numbers of left and right sided stimulations were performed for each sub-group) stimulation study was performed using material that was either verbal (Sherlock Holmes story, N=6), non-verbal (tone sequences, N=8; or chord pairs, N=4) or both. Subjects described in detail their strategy for stimulus identifications and were paid in proportion to their accuracy on a subsequent test that was given for each task.

Control studies demonstrated cerebral metabolic (left/right) symmetry with eyes open (previous studies with ears open, eyes closed or both open have been symmetric) but when both the eyes and ears were closed there was relative left sided hypermetabolism, particularly frontal ($p < 0.015$), perisylvian ($p < 0.001$) and associative visual ($p < .04$) cortex. All auditory stimulations resulted in symmetric (L/R: $p < .05$) bilateral activation of the posterior transverse temporal surfaces, with the left-sided activation being more posteriorly placed and more extensive in terms of the surface area activated. This pattern corresponds to previous reported human morphological asymmetries of this region. Verbal stimulation caused bilateral posterior temporal (l>R, $p<.01$), left inferior frontal ($p < .01$) and left associative visual ($p < .001$) cortical activations. Stimulation with chords resulted in bilateral inferior panetol ($p < .01$) and right posterior temporal ($p < .01$) and frontal ($p < .001$) cortical activations. The results of the tone sequence task depended on the subjects, task strategy and musical sophistication. Subjects who used non-analytical methods and were musically naive (N=5) had right sided activations particularly of the posterior temporal ($p < .001$), and frontal ($p < .001$) cortex. Analytical or musically sophisticated subjects (N=3) had left sided activations of the posterior temporal ($p < .01$) and inferior frontal ($p < .001$) cortex. These results will be discussed in terms of functional neuroanatomy and cerebral dominance.

79.1 DISRUPTION OF THE SLEEP-WAKING CYCLE AFTER PONTINE TEGMENTAL LESIONS AS REVEALED BY COMPUTER GRAPHICS ANALYSIS. Lee Friedman*, and Barbara E. Jones. Lab. of Neuroanatomy, Montreal Neurological Inst., Dept., Neurology and Neurosurgery, McGill Univ., Montreal, Quebec, Canada H3A 2B4.

Previous research has shown that large lesions involving the pontine gigantocellular tegmental field (FTG) and the lateral tegmental field (FTL) eliminate the state of paradoxical sleep (Jones, Neurosci. Lett., 13:285-293, 1979). The aims of the present research were to quantify and analyze more accurately this effect on the sleep-waking electrographic variables and cycle organization and to delimit the effective lesion site.

The electrographic variables were recorded from chronically implanted cats on a Grass Model 78D Polygraph. For quantification the respective filtered amplitudes of EEG, EMG, EOG and olfactory bulb spindles (OBS) were integrated and digitized and PGO spikes were automatically detected and counted on a Buxco Electronics Data Logger for successive one minute epochs over 24 hours. One day's data were collected and transferred to a PDP 11/60 computer for analysis. Bivariate frequency histograms based on amplitude (EEG, EMG, EOG, OBS) or number (PGO spikes) of electrographic variables per minute epoch were displayed for baseline and post-lesion days on a color Lexidata System 3400 Image Processor.

In 24 hour baseline records, bivariate histograms reliably manifested three major clusters which corresponded to waking, high amplitude sleep and paradoxical sleep. One to three weeks after radiofrequency lesions of the pontine FTG and FTL, the histograms showed a complete alteration of the association among parameters and revealed a transformation of the cluster configuration. Graphics analysis of state clusters demonstrated that paradoxical sleep was absent for the three week post-lesion survival period. In examination of individual variables, it was obvious that relative to baseline amounts, there was a downward shift in EEG amplitude, an upward shift in EMG amplitude, and a loss of epochs with a high rate of PGO spiking. The sequential display of data points on the bivariate histograms showed underlying truncation of the sleep-waking cycle after the lesion, such that only the initial segment between waking and moderate EEG amplitude sleep was evident. Smaller lesions restricted to either the FTG or the FTL of the pontine tegmentum were not associated with the same longterm cycle disruption. In conclusion this analysis demonstrates that the pontine tegmentum, including both gigantocellular and lateral tegmental fields, is essential not only for the state of PS but moreover for the full coordination and concatenation of electrographic parameters that underlie the sleep-waking cycle.

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79.2 BEHAVIORAL STATES AFTER BRAINSTEM TRANSECTION AT THE MEDULLARY LEVEL

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We have attempted a) to determine which elements of REM sleep survive in cats which have had their brainstem transected just rostral to the level of the 6th nerve nucleus and b) to characterize the physiological periodicities and behavioral states in each half of the preparation. Unit activity on both sides of the cut as well as standard EEG, EKG, PGO, and respiration measures were recorded. Surgical section of the 3rd and 4th nerves on one side and the 6th nerve on the other side allowed the separate observation of eye movements controlled by the forebrain and brainstem. Cats were maintained in stable physiological condition for as long as 30 days.

Forebrain States: Three distinct states were seen. 1) a state with desynchronized EEG and no PGO spikes. 2) A state with intermittent synchronized EEG and no PGO spikes. 3) A state with continuous synchronized EEG and isolated, high amplitude PGO spikes. This latter state resembled the state of transitional SWS seen in the intact cat. No state with PGO spikes and desynchronized EEG was seen. Neuronal activation resembling that of REM sleep was not observed. Synchronized EEG periods were accompanied by lower but more irregular rates of midbrain unit activity than desynchronized periods.

Medulla States: In the isolated medulla, three states could be recognized. 1) A quiescent state. At this time, heart rate and unit activity were slow and regular and muscle tone was very low, although not completely absent. 2) Strong stimulation could produce a second state that can be described as tonic activation. In this state, EMG amplitude, heart rate, and brainstem unit activity were all high. Respiration was rapid and gross body movements often occurred. A brief stimulus could precipitate a tonic activation period which lasted several hours, terminating with a gradual return to the quiescent state. 3) The third state, can be described as phasic activation. It occurred periodically in the undisturbed cat and consisted of a brief, one to three minute activation of EKG, EMG and respiration.

79.3 PGO WAVE ACTIVITY AND CORTICAL EEG IN THE RESERPINIZED, ANESTHETIZED CAT. P. J. Morgane, J. D. Bronzino and M. M. Kennard*. Worcester Foundation for Expt. Biology, Shrewsbury, MA 01545.

In order to study PGO spike waves in relation to cortical EEG activity we have quantitatively studied PGO spike rhythms and the relation of PGO spikes to degrees of cortical EEG synchrony in the reserpinized, anesthetized cat. In waking cats it has been repeatedly shown that numerous drugs which act upon brain monoamines can dissociate the appearance of PGO waves from REM sleep. In the reserpinized, anesthetized cat we still see a recurring PGO rhythm and a clear relation of PGO waves to specific patterns of EEG activity in the cerebral cortex. It has been reported that in unanesthetized cats there is a desynchronizing tendency of the PGO wave mechanism. Under reserpine PGO waves occur with a periodic character having a basic rhythm of approximately 30-35 minutes within the range of a sleep EEG cycle (24-30 minutes) in the cat though longer than the normal REM cycle (18-22 minutes) in this species. After peaking in each cycle PGO activity rates either dropped to zero or fell to very low values. Cortical EEG activity, particularly in the frontal cortex, was clearly related to the density of PGO waves. In periods of dense PGO waves the frontal cortex was highly synchronized and the degree of synchrony diminished as the PGO wave rate fell. During periods of EEG desynchronization no PGO waves were present. Coherence values between frontal and occipital cortices were greatest during high PGO spike activity. It is noteworthy that the changes in PGO wave discharge always preceded the EEG changes whether proceeding toward synchrony or desynchrony (PGO waves becoming more or less dense, respectively). We found routinely that when there were more PGO waves there was more power in the lower frequency ranges of the EEG. In these preparations we also found that the most dramatic EEG changes from synchrony to desynchrony and the reverse were seen in the frontal cortex while the occipital cortex exhibited far less fluctuations in this regard. Even when there was extreme synchrony in the frontal cortex with dense PGO wave activity there appeared in most instances fewer changes in the occipital cortex. On some occasions when we saw the most extreme density of PGO waves we found hypersynchrony in both the frontal and occipital cortex. These studies indicate that PGO waves in the reserpinized, anesthetized cat are under control of a periodic generator and bear an unvarying relation to cortical EEG activity. Thus, in this preparation we have shown that there is not a desynchronizing tendency of PGO waves. Reserpine does not block cortical EEG synchronization but does eliminate any mutual exclusivity of PGO waves and cortical synchronization such as appears in normal sleep. (Supported by NSF grant 79-22507).

79.4 THE EFFECTS OF SLEEP ON THE STRENGTH OF NEURAL ENTRAINMENT IN DIFFERENT LAYERS OF THE SOMATOSENSORY CORTEX. G. Gücer. Department of Neurosurgery and Physiology, Johns Hopkins University, Balto.MD 21205

Somatosensory neurons show a decrease in the strength of entrainment during different stages of sleep. The transition from waking to slow wave sleep is accompanied by a decrease in the amplitude of the post stimulus histograms (PSH) while entrainment to the peripheral vibratory stimulus is unchanged. During desynchronized sleep with REM'S the amplitude of the PSH is further reduced to 9 ± .98% of waking. However some neurons do not show a loss in the strength of entrainment during slow wave sleep but show a loss during desynchronized sleep with rapid eye movements (Gücer, G., Exp. Brain Res., 34:287, 1979).

The purpose of this study was to determine how the effects of sleep as seen by a change in the strength of entrainment are distributed across the six layers of the somatosensory cortex. Twelve macaque monkeys were trained to fall asleep in a primate chair while the head was restrained. A gentle vibratory stimulus was delivered to the glabrous skin of the hand; it did not alter the normal sleep cycle of the macaque. Single unit studies of the post central gyrus neurons was performed with extracellular recording. One hundred and twenty three neurons could be entrained to peripheral sinusoidal mechanical stimuli and studied through a sleep cycle. Thirty nine neurons were studied through a sleep cycle which included a desynchronized sleep epochs with rapid eye movements.

The strength of entrainment of neurons located in the supragranular layers of the cortex was decreased during slow wave sleep as well as during desynchronized sleep. The strength of entrainment of neurons located in Layer 4 was unaffected by slow wave sleep however a decrease in the strength of entrainment during desynchronized sleep to 35% ±1.5was observed. This was even further reduced during rapid eye movement epochs of desynchronized sleep to 9% ±.98.

Neurons in the input layers of the somatosensory cortex are strongly linked to sensory drive and not affected by slow wave sleep. However, as information transmission to the cortex is decreased by brainstem inhibition of relay nuclei during rapid eye movements of desynchronized sleep, even neurons in the input layer show a decrease in strength of entrainment but not in the fidelity of entrainment.

79.5 HYPNOGENIC-CENTER THEORY OF SLEEP: NO SUPPORT FROM 2-DEOXYGLUCOSE STUDIES IN MONKEYS. R.K. Nakamura, C. Kennedy, J.C. Gillin, S. Suda*, M. Ito*, F. Storch*, W. Mendelson, M. Mishkin, L. Sokoloff
National Institute of Mental Health, Bethesda, MD 20205.

The hypnogenic-center theory postulates that sleep is initiated and maintained by an active process in some cerebral structure (cf Bremer, Ann. Neurol., 2:1-6, 1977). We examined this theory by applying the 2-deoxyglucose (2DG) technique in monkeys that were either awake (n=4) or in slow wave sleep (n=4). All animals were adapted to sleeping in a restraint chair within a dark booth with 70 db of masking white noise. EEG, EOG, and EMG were monitored throughout, and behavior was observed via an infrared TV camera. The experimental animals were allowed to enter slow wave sleep (SWS), whereas the control animals were awakened (and kept awake) by light taps on, and movements of, the booth. Then 2DG was given, and 30 minutes later the animals were killed. In all cases, the electrographic and behavioral evidence confirmed that the desired states were maintained for a minimum of 27 minutes.

After autoradiographic development of the brain sections, calculations were made in each monkey of rates of local cerebral glucose utilization (LCGU) for 74 structures, including more than a dozen that have been suggested as possible hypnogenic centers. In none of the 74 structures was the mean value for LCGU higher in SWS than in waking. In fact, in 43 structures, LCGU was significantly lower in SWS. Overall there was a 30% reduction. Besides the structures analyzed quantitatively, others scanned visually likewise yielded no suggestion of a significant increase in SWS.

Since the control animals were awakened and had to be regularly disturbed in order to be kept awake, a hypnogenic center may have been active in these monkeys despite their awake state. We therefore made further comparisons with six monkeys that had been studied earlier with the 2DG technique while they were fully alert. Out of the 74 structures examined only two, the cochlear nucleus and inferior colliculus, had significantly higher metabolic activity in either the sleeping or control animals than in the fully alert animals, a finding that was presumably due to the use of the masking noise in the present study. Thus, the comparison of the sleeping monkeys with either the control or the fully alert monkeys gives no support to the notion of a hypnogenic center that actively maintains sleep. In addition, the absence of a metabolic hot spot in drowsy as compared with alert animals weakens the possibility of a center that triggers sleep.

A hypnogenic center could have been missed if its mechanism involves: a) a small or brief increase in activity, b) a change in the temporal pattern of activity, or c) a closely intermixed set of reciprocally related cells. At present, however, the 2DG evidence appears to favor a deactivation theory of sleep.

79.6 SEQUENCING OF SLEEP STATES IS ALTERED IN INFANTS AT RISK FOR THE SUDDEN INFANT DEATH SYNDROME. R. M. Harper, B. Leake*, T. Hoppenbrouwers*, M. Sterman and J. Hodgman*. Dept. of Anatomy and the Brain Research Institute, UCLA, Los Angeles, CA 90024, and LAC-USC Med Center, Los Angeles, CA 90033.

Infants normally exhibit a sequence of active and quiet sleep states which is interrupted by episodes of waking during a night recording. A periodicity appears in the temporal organization of states such that epochs of quiet sleep and active sleep follow each other at hourly intervals; this periodicity requires 3 to 4 months to stabilize and provides a valuable neurological indicator of development. The development of state sequencing is disturbed in infants at risk for the Sudden Infant Death Syndrome (SIDS), and this disturbance persists until at least the sixth month of life.

25 control infants and 25 age and socio-economically matched siblings of SIDS victims, who are at 5-fold higher risk for the syndrome, were recorded at 1 week and at 1, 2, 3, 4 and 6 months of age. 12 hr all-night sleep recordings were obtained using EEG, EKG, eye movement, EMG activity, somatic movement and respiratory signals to indicate sleep state. Each successive minute of the all-night recording was classified as quiet sleep (QS), active sleep (AS) or waking (AW) and transformed into a binary series of 0 (representing presence of a particular state) or 1 (representing absence of that state). These binary sequences were then smoothed with a digital filter and subjected to spectral analysis to examine the temporal sequence. ANOVA procedures were used to provide statistical assessment of trends.

As described earlier (Harper, R.M. et al., Science, In Press), the organization of sleep states is disturbed even by the first week of life. This larger group of infants additionally demonstrates that risk infants show a decreased 1 cycle/hr activity in AS and AW at 3 months and in AS at 6 months.

These changes in periodicity of state organization were reflected in binary plots of state epochs as a combination of fewer short-term wakings, sustained inter-awake intervals as well as long inter-AS intervals. Infants at risk for SIDS appear to have difficulty in establishing the normal waking patterns that occur, often momentarily, during a night's sleep. Sudden Infant Death may reflect a "failure to arouse" to events which might compromise an infant during sleep.

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80.1 THE AMNESIC PATIENT H.M.: CLINICAL OBSERVATIONS AND TEST PERFORMANCE 28 YEARS AFTER OPERATION. Suzanne Corkin, Edith V. Sullivan, Thomas E. Twitchell*, and Elizabeth Grove*. Dept. Psychol., Mass. Inst. Tech., Cambridge, MA 02139.

In 1953, at the age of 27, H.M. underwent an experimental operation for intractable epilepsy in which the uncus, amygdala, and anterior portions of the hippocampus and parahippocampal gyrus were removed bilaterally. The severe and lasting global amnesia that followed was described initially by Scoville and Milner (1957), and a 14-year follow-up study of H.M. was conducted by Milner, Corkin, and Teuber (1968). We now document H.M.'s condition at the age of 55, 28 years after operation.

The most striking finding with H.M. is the stability of his symptoms over the 28 years. He still exhibits a profound anterograde amnesia, and does not know where he lives, who cares for him, or what he ate at his last meal. His guesses as to the current year range from 1958 to 1993, and, when he does not stop to calculate it, he estimates his age to be 10 to 26 years less than it is. Nevertheless, he has islands of remembering, such as knowing that an astronaut is someone who travels in outer space, that a public figure named Kennedy was assassinated, and that rock music is "that new kind of music we have." He can still draw an accurate floor plan of the house in which he had lived from 1960 to 1974; moreover, he believes that he still lives there. A typical day's activities include doing crossword puzzles and watching television.

Neurological examination of H.M. reveals ataxia of gait, polyneuropathy, and a left ulnar neuropathy. These signs are identical to those found in 1966, except for progression of the polyneuropathy since 1970 and the appearance of the ulnar neuropathy in 1977. H.M.'s CT scan shows postsurgical changes in both anterior temporal lobes, a slight increase in Sylvian fissure size, and significant cerebellar atrophy.

Formal neuropsychological testing showed a drop on several measures between 1977 and 1980 (Wechsler Intelligence Scale, verbal fluency, expressive and receptive language capacities, recency discrimination). Over the same time, however, there was no change on many other tests (Wechsler Memory Scale, Hidden Figures Test, Porteus Maze Test, nonverbal fleuncy, copy of the Rey figure, speed of tapping, short-term retention, continuous recognition of verbal and nonverbal material). In addition, H.M. failed to show the depth-of-processing effect when given 30 words at a time but did show it when given 6. Our findings reflect the interaction of mnemonic processes, other cognitive mechanisms, and age-related changes in the brain.

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80.2 THE AMNESIC PATIENT H.M.: LEARNING AND RETENTION OF A COGNITIVE SKILL. Neal J. Cohen and Suzanne Corkin. Dept. Psychol., Mass. Inst. Tech., Cambridge, MA 02139.

The patient H.M. has exhibited a profound amnesia since sustaining a bilateral removal in the medial temporal-lobe region for relief of chronic epilepsy in 1953. His disorder of learning and memory affects both verbal and nonverbal stimulus material irrespective of the modality of stimulus presentation. Nonetheless, H.M. has been able to learn and retain certain perceptual-motor skills (Milner, Physiologie de l'Hippocampe, 1962, p. 257; Corkin, Neuropsychologia, 1968, 6, 255), although he could not recall his experience with the task. More recently, other amnesic patients exhibited normal learning and 3-month retention of a mirror-reading skill, despite markedly impaired memory for the words that had been read and for the training experience itself (Cohen and Squire, Science, 1980, 210, 207). This work suggested a dissociation among memory systems, such that the learning of skills involving procedural, rule-based knowledge is spared in amnesia, whereas learning and memory of declarative, data-based knowledge (comprised of specific episodes and facts about the world) is markedly impaired.

New work with H.M. provides further support for this view: H.M. was tested on the Tower of Hanoi puzzle, a well-studied problem domain involving a number of pegs and a number of blocks of differing sizes. To begin the problem, all of the blocks are arranged on the "source" peg in size order, with the smallest on top and the largest on the bottom. The task of the subject is to move the blocks, one at a time, onto the "goal" peg, reestablishing the size order while never placing a block onto one smaller than itself. Normal control subjects require a decreasing number of moves for solution across trials. H.M. was tested four times per day on four consecutive days and, one week later, on another four consecutive days. Despite his inability to remember particular moves and whether they advance or retard solution, H.M.'s performance improved systematically across days, from a mean of 46.3 moves per solution on the first day of testing to a mean of 32 moves per solution on the eighth day of testing (minimum number of moves=31). By the seventh and eighth days of training, despite near-perfect performance, his commentary during each trial always sounded as if he were solving the puzzle for the first time. In short, H.M. was able to learn the procedures or operations necessary for the successful performance of this cognitive skill, despite markedly impaired declarative memory for the task. This finding is corroborated by our results for patients with amnesias of other etiologies.

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80.3 THE AMNESIC PATIENT H.M.: LEARNING AND RETENTION OF PERCEPTUAL SKILLS. Mary Jo. Nissen*, Neal J. Cohen, and Suzanne Corkin (SPON: Edith V. Sullivan). Dept. of Psychol., Mass. Inst. Tech., Cambridge, MA 02139.

Following a bilateral resection in the medial temporal region, H.M. has demonstrated a severe amnesia for new declarative information, including both verbal and nonverbal material. He nevertheless is able to learn and retain new perceptual and perceptual-motor skills. We have now replicated an earlier demonstration (Milner, Corkin, and Teuber, Neuropsychologia, 1968, 6, 215) that H.M.'s ability to identify fragmented pictures is facilitated by prior experience with the stimulus set. In addition, we have extended the analysis of his perceptual-learning capacity by testing the effect of experience on the identification of words presented briefly. The present paradigm allowed us to determine whether his perceptual learning is limited to the specific stimulus material presented previously or whether it generalizes to new material.

Words were presented in blocks of 40, of which half were common to each block of the experiment (repeated words), and half were unique (non-repeated words). H.M. identified more of the repeated words than the non-repeated words. In addition, he showed a more general perceptual learning: He identified an increasing number of non-repeated words over the course of the experiment. Both of these effects persisted after delays of 1 hour and 1 day, the same delays over which facilitation was observed in the fragmented pictures test. Despite his perceptual learning in both of these tasks, H.M. did not remember having performed the task, a finding consistent with observations made in previous reports of impressive learning by amnesic patients. Moreover, H.M.'s recognition-memory performance after the experiment for the words that had been presented during the experiment was at chance.

The present results corroborate the findings of intact learning and long-term retention of certain skills in amnesic patients, despite impaired memory for the details of the experimental sessions and for the specific stimuli that had been presented (Milner, Physiologie de l'Hippocampe, 1962, p. 257; Corkin, Neuropsychologia, 1968, 6, 255; Cohen and Squire, Science, 1980, 210, 207). These findings provide further support for a dissociation among memory systems. In particular, they support the distinction between acquisition of procedural and declarative knowledge.

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80.4 THE AMNESIC PATIENT H.M.: DIMINISHED ABILITY TO INTERPRET AND REPORT INTERNAL STATES. Nancy Hebben, Karen J. Shedlack*, Howard B. Eichenbaum, and Suzanne Corkin. Dept. Psychol., Mass. Inst. Tech., Cambridge, MA 02139, and Dept. Biol., Wellesley Coll., Wellesley, MA 02181.

H.M. became amnesic after a bilateral resection in the medial temporal region for epilepsy. Since then it has been noted that he rarely comments on such internal states as hunger, thirst, pain, and fatigue. In order to document these clinical observations, we examined his thermal pain perception in relation to his other somatosensory capacities, and his reports of hunger before and after meals of normal and twice normal quantity.

H.M.'s thermal pain perception was evaluated using a Hardy-Wolff-Goodell dolorimeter. On three occasions, the radiant-heat stimuli were self-administered to the volar surface of both forearms. Subsequently, the same stimuli were self-administered to the chest, in case the results for the forearms had been affected by his known peripheral neuropathy. During each test session, 144 stimuli, 24 at each of 6 intensities (0, 90, 180, 270, 320, and 370 mcal), were presented pseudorandomly with respect to intensity. H.M. was instructed to assign each sensory experience to one of 11 categories ranging from "absolutely nothing" to "very painful" and "withdrawal." The data were analyzed using the methods of sensory decision theory, providing both an index of discriminability, P(A), and of report criterion, B. At all three stimulus sites, H.M. had significantly poorer discriminability scores for all intensity levels than did a group of normal control subjects. He also had higher criterion scores than did normal subjects, particularly at the three highest stimulus intensities. In fact, H.M. never gave reports of pain for the most intense stimulus applied either to his forearms or chest, and, in contrast to most other subjects, he did not withdraw the stimulus before the end of the 3 sec. stimulus duration.

A similar neglect of internal states is seen in H.M.'s reports of hunger. On a scale of 0 to 100, where 0 is "famished" and 100 is "too full to eat another bite," he consistently reported 50, whether he was about to eat or had just eaten. On one occasion we attempted to influence his report of satiety by supplying him with as much food as he could eat at one sitting. After he consumed one full dinner, his empty tray was removed, and without explanation another full dinner was provided. He did not behave as if anything unusual had happened, and only after having eaten the second dinner, except for the salad, did he report simply that he was "finished." At the time, he rated his satiation at 75. We conclude that medial temporal structures participate in the monitoring of pain and hunger.

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80.5 THE AMNESIC PATIENT H.M.: SEVERE, SELECTIVE OLFACTORY-DISCRIMINATION DEFICIT FOLLOWING BILATERAL MEDIAL TEMPORAL LOBECTOMY. Howard B. Eichenbaum, Harry Potter*, and Suzanne Corkin. Dept. Biol., Wellesley Coll., Wellesley, MA 02181, and Dept. Psychol., Mass. Inst. Tech., Cambridge, MA 02139.

Following bilateral resection in the medial temporal region, the patient H.M. has suffered from global anterograde amnesia. This man has been studied extensively because of the purity of his amnesia, a near-total inability to learn, in the absence of other serious cognitive, perceptual, or emotional impairments. Despite the well-known intimacy of olfactory and limbic brain structures, however, H.M.'s olfactory capacities have not been measured formally until now.

A series of measures, including threshold adaptation, intensity and quality discrimination, and odor identification, were used to compare the olfactory capacities of H.M., age-matched control subjects, patients with Korsakoff's disease, and patients with unilateral frontal-cortex lesions. H.M.'s thresholds for almond, ethanol, lemon, and acetone, using a forced-choice descending-staircase procedure, were comparable to controls'. In a standard signal-detection paradigm, H.M.'s detection of n-butanol and phenethyl alcohol was at least as good as controls', and, in the same paradigm, he demonstrated normal adaptation and cross-adaptation.

H.M. also performed normally on an intensity-discrimination task, where subjects were forced to choose the stronger of two odor intensities. The most intense of each of four odors was paired with each of nine others of less intensity. On this task, H.M.'s performance was superior to the mean scores of control subjects.

In striking contrast to his ability to detect and discriminate odor intensities, H.M. demonstrated virtually no ability to discriminate odors by quality, nor could he identify common odors: H.M.'s d' on a same-different signal-detection task was near-zero, even on a discrimination that normal subjects found easy. In a matching-to-sample task on which the performance of normal subjects is nearly perfect, H.M.'s score was at chance (except perfect detection of a blank stimulus), and he could not name any of the common odors correctly. H.M.'s perceptual difficulties seem to be restricted to the olfactory mode, since he performs well on a color-discrimination signal-detection task, and he can identify by vision or touch the same common foods he cannot name by smell. After naming a lemon by vision, he remarked, "Funny, it doesn't smell like a lemon."

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80.6 RECOGNITION IMPAIRMENT AFTER MEDIAL THALAMIC LESIONS IN MONKEYS. J.P.Aggleton* and M.Mishkin (SPON: L. Ungerleider). Lab of Neuropsychology, NIMH, Bethesda, MD 20205.

Although it has been known for nearly a century that some amnesic syndromes are associated with damage to the diencephalon, the locus of the critical lesion has remained uncertain. One region, however, that has been implicated by neuropathological evidence, both from amnesic patients with Korsakoff's psychosis and from those with third ventricle tumors, is the medial thalamus. In this experiment we tested whether or not surgical lesions of the medial thalamus in monkeys would impair memory.

Six cynomolgus monkeys (Macaca fascicularis) were trained preoperatively on a one-trial visual recognition task in which they were required to distinguish a sample object, presented 10 sec previously, from one that was completely novel (delayed nonmatching-to-sample). After reaching a criterion of 90% correct responses, three of the monkeys received thalamic lesions and three were kept as unoperated controls. The thalamus was approached from its dorsal aspect through a slit in the corpus callosum, after which the entire extent of the interthalamic connection, or massa intermedia, was transected along the midline. A sagittal strip of tissue extending 1-2 mm lateral to the midline was then removed from the massa intermedia on either side of the transection. The thalamic structures within the area of removal include the medial portion of n. medialis dorsalis, n. parataenialis, n. anterior medialis, n. reuniens and all of the midline nuclei.

This surgery had a profound effect on performance of the recognition task. Whereas the control animals reattained the preoperative criterion within a maximum of 80 trials, the best operated monkey needed 640 trials. The other two operated monkeys required over 1,000 trials and then could reach criterion only when the sample object was presented twice prior to the recognition test. Imposition of gradually increasing delays, from 10 to 120 sec, between sample and test, reinstated this deficit: whereas the normal monkeys' performance at the 120 sec delay remained high (range, 89-92%), that of the operated animals fell sharply (range, 67-71%). Subsequently, when the same monkeys were tested for visual discrimination learning, no evidence of impairment was found on either of two pattern discrimination. These preliminary results indicate that medial thalamic lesions in monkeys may impair memory selectively, and thus may provide an animal model of diencephalic amnesia.

80.7 THE ANATOMY OF AMNESIA: AMYGDALA-HIPPOCAMPUS VS. TEMPORAL STEM. Stuart Zola-Morgan* Larry R. Squire and Mortimer Mishkin, Veterans Administration Medical Center, San Diego, CA, Dept. of Psychiatry, UCSD School of Medicine, and NIMH, Bethesda, MD.

There has for some time been a lack of correspondence between the brain regions presumed critical to human amnesia and the findings in experimental animals with lesions in those same brain regions. Results from new studies have suggested that the specific brain structures most often linked to human amnesia and consequently most frequently investigated in animal experiments may have been misidentified, at least in part. Thus, Horel has suggested that damage to the temporal stem (TS) and not damage to the hippocampus itself is responsible for the deficit in human amnesia. TS contains afferents and efferents of temporal neocortex and more medial brain structures. Alternatively, Mishkin has demonstrated that conjoint damage to amygdala (A) and hippocampus (H), but not separate lesions of A or H, mimic some of the critical features of human amnesia. We have addressed these two views of amnesia by comparing the effects of TS damage with the effects of conjoint damage to A and H.

Monkeys with A+H lesions, those with lesions of TS, and a group of normal control monkeys were given two tasks: 1) the trial-unique delayed non-matching to sample task. This task can be easily mastered by monkeys who have had no previous test experience, and tests memory in much the same way that forced-choice recognition does in humans. We used intervals of 5 sec to 10 min between the acquisition and retention trials, permitting measurement of forgetting over relatively long delays. Impairment in delayed recall or recognition is a hallmark of human amnesia; 2) two-dimensional pattern discrimination. This task is known to be sensitive to temporal neocortical damage.

A double dissociation was found: animals with A+H lesions were severely impaired on the non-matching task and the severity of their impairment correlated with the length of delay. They were only mildly impaired on the pattern discrimination task. By contrast, monkeys with TS lesions were severely impaired on the pattern discrimination task, but were not different from normal monkeys on the non-matching to sample task.

The results will be discussed in terms of their implications for the neuropsychology of memory.

80.8 DIFFERENCES BETWEEN LIMBIC AND NONLIMBIC RETENTION PROCESSES. B. L. Malamut* and M. Mishkin. LN, NIMH, Bethesda, MD 20205.

Monkeys with combined amygdalo-hippocampal ablations cannot recognize objects they have seen only a minute or two before; yet they readily learn to select the baited object of a pair even when successive trials with that pair are separated by 24-hour intervals (Malamut et al., Neurosci. Abstr., 6:191, 1980). This successful learning in the face of rapid forgetting indicates the existence of a powerful mechanism for retention of information that is independent of the limbic system. New experiments characterize further the essential difference in function between the limbic and nonlimbic retention mechanisms.

Rhesus monkeys with amygdalo-hippocampal lesions (Group A+H) and their unoperated controls (Group N) were tested on Gaffan's object-reward association task involving the principle of win-stay, lose-shift (Learn. & Motiv., 10:419, 1979). In Experiment I, a list of 20 objects, half baited and half unbaited, were presented one at a time for acquisition. The baited and unbaited objects were then paired in a series of ten test trials. This series was repeated up to 4 times each day until the animals reached 90% performance on it twice. On ten such lists, each with different objects, Group A+H required an average of 6 trials to reach criterion, significantly longer than Group N, which averaged only 2.5 trials. On the first test trial of each list, however, the two groups did not differ significantly, Group A+H averaging 62%, and Group N, 71%. In Experiment II, the animals were tested on 50 new lists of 20 objects each, but the test trials for each list were given only once. Again, there was little difference in trial-one performance, Group A+H averaging 63%, and Group N, 68%. Finally, in Experiment III, the number of objects in each list was reduced from 20 to 2, with only one test trial per pair as before. In this experiment, Group A+H still averaged 63%, showing no evidence of improvement over the course of 1000 trials. Group N, by contrast, achieved 90% performance after an average of 460 trials.

Thus, an intact limbic system yielded no advantage on trial-one performance of the first two experiments, a small advantage when the object pairs were repeated within sessions in Experiment I, and a decisive advantage on trial-one performance in the third experiment. Apparently, the limbic system is critical for high levels of retention of object-reward associations after a single acquisition trial with short lists, or after two or three repetitions with long lists but short intertrial delays. With greater repetition, however, retention of object-reward associations can be mediated by a nonlimbic mechanism (Experiment I), and this retention appears to be independent of both list length and delay (Malamut et al., op cit).

80.9 ROLE OF THE AMYGDALA AND HIPPOCAMPUS IN TACTUAL MEMORY. Elisabeth A. Murray and M. Mishkin. Lab. of Neuropsychology, N.I.M.H., Bethesda, MD 20205

A severe deficit in the visual recognition memory of monkeys follows combined bilateral removal of the amygdala and hippocampus (Mishkin, Nature, 273: 279, 1978). To determine whether the memory impairment following this ablation is restricted to vision, possibly as a result of inadvertent damage to the visually related white matter and cortex of the temporal lobe, we examined the effects of combined lesions of the amygdala and hippocampus on monkeys' tactual memory.

One rhesus and three cynomolgus monkeys were trained preoperatively on a delayed nonmatching-to-sample task in the dark. In this paradigm the monkey is presented with a baited sample object and, subsequently, is rewarded for choosing the one of two simultaneously presented objects that does not match the sample. The animals were tested for 20 trials per day with a fixed set of 40 tactually distinctive objects that were randomly re-paired daily. The objects were mounted on corks which fit snugly into the wells of the testing board so that the monkeys had to grasp and lift the objects in order to obtain the reward. After achieving the criterion of 90% correct responses in 100 trials with 10 sec delays between sample presentation and choice, the animals' performance was measured in blocks of 100 trials each with delays of 30, 60, and 120 sec. The monkeys then underwent bilateral removal either of the amygdaloid complex, the hippocampal formation, or both. Following reattainment of criterion at 10 sec delays, the animals' performance was again measured at the longer delays.

Preoperatively, the monkeys averaged greater than 90% correct responses at all delays. Postoperatively, two monkeys (one rhesus and one cynomolgus) with the combined amygdalectomy and hippocampectomy eventually reattained an average of 87% correct responses at 10 sec delays but then were severely impaired at the longer delays, averaging 60% at 60 sec and 56% at 120 sec. A hippocampectomized monkey performed at better than 90% accuracy across all delays, while an amygdalectomized monkey's performance dropped to approximately 80% at 120 sec delays.

These preliminary results suggest that the amygdala and hippocampus are necessary for memory of tactual as well as visual and, perhaps, other stimuli and support the view that only the combined removal of these structures produces a severe memory impairment.

80.10 HIPPOCAMPAL ABLATION CAUSES SPATIAL REFERENCE MEMORY DEFICIT IN THE RAT. R.G.M.Morris*,P.Garrud* and J.N.P.Rawlins* (SPON: W.J.Heitler). Psychology Laboratory,University of St.Andrews, Scotland and Dept.Exptl.Psychology,Univ.Oxford,England.

The purpose of this behavioural study was to test Olton et al's(Behav.Brain.Sci.,1979) claim that spatial reference memory is unimpaired by hippocampal damage. Rats with total lesions of the hippocampus, superficial cortical lesions or sham control surgery (Ns=13,11,8 respectively) were allowed to escape from opaque water (26-1°C) onto a small platform (8.7 cm diameter) hidden (1 cm below water surface) at a fixed position in a large (132 cm diameter) pool (See Morris, Learn & Motiv,1981, for behav.procedure). Spatial room cues were readily available but no local cues marked the position of the platform. Hippocampally lesioned rats only were impaired throughout training ($p<0.001$) measured in terms of both escape-latency and path-length. On a subsequent "probe" trial during which the hidden platform was removed from the apparatus, control and cortical rats searched persistently in the previous vicinity of the platform; hippocampals did not. When a visible platform (1 cm above water surface) was then used for escape, again in a fixed place, the hippocampals soon showed rapid directional escape behaviour onto the platform indicating that their deficit was not due to any motor, motivational or reinforcement aspects of the task. A second identical "probe" test was conducted and the hippocampals again, unlike the controls and corticals, did not show directional search, so calling into question Moore's (Physiol.Psych.,1980) claim that damage to this brain area may result in greater attention to redundant cues. Finally, transfer of the rats back onto the "pure" spatial task resulted in the hippocampal deficit reappearing ($p<0.001$) so demonstrating that the original learning difficulties were not caused by interference from inflexible motor strategies. This pattern of results calls into question Olton et al's (1979) hypothesis. The effective performance of the control and cortically lesioned rats may be based on their learning a "spatial map" (O'Keefe and Nadel,1978) of the environment and encoding the position of the hidden platform. The poor performance of the hippocampally lesioned rats is caused either by the outright lack of such a map, an inability to use it, or by a severe impairment in spatial "acuity". A short film will be presented.

80.11 DISRUPTION OF SINGLE ALTERNATION LEARNING IN RABBITS BY HIPPOCAMPAL LESIONS. W. R. Salafia, K. M. Cardosi*, D. Marini*, J. M. Hogan*, D. R. Olson* and E. J. Pollack*. Dept. of Psychology, Fairfield Univ., Fairfield, CT 06430.

O'Keefe and Nadel (Behav. Brain Sci., 2:487-533, 1979) have argued that the hippocampus is responsible exclusively for the processing of spatial information. Hippocampal lesions are thought to disrupt the construction, storage and/or use of spatial maps. The purpose of the present experiments was to evaluate the effects of hippocampal lesions on a Pavlovian conditioning task, which required for performance, the processing of fundamentally temporal information. The task chosen was single-alternation (SA) pattern learning in which each SA trial consisted of presentation of a CS-US or reinforced (R) trial followed after 3 sec by a CS-alone or non-reinforced (N) trial. This sequence was repeated each 30 sec for a total of 50 SA trials per session.

Initially, rabbits were conditioned, at interstimulus intervals (ISIs) of either 250 or 1000 msec, to make nictitating membrane CRs. At each ISI, there were 3 groups, viz., dorsal hippocampal lesion (H), cortical lesion (C) and unoperated (U). After conditioning, animals were shifted to the SA pattern. At the 250-msec ISI, all animals appeared to learn the SA task, i.e., to respond more on R trials than on N trials, but the level of proficiency was low. At 1000-msec ISI, however, the C and U groups performed very well (90% CRs on R trials vs 15% CRs on N trials) while considerably poorer performance was in evidence for Group H.

A second phase was run to determine if some of the apparent SA-pattern learning may have been generated solely by the reinforcement schedule which was 50% by definition. In this phase normal (unoperated) animals were conditioned, then shifted to a quasi-alternation (QA) task which consisted of the same pattern of trials as previously i.e. one trial (A) followed after a 3-sec interval by another (B), followed by a 30-sec interval, etc. However, for the QA task, the CS was paired with a US on a 50% schedule randomly distributed across A and B trials. An overall tendency was seen at both ISI values, for there to be fewer CRs on B trials than on A trials, an effect which was particularly pronounced at the 1000-msec ISI. This indicated that in the previous SA phase, most of the learning displayed by Group H at 1000-msec ISI was not SA-pattern learning and therefore, that the disruption of this type of learning by hippocampal lesions was virtually complete. These data are incongruent with the spatial information processing hypothesis of hippocampal functioning.

80.12 MEMORY FOR LISTS OF ITEMS IN RATS: ROLE OF THE HIPPOCAMPUS. Raymond P. Kesner and Jeanne M. Novak*. Dept. of Psychol. Univ. of Utah, Salt Lake City, UT. 84112.

The present study was designed to examine whether similar to humans rats (a) can remember a list of 8 items, (b) can display a serial position curve, (c) do not show the "recency" component of a serial position curve when tests are delayed, (d) do not show the "primacy" component of a serial position curve after the hippocampus is ablated.

Rats were trained on an eight arm radial maze for Froot Loop reinforcement. After extensive training each animal was allowed on each trial (one per day) to visit all eight arms in an order that was randomly selected for that trial. The sequencing of the eight arms was accomplished by sequentially opening of Plexiglas doors (one at a time) located at the entrance of each arm. Thirty seconds after the animal had received reinforcement from the last of the eight arms, the test phase began. Only one test was given for each trial and consisted of opening two doors simultaneously. On a random basis either the first and second, fourth and fifth, or seventh and eighth doors that occurred in the sequence were selected for the test. The rule to be learned leading to an additional reinforcement was to choose the arm that occurred earlier in the sequence. Based on 36 tests per animal all rats displayed a serial position curve, e.g., excellent retention (better than chance performance) for 1-2 and 7-8 positions, but no retention (chance performance) for 4-5 position. Delaying the test phase by 10 min eliminated retention of the "recency" (7-8) component of the serial position curve without markedly altering the "primacy" (1-2) component. Dorsal hippocampal lesions impaired retention of the "primacy" (1-2) component of the serial position curve without markedly altering the "recency" (7-8) component. These data are remarkably similar to memory performance seen with normal humans and humans with presumed hippocampal damage. It is concluded that the hippocampus of both rats and humans plays an important role in encoding of mnemonic information in episodic long-term but not short-term memory.

80.13 EFFECTS OF FRONTAL- AND TEMPORAL-LOBE LESIONS ON PERFORMANCE OF SELF-ORDERING TASKS. M. Petrides* and B. Milner. Dept. of Psychology and the Montreal Neurological Institute, McGill Univ., Montreal, Quebec, Canada, H3A 2BA.

Patients with excisions of the left frontal (LF) or right frontal (RF) lobe, left (LTh) or right (RTh) temporal lobe involving little or no damage to the hippocampal region, and left (LTH) or right (RTH) temporal lobe with extensive damage to this region were tested on four self-ordering tasks. In these tasks, the subject had to organize and carry out a sequence of responses rather than merely reproduce a sequence imposed by the experimenter. On any given trial, the subject was required to touch a set of stimuli, one at a time and in any order he liked but without touching any stimulus more than once. The four self-ordering tasks differed only in the type of stimulus-material: abstract designs (AD), representational drawings (RD), high-imagery (HI) or low-imagery (LI) words.

The LF group was impaired on all four tasks, whilst the RF group was impaired only on the two nonverbal tasks. The deficits appeared to be due both to poor organizational strategies and poor monitoring of responses. In addition, a deficit was observed in the LTH group on the more difficult verbal task (LI) and in the RTH group on the two nonverbal tasks (AD and RD). The LTh and RTh groups showed no impairment on any of these tasks. The material-specific deficits of the temporal-lobe groups with extensive involvement of the hippocampal region can be ascribed to the memory-demands of the tasks. These results as a whole point to an interaction between the frontal neocortex and the hippocampal region in self-ordering behavior.

81.1 READINESS-POTENTIALS (RP's) CORRELATED WITH MENTAL "PLANNING" VS. "SPONTANEITY", WHEN PERFORMING SELF-PACED MOTOR ACTS. B. Libet, E.W. Wright, Jr.*, and C.A. Gleason*, Dept. Neurosci.-Neurol.Inst., Mt. Zion Hospital, and Dept. Physiol., University of California, San Francisco, CA. 94143.

The slow negative RP's that precede self-paced, quick flexions of fingers can exhibit durations from 0.3 to > 1.4 sec, as recorded at the vertex and averaged for 40 trials. These formed two groups: I) durations clustered close to 0.5 sec (0.4-0.6); II) durations usually considerably longer than 0.6 sec. A given subject (S) may exhibit both types.

(a) After a type-II RP, questioning S almost invariably revealed S was aware of a plan or intent to act some variable time up to 1 sec or more before at least some of the acts in the 40-trial series. (b) When S was instructed to "try to let the urge to act come on its own, no matter how long it may take, without concentrating on and/or pre-planning the act" - (i) a type-II RP in a preceding uninstructed trial series could "switch" to a type-I RP in the next series; (ii) RP's of all series with such instructions were predominantly type-I. (c) Conversely, if a condition for pre-planning was introduced deliberately, the potentials appearing before the acts (averaged for 40) resembled type-II RP's, though they were generally larger and longer. In this condition, S's were instructed to act at a predetermined "clock-time" whose impending arrival was displayed to S. Such "planning potentials" did not appear when S expected to receive (and subsequently report) a sensory stimulus to the hand rather than being required to act, at similarly predetermined times; these potentials are therefore a function of neural preparation for a motor act, not one of anticipation of the occurrence of any event.

We conclude (1) that duration and amplitude of RP's may be altered in variable but potentially large amounts by a factor related to pre-planning to act; and (2) that substantial RP's of more uniform durations (~0.5 sec) appear when the immediate urge, felt by S to perform the self-paced act, arises suddenly and "spontaneously".

81.2 DYNAMIC BRAIN POTENTIAL PATTERNS OF HUMAN HIGHER COGNITIVE FUNCTIONS. Alan S. Gevins, Joseph C. Doyle*, Brian A. Cutillo*, Robert E. Schaffer*, Robert S. Tannehill*, EEG SYSTEMS LABORATORY, Univ. California Sch. of Med., SAN FRANCISCO, CA 94143.

A new technique has been developed to identify dynamic spatiotemporal electrical patterns of the human brain during purposive behaviors. In this method single-trial time-series correlations between brain macropotentials recorded from different scalp sites are analyzed by distribution-independent mathematical pattern recognition. Dynamic patterns of correlation clearly distinguished two brief visuomotor tasks differing only in type of mental judgment required (spatial or numeric). These complex patterns shifted in the anterior-posterior and left-right axes between successive 175 msec intervals, indicating that many areas in both cerebral hemispheres were involved even in these simple judgments. These patterns were not obtainable by conventional analysis of averaged evoked potentials or by linear analysis of correlations, suggesting that this method will advance the study of human brain activity related to cognition and goal-directed behaviors.

81.3 FUNCTIONAL 2-DEOXYGLUCOSE MAPPING IN ASSOCIATION CORTEX: PREFRONTAL ACTIVATION IN MONKEYS PERFORMING A COGNITIVE TASK. N.M. Bugbee and P.S. Goldman-Rakic. Section of Neuroanatomy, Yale Univ. School of Medicine, New Haven, CT 06510.

Surveys of local cerebral metabolism using 2-deoxyglucose have been successful in identifying central correlates of sensory and motor stimulation in behaving animals. We now demonstrate that this method also reveals metabolic changes associated with the performance of a cognitive task.

Ten rhesus monkeys, ranging in age from 3-48 months, served as subjects. Six animals were trained on a delayed response (DR) task, a behavioral paradigm known to be dependent upon prefrontal association cortex. In this task, the monkey was required to remember the location of a target over a 12 second delay period. Correct responses were rewarded with food. Four control (CON) animals were tested under identical circumstances, with the same number of trials and reinforcements, except that no delay was interposed; these subjects were allowed to respond as soon as the target was presented. To insure that motor activity and auditory input were similar for both groups, all subjects were restrained in primate chairs and exposed to amplified white noise. At the beginning of its final testing session, each monkey received an i.v. pulse of ^{14}C 2DG (100 μCi/kg) via a femoral catheter; arterial samples were collected throughout the session. Subjects worked steadily over the 45 minute period of 2DG administration, with DR animals performing at 90% correct.

Local cerebral glucose utilization (LCGU) quantified according to the procedures described by Sokoloff et al (J. Neurochem., 28, 897, 1977), was measured in eight cortical regions: prefrontal, motor, auditory, striate, extrastriate, entorhinal, and superior and inferior temporal gyrus. To compensate for individual variations in absolute LCGU, data for each animal was expressed as ratios. LCGU of each of the above cortical regions was compared to that of primary auditory cortex, a region in which sensory input was constant for all subjects. Statistical comparisons demonstrated that only the prefrontal ratio differed for the DR and CON groups ($p < .05$, t test, two-tailed). None of the other cortical areas examined could be differentiated according to behavioral task.

These findings based on metabolic mapping add to a large body of behavioral and electrophysiological evidence demonstrating the involvement of prefrontal cortex in DR performance. Whether these prefrontal metabolic changes are specific to the spatial-mnemonic aspects of the task, or whether they reflect the more general attentional demands of any task, the results indicate that the 2DG method can be used to study neural systems associated with cognitive processes. (Supported by NS16666 and MH07790).

81.4 ONLINE CLASSIFICATION OF INDIVIDUAL VISUAL EVOKED POTENTIALS. H. Galin*, D.R. Crapper McLachlan. (SPON: J.T. Murphy). Dept. of Physiology, Univ. of Toronto, Toronto, Ontario. M5S 1A8 (Canada).

Human visual evoked potentials were recorded in response to nine different randomly presented stimuli generated by an 8x8 matrix of yellow light emmitting diodes. Stimuli, subtending about 4^{o}, were presented for 5 msec. at an intensity of 8 to 14 cd/m^2. Stimulus presentation, data collection and the subsequent processing of the evoked potential were controlled by a program running on a PDP 11/35 computer and interacting with a CAMAC crate. Data sampled at 1 kHz, consisted of a 50 msec. pre-stimulus period and a 462 msec. post-stimulus period. The post-stimulus period was divided into nine segments and the slope and the logarithm of the residual sum of squares was calculated for each segment. These eighteen parameters were stored for each presentation until a sufficient number of repetitions for each stimulus had been accumulated to allow the calculation of a co-variance matrix and its inverse. A discriminant analysis was then performed in order to generate a set of classification criteria for the stimulus sub-types. These classification criteria were then applied to the previously collected response vectors and the set of posterior probabilities calculated. Based on the error rate of this criterion check either these criteria were utilized to process subsequent novel presentations or these criteria were rejected and additional stimuli were accumulated.

In eight experimental sessions involving three subjects, over 90% of the approximately 2000 presented stimuli have been successfully predicted based on the calculated posterior probabilities.

81.5 METABOLIC AND STRUCTURAL COMPARISONS TO LANGUAGE FUNCTION IN APHASIA, E. J. Metter*, W.Riege, W.Hanson*, L. Camerus*, M. Phelps , D. Kuhl (SPON: N. P. Rosenthal). Dept. of Nuclear Medicine, UCLA Med. Ctr., Los Angeles 90024.

Tomographic images of brain metabolism in post-stroke aphasics using ^{18}fluorodeoxyglucose positron emission computed tomography (FDG PECT) showed areas of metabolic depression extending beyond the zones of infarction determined by x-ray CT. To evaluate the relationship between language abnormalities and anatomic or metabolic lesions, eleven right handed aphasic patients had FDG PECT, x-ray CT, and the Boston Diagnostic Aphasia Examination (BDAE). Local cerebral metabolic rates for glucose (LCMRglc) were determined for thirteen brain areas in the damaged hemisphere and were compared to the opposite hemisphere. Corresponding areas on x-ray CT scans were graded by a neuroradiologist on a five point scale for severity of damage. The BDAE subtests were reduced to eleven mean scores, and were compared to either metabolic or CT data using correlational analysis. At $p<0.01$, the LCMRglc of the posterior middle-inferior temporal ($r\sim0.80$) and the parietal regions ($r\sim0.82$) were significantly correlated with auditory comprehension, naming, oral reading, and repetition. X-ray CT did not show these correlations but showed correlation of left frontal damage with writing ($r=0.74$), left parietal damage with repetiton ($r=0.70$), damage of Wernicke's area with oral reading and repetition ($r\sim0.77$), and posterior middle-inferior temporal area damage with automatic speech ($r=0.87$) and repetition ($r=0.69$). These findings suggest that language correlations with structural damage may be deceptive by ignoring abnormal function in remaining cerebrum. The correlations from metabolic rates, suggest that areas posterior, inferior, and superior to the traditional Wernicke's area may be important in the abnormal language of these aphasics.

81.6 MONKEY VOCALIZATION: EFFECTS OF SUPPLEMENTARY MOTOR DAMAGE. D. Sutton, R.E. Trachy* and R.C. Lindeman*. Virginia Mason Research Center, Seattle, WA 98101.

Two rhesus monkeys were trained to produce a vocal call (coo) or a lever press to distinctive visual cues. Measures of latency, discrimination index and efficiency were obtained for each response. In addition, acoustic properties of the coo were analyzed. Bilateral lesions of the supplementary motor area (SMA) were imposed after the animals had achieved a stable performance level reflected by high discrimination and short-latency responding. Following a one-week recovery period, retesting was carried out daily for one month.

SMA lesions failed to disturb lever press measures. By contrast, vocalization performance underwent sustained increases in latency on the part of both monkeys. There was little influence of lesions upon other vocal performance measures.

Histologic study of brain tissue from the two animals revealed damage confined largely to superior frontal gyrus (areas 6 & 8), superior to the cingulate sulcus.

The results suggest that supplementary motor area participates in the process of initiating voluntary phonation.

81.7 PROLONGED VISUAL RECOGNITION MEMORY IN SQUIRREL MONKEYS. William H. Overman, Jr. University of North Carolina at Wilmington, N.C. 28401

Memory capabilities of non-human primates are often assessed by the procedure of delayed-match-to-sample in which a sample object is presented, removed, and after a delay, presented again together with a second object. The animal's task is to respond to (match) the object previously seen. When the same pair of objects is used repeatedly, one or the other serving as the sample, macaques have difficulty making the match after a few seconds; however, when a new pair of objects is used for each trial, macaques can make reliable matches after delays of hours or days (Overman and Doty, Neuroscience 5: 1825, 1980). Moreover, when tested by the trial-unique procedure, macaques can accurately remember lists of ten items (ten consecutive samples) after delays of several minutes (Gaffan, J. Comp. Physiol. Psychol. 86: 1100, 1974). Recently, the serial position curves of errors on lists of ten items have been shown to be quite similar for man and macaques (Sands and Wright, Science 209:938, 1980).

In the present study the trial-unique procedure of matching was extended to a species of new world primates (Saimiri sciureus) It was found that squirrel monkeys, like old world monkeys (Mishkin and Delacour, J. exp. Psychol.:An. Beh. Proc. 1:326, 1975) tend to respond preferentially to novel objects. Thus, one group (N=5) learned a trail-unique, non-match-to-sample task with an average of 216 errors while another group (N=5) learned a trial-unique match-to-sample task with an average of 587 errors. Once either the match or non-match group learned, however, the animals could reliable remember lists of 3, 5,or 10 items significantly better than chance.

The non-match group was given 40 days training on lists of 20 items. When the groups errors were computed for each of the 20 serial positions, there were strong recency and primacy effects. A quadratic curve fitted to these data was highly significant.

Thus, it is concluded that visual recognition memory operates by similar mechanisms in a variety of primates including man, old world monkeys and according to the present data, a species of readily available new world primates, squirrel monkeys.

81.8 RANDOM DOT KINEMATOGRAMS: DISPLACEMENT LIMIT DEPENDS ON STIMULUS AREA. Curtis L. Baker, Jr. and Oliver J. Braddick*, Departments of Physiology and Experimental Psychology, Cambridge University, Cambridge, U.K.

The sensation of a smoothly moving textured surface elicited by 'random dot kinematograms', in which a contiguous patch of dots is uniformly displaced from one frame to the next, illustrates a 'correspondence problem' for motion similar to that which is recognised for random dot stereograms. Braddick (1974) showed that the perceptual segregation of a displaced patch in such patterns depended on a 'short-range' motion process which could detect displacements of less than 15 min.arc, regardless of the number of element positions this entailed.

We have now shown that this displacement limit increases with the area of the displaced patch (measured in retinal angle). (This would be analogous to the maximum disparity for stereopsis increasing with the area of a random dot stereogram.) Changing patch area over a range of 0.25-12.0 deg^2 results in displacement limit variation ca 4-15 min.arc. The limit for directional discrimination shows a similar area dependence to that for segregation. Experiments varying dot density show that the number of dots in the patch is not a confounding variable, and in fact has surprisingly little effect over a large range. The displacement limit also increases slightly with eccentricity, but not enough to account for the observed effects. The result is not an artifact of changes in surround size.

Displacement limit dependence on patch area, but not on dot density, is consistent with a motion analog of the cooperative stereopsis model of Marr and Poggio (1976).

Psychophysics experiments on random dot kinematograms delineate a low level motion-detecting process with well-defined dependence on displacement and other stimulus variables. We therefore believe that they provide a promising correlate for potential neurophysiological investigations, which may lead to better understanding of the neural basis of motion detection, the solution of correspondence problems, and figure-ground segregation.

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Braddick, O.J. Vision Res. 14: 519-527, (1974).
Marr, D. and Poggio, T. Science 194: 283-287, (1976).

81.9 ELECTROPHYSIOLOGIC ASYMMETRY AND BRAIN CHANGE IN PRIMARY DEGENRATIVE DEMENTIA. B. Reisberg,* S.H. Ferris, H. Ahn and E.R. John. Dept. of Psychiatry, N.Y.U. Med. Ctr., New York, N.Y. 10016.

We studied progressive EEG and auditory evoked potential (AEP) changes accompanying cognitive decrement in 12 outpatients 60 to 85 years of age with Primary Degenerative Dementia (PDD).

Electrophysiologic data were gathered using an automated digital electrophysiological data acquisition and analysis system which gathered data simultaneously from all 19 electrodes of the international 10/20 system, monopolar, referenced to linked earlobes. Bipolar derivations were constructed by computation. An automatic artifact rejection algorithm rejected data contaminated by eye movements. Spectral computations for the one minute eyes-closed EEG condition were performed by numerical filtering. These analyses yielded relative power in each of 4 frequency bands: delta (1.5-3.5Hz), theta (3.5-7.5Hz), alpha (7.5-12.5Hz) and beta (12.5-25Hz). Power asymmetry in the fronto-temporal and temporal brain regions for delta, theta and alpha wavebands increased significantly ($p<.05$) with cognitive decrement assessed on a global deterioration scale (GDS). Some of these relationships were particularly strong. Most notably, theta asymmetry in the fronto-temporal region correlated with global assessments of deterioration ($r=.81$, $p<.001$).

AEP measures for regularly produced auditory stimuli were examined for the anterior temporal regions (T3 and T4). The stimuli consisted of clicks with a duration of .1 msec, presented 8 feet from the patient with an intensity of 60 db at a rate of 1/sec over a 1-minute period. Fifty evoked potentials were averaged to produce the AEP waveform which we studied. We measured the latency of successive negative and positive peaks whick deviated more than 0.5 μv from the baseline. These peaks were labeled in an ordinal fashion, successively being designated N_1, P_1, etc. We constructed ratios of the longer of the two hemispheric latency measures to the shorter of the two hemispheric latency measures. Increased asymmetry of N1 and N3 correlated significantly with GDS assessments of deterioration ($r=.67$ and .60 respectively, p's<.05).

We conclude that although PDD is customarily thought of as a symmetric degenerative process, occurring to approximately the same extent in both brain hemispheres, it is logical to postulate that subtle asymmetric neurophysiologic changes do indeed occur. The electrophysiologic manifestations of these asymmetries appear to relate strongly to the severity of the disorder. These results also suggest that electrophysiologic asymmetries may be related to general impairments of cognitive processing accompanying PDD.

81.10 A CORRELATIONAL INVESTIGATION BETWEEN POWER SPECTRAL BANDS FROM MULTI-CHANNEL EEGs IN THE CAT. J. H. Stramler. Department of Biology, Texas A&M University, College Station, TX 77843.

The coherence measure represents, in the frequency domain, the degree of linear relationship at a given frequency between two channels of time series data. It has been observed that for EEG data the coherence measure is rather varient over time, and values are generally low, indicating the lack of such a relationship. However, this technique is not capable of comparing relationships between different frequencies. To explore a possible means of determining whether one frequency in one channel may correlate with another frequency in a second channel, data were used from an experiment which recorded multi-channel EEG activity from cats in both normal and drugged (physostigmine, scopolamine, amphetamine) states. The usual power, phase and coherence spectra were computed, but additionally correlation matrices were constructed from normalized power spectral intensities divided into a number of bands over the range of frequencies from 0-50 Hz. The resulting matrices were presented for visual examination using a Fortran subroutine PRTCM (Stramler, J. H. and Lewis, J. L., Behav. Res. Meth. Instr., 12: 471-2, 1980). In general, the linear correlations obtained with this technique were low, as are most coherence values, but a greater number of bands show significant correlations than would be expected by chance at the .01 level. The pattern of these correlations changes with state.

81.11 EEG CORRELATES OF INTELLIGENCE IN CHILDREN. Robert W. Thatcher, Mike L. Lester, Rebecca McAlaster and Richard Horst. Applied Neuroscience Inst., University of Maryland Eastern Shore, Princess Anne, MD 21853.

Two minutes of eyes closed EEG was obtained from 19 scalp locations (O1, O2, P3, P4, T5, T6, T3, T4, C3, C4, F3, F4, F7, F8, Fp1, Fp2, Fz, Cz, Pz) and a eye monitor channel in 196 children age 5 to 16. Power spectral analyses were performed on artifact free EEG samples yielding measures of absolute power, relative power; interhemispheric amplitude symmetry, and interhemispheric coherence in the frequency bands of delta (1.5 to 3.5 hz), theta (3.5 to 7.0 hz), alpha (7.0 to 13 hz) and beta (17 to 22 Hz). Psychometric tests were administered to each child which included the WISC-R, WRAT, Purdue Pegboard, The Stott, Moyers and Henderson test for Motor Impairment and measures of handedness. The psychometric statistics for the sample were full scale I.Q. (mean = 110.449, range 66 to 150), WRAT reading (mean = 112.659, range = 58 to 156), WRAT spelling (mean = 107.116, range 56 to 151), and WRAT arithmetic (mean = 98.572, range 63 to 149).

Hierarchical regression analyses in which age was regressed out yielded significant relations between WISC and WRAT scores and measures of interhemispheric coherence. A quadratic function (inverted "U") significantly fit the relation between interhemispheric coherence and I.Q. in the delta ($P < .01$) and theta ($P < .01$) frequency bands. A linear function was significantly related to I.Q. and interhemispheric coherence in the alpha ($P < .0002$) and beta ($P < .01$) bands. The former relationship was found in widespread regions of the scalp while the latter occurred strongest in frontal derivations. To test the strength of these relations "effect size" was calculated and split-half replications (BMDP P9R) were performed. The effect size power was .9995 and the split half replications were also significant.

81.12 AUDITORY BRAINSTEM EVOKED RESPONSES IN ATTENTION DEFICIT DISORDER WITH HYPERACTIVITY. Janet A. Camp* and Bertrand G. Winsberg* (SPON: Henri Begleiter). Long Island Research Institute, State University of New York at Stony Brook, Stony Brook NY 11794.

Although a variety of data provide circumstantial evidence that children with Attention Deficit Disorder with Hyperactivity (ADD/HA) are stigmatized by central nervous system (CNS) dysfunction, there is little direct data to support this notion. The present study addressed the question of CNS integrity in this population by asking whether 1) ADD/HA children differ from normal controls with respect to auditory brainstem evoked responses (BER), 2) the BER correlates with psychological/behavioral parameters used in diagnosing ADD/HA, and 3) methylphenidate (Ritalin) normalizes the BER.

Subjects were boys aged 67-154 months with IQ ≥86. The clinical sample (N=20) had been diagnosed as ADD/HA according to DSM-III criteria, and in addition all had received scores >1σ above the standardized mean on both the Attention and Hyperactivity factors of the Conners' Teacher Rating Scale; normal controls (N=20) all had received factor scores below this mean. A similar dichotomization was used with parental ratings of the child's behavior. Monaural BERs to left and right ear 60 dB clicks were recorded between the vertex and the ipsilateral mastoid, with the contralateral mastoid as ground. Data were recorded on tape, and tapes were edited to eliminate movement artefacts. All children were tested without sedation and were free of all other medications at the time of testing.

ADD/HA children showed significantly longer latencies for BER Wave I and Wave V, while brainstem conduction times did not differentiate the two groups. Since IQs for the control sample were significantly higher, a subsample of 10 children per group were matched for age and IQ; identical group differences were obtained from this subsample. Regarding behavioral parameters, no aspect of the BER correlated with any psychological or rating scale data obtained from the ADD/HA children. Finally, when half the ADD/HA children were reexamined under methylphenidate, both Wave I and Wave V latencies were significantly decreased.

Since the putative generators of both Wave I and Wave V of the BER receive efferent inhibitory inputs from more central CNS structures, it may be that the longer BER latencies in the ADD/HA children represent a peripheral reflection of a dysfunctional CNS inhibitory process. The fact that methylphenidate appears to correct this electrophysiological problem suggests that the drug may be acting on those structures involved in providing this inhibition.

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82.1 PRINCIPLES OF USE OF FREEZE-SUBSTITUTION FOR FINE-RESOLUTION (2-DEOXY-D-GLUCOSE) GLUCOGRAPHY OF BRAIN. S.J. Schein and F.M. de Monasterio*. Clinical Branch, National Eye Institute, Bethesda, MD 20205.

Fine-resolution glucography, employing 2-deoxy-D-glucose (2-DG) to map rates of glucose utilization, promises discrimination of small structures and high signal-to-background ratios. The accumulated intracellular label, 2-deoxy-D-glucose-6-phosphate (2-DG-6-P), is water soluble, imposing severe constraints on how the tissue may be processed. The large size of most central nervous system material, from a rat brain (∿1.5 ml) to a cynomolgus monkey brain (∿60 ml) imposes an additional difficulty. Freeze-substitution has been used to retain localization of water-soluble molecules in thin material. We show here that freeze-substitution may also be used with large structures.

First, we discuss the physical principles of freeze substitution. The rates of withdrawal of water from a rat hemisphere or a gelatin block fall exponentially with time and are proportional to the surface area. The surface-normalized initial rate of withdrawal of water (R) is the same for a rat hemisphere as for a block of gelatin. These findings implicate diffusion as the mechanism of water withdrawal. For a given solvent, R declines as the temperature declines. The temperature dependence is not fit by a single line on an Arrhenius plot. Instead, the effect of lower temperature is to lower the solubility of water in solvent, and R falls proportionately. The nature of the solvent also plays a role: In tetrahydrofuran R is 0.016 gm/day·cm^2 for each percent water solubility. In pyridine, acetone and dimethylformamide, R is less by the factors 0.94, 0.38 and 0.26, respectively. These additional findings permit us to present a detailed description of the physical process of freeze-substitution, including the factors which affect it. Given these data, appropriate conditions of solvent, temperature and time may be determined for freeze-substitution of tissue of any size, even tissue as large as a monkey brain.

Second, using both ^{14}C- and ^{3}H-2-DG, we will demonstrate that preservation of strict localization of 2-DG-6-P may be obtained with freeze-substitution of brain.

82.2 THE INTERACTION OF BLOOD-BRAIN BARRIER TRANSPORT AND BRAIN PHOSPHORYLATION OF GLUCOSE AND 2-DEOXYGLUCOSE: A REEVALUATION OF THE LUMPED CONSTANT. W.M.Pardridge, P.D.Crane* and W.H. Oldendorf. UCLA School of Medicine, Los Angeles, CA 90024

The quantitation of regional brain glycolysis with the 2-deoxyglucose (2-DG) technique requires the use of a correction factor, the lumped constant (LC), which expresses the relative rates of phosphorylation of the two hexoses. Since LC is a function of λ (the relative brain volumes of distribution of the two sugars), it is to be expected that LC will not be constant, but will vary with the given physiological condition. For example, when brain phosphorylation rates are slow relative to blood-brain barrier (BBB) transport rates (e.g., the conscious or anesthetized states), the LC will approximate 0.4 (Sokoloff, 1977) and this reflects the greater affinity of hexokinase for glucose relative to 2-DG. However, when brain phosphorylation is limited by BBB transport (e.g., hypoglycemia or states of accelerated demand such as ischemia) then LC will equal or exceed values of 1.0 (Crane et al, J. Neurochem. 36:1601, 1981) and this reflects the greater rate of 2-DG transport through the BBB relative to glucose (Oldendorf, 1971). Although LC is not constant, this parameter can be reduced to absolute constants. Given these absolute constants, then LC can be predicted from data on only plasma (Ca) and brain (C_B) glucose. The absolute constants needed to predict LC from Ca and C_B are (i) the K_m, V_{max}, K_D of BBB transport of glucose and 2-DG and (ii) the k_3^*/k_3 ratio which is the ratio of fractional rate constants of hexokinase phosphorylation of 2-DG (k_3^*) and glucose (k_3). Therefore, in the present studies we determined for seven regions of the barbiturate-anesthetized rat brain the K_m, V_{max}, K_D of hexose transport and the k_3^*/k_3 ratio of hexose phosphorylation. Parameters for two regions are:

Hexose	Parameter	Hippocampus	Hemisphere
Glucose	K_m (mM)	5.5 ± 1.6	11.2 ± 2.7
	V_{max} (μmol/min.g)	0.47 ± 0.18	1.14 ± 0.37
2-DG	K_m (mM)	10.1 ± 3.8	7.7 ± 0.4
	V_{max} (μmol/min.g)	1.45 ± 0.76	1.15 ± 0.13
	k_3^*/k_3	0.26 ± 0.06	0.38 ± 0.16

Given known values for Ca and C_B, the calculated λ is 1.65 and 1.39, and the calculated LC is 0.43 and 0.53, for the hippocampus and hemisphere, respectively. However, under conditions of hypoglycemia, e.g., hypothetical values of Ca = 1.0 mM and C_B = 0.1 μmol g^{-1}, then the calculated values for λ and LC are 2.70 and 1.03 respectively, for the hemisphere. In conclusion, the lumped constant varies with changing plasma and/or brain glucose and the magnitude of LC can be predicted from known constants of BBB hexose transport and brain phosphorylation.

82.3 A COMPARISON OF THE BLOOD-BRAIN BARRIER TRANSPORT AND HEXOKINASE KINETICS OF 2-FLUORO-2-DEOXY-D-GLUCOSE AND 2-DEOXY-D-GLUCOSE. P.D.Crane*, W.M.Pardridge, L.D.Braun*, and W.H.Oldendorf (SPON:M.Philippart). Depts. of Neurology and Medicine, UCLA Sch. of Med.,and Brentwood VA Med. Ctr., L.A., CA 90073

The kinetics of transport across the blood-brain barrier (BBB) and metabolism in brain (hemisphere) of ^{14}C 2-fluoro-2-deoxy-D-glucose (FDG) were compared with that of ^{3}H 2-deoxy-D-glucose (DG) and D-glucose in the adult pentobarbital anesthetized rat. Saturation kinetics of transport across the BBB, measured with the brain uptake index (BUI) method (Oldendorf,W.H., Brain Res. 24:372, 1970) indicated a BUI of 56 ± 2 with a Km = 9.1 ± 1.5 mM, Vm = 2.6 ± 0.5 umol/min/g and the Ki of glucose inhibition of FDG transport was 10.7 ±4.4 mM. The kinetic constants of influx (k_1), efflux (k_2), and metabolism (k_3) for FDG were calculated from the Km, Vm, and plasma and hemisphere glucose concentrations, 2.51 umol/g and 9.7 mM, respectively. The lumped constant (LC) was obtained with the equation of Sokoloff et al. (J.Neurochem. 28:897, 1977), $LC = (k_1^*/k_1)(k_3^*/k_3)[(k_2+k_3)/(k_2^*+k_3^*)]$. The calculated LC for DG = 0.47 and for FDG = 0.71. We have predicted (Crane et al., J. Neurochem. 36:1601, 1981) that the LC will rise under conditions of transport limitation (hypoglycemia) or elevated glycolysis (ischemia, seizures) to a maximum = k_1^*/k_1 (1.2 for DG, 1.79 for FDG) and drop to a minimum = k_3^*/k_3 (0.34 for DG and 0.47 for FDG) during phosphorylation limitation in extreme hyperglycemia. Conclusion: The fluctuation of the LC can be predicted on the basis of the known Km and Vm of FDG and glucose transport across the BBB. Knowledge of this fluctuation in the LC is crucial in interpreting data obtained from ^{18}FDG analysis of regional glucose utilization in human brain in pathological states. (This study was funded by the American Diabetes Association, St. Louis Chapter, by NIAAA-AA03513, and by the Veterans Administration.)

82.4 RAPID TRANSIENT DROP IN BRAIN FREE GLUCOSE AFTER INTRAVENOUS PHLORETIN. W.H. Oldendorf, P.D.Crane*,and L.D.Braun*. Research Service, Brentwood VA Medical Center, Los Angeles, CA 90073 and Department of Neurology, UCLA School of Medicine, Los Angeles, CA 90024.

Phloretin, the aglycone of the glycoside phlorizin, was injected into the tail vein of awake adult Wistar rats to assess its effect on brain free glucose (BFG). Dosage range was 30-90 mg/kg in 0.5 ml propylene glycol. Injection of the vehicle alone as a control had no measurable effect on plasma or brain glucose. Phloretin is an inhibitor of many transport systems and other enzymes but has an especially high affinity for non-sodium dependent glucose transport. When present in blood, it competes for blood-brain barrier (BBB) transport of glucose, diminishing influx of glucose into brain and BFG falls. At intervals up to 20 minutes after injection, the rat brain was brought rapidly (2 sec) to 90-100C by exposure to high intensity microwaves (12.4 cm mavelength, Gerling-Moore "Metabostat"). After cooling, the cerebral cortex was dissected free and its BFG determined enzymatically. Control rat BFG was 2.2 umol/g. By one minute after injection of 90 mg/kg, BFG was 0.8 umol/g, falling by 4 minutes to a minimum of 0.65 umol/g. After a single injection of phloretin, the BFG had returned to control levels by 15 minutes. One rat receiving 200 mg/kg died within 3 minutes, BFG not done. Although plasma glucose remained within normal limits, the animals exhibited behavior compatible with hypoglycemia (shivering, piloerection) during the periods when BFG was depressed. This approach provides a means of rapidly and transiently dropping BFG and may find application in experimental brain ischemia studies in which the influence of a lowered BFG might be of interest. These studies also suggest that the traditional symptoms and signs of hypoglycemia are related to lowered BFG and not to systemic hypoglycemia. (This study was funded by the Veterans Administration, the American Diabetes Association, St. Louis Chapter, and by NIAAA-AA03513.)

82.5 [^{3}H]2-DEOXYGLUCOSE TRANSPORT BY BRAIN SLICES: APPARENT ENERGY DEPENDENCE. Jayne T. Kyle and Barry I. Gold. Dept. of Pharmacol. Uniformed Services Univ., Sch. of Med., Bethesda, MD 20014.

Glucose transport by brain is thought to proceed by facilitated diffusion. Several investigators have, however, reported high affinity uptake of the glucose analogue 2-deoxyglucose (2DG) by synaptosomes. This process is sensitive to inhibition by the mitochondrial uncoupler 2,4-dinitrophenol (DNP). We have begun to study the uptake of [^{3}H]2DG by brain slices to explore the nature and apparent energy dependence of the glucose transport system in brain.

Rats were decapitated and the brains rapidly removed to a 0.9% saline-moistened filter paper atop an ice-filled petri dish. Cerebral cortex was dissected free of basal ganglia and brain stem and was cut into pieces (~50 mg). Brain slices were prepared with a Sorvall tissue chopper to an indicated thickness of 225 μ. Slices were transferred to vials containing a modified Krebs-Ringer-bicarbonate medium which was bubbled with O_2/CO_2 (95:5) immediately before the addition of slices. After a 5 min equilibration, [^{3}H]2DG or L-[^{3}H]glucose (both 0.1 μCi/μmole) was added to a final concentration of 0.5 mM. Transport was terminated 5 min later by rapid filtration over GF/B filters. The slices were rinsed, tissue was digested in Protosol, and radioactivity was estimated by liquid scintillation spectrometry.

Net transport, defined as the difference between [^{3}H]2DG uptake and the uptake of L-[^{3}H]glucose (the diffusion component) in parallel samples, was linear for at least 3 min and exhibited half-maximal saturation at 2.0 mM 2DG. Singular deletion of Na^+, K^+, Ca^{2+}, Mg^{2+}, or Cl^- from the incubation medium had no effect on net [^{3}H]2DG transport. Incubation of the slices in the presence of 4.0 μM A23187 or 5.0 μM tetrodotoxin did not alter net transport; preincubation in 1.0 mM ouabain was also without effect. DNP at 1.0 mM significantly inhibited net [^{3}H]2DG uptake whereas the glucose transport inhibitors phlorizin and phloretin in concentrations up to 0.1 mM did not affect net [^{3}H]2DG transport. The temperature coefficient (Q_{10}) of the transport system between 4° and 25° was estimated graphically to be 2.1. Preliminary results demonstrated decreased net [^{3}H]2DG uptake as a function of preincubation time in the absence of an energy source; this decrease was more pronounced in the presence of DNP.

These results are consistent with the hypothesis that glucose transport by brain slices proceeds, in part, by an energy-dependent mechanism different from glucose transport in other tissues.

82.6 PATTERNS OF ^{14}C DEOXYGLUCOSE UPTAKE IN THE BRAIN OF THE HIBERNATING GROUND SQUIRREL. T.S. Kilduff, F.R. Sharp and H.C. Heller*. Dept. of Biological Sciences, Stanford University, Stanford, CA and Dept. of Neurosciences, UCSD Medical Center, San Diego, CA.

The autoradiographic ^{14}C 2-deoxyglucose (2DG) method of labelling tissues as a function of their glucose utilization offers a global perspective on brain function by providing a pictorial representation of simultaneous metabolic activity. We have utilized this technique to describe changes in the metabolic activity of neural structures between euthermia and the different phases of hibernation. Golden-mantled ground squirrels (Citellus lateralis) were implanted with chronic jugular catheters and subcutaneous thermocouple re-entrant tubes. After injecting ^{14}C 2DG via the catheter (150uCi/kg body weight) and allowing an appropriate incubation time, the brain and spinal cord were removed, frozen, sectioned in a cryostat and exposed to X-ray film for 7 days. On the resultant autoradiographs, we measured the optical densities (OD) of the images of 85 neural structures. The relative 2-deoxyglucose uptake (R2DGU) of each structure was calculated as the ratio of the OD of the structure to the OD of the optic tract. The autoradiographs of brains of hibernators were remarkably homogeneous in comparison to euthermic animals; hence, the R2DGU decreased in almost all structures. Two categories of structures were identified as functionally important during hibernation: (1) structures which had the highest R2DGU during hibernation and (2) structures that underwent the smallest reduction in R2DGU when compared to the euthermic group. The former category includes several sensory nuclei which receive primary afferent projections (cochlear nuclei and superior colliculus), which may reflect the sensitivity of the hibernator to environmental changes, and structures implicated in sleep and circadian organization (locus coeruleus, dorsal tegmental nucleus and the suprachiasmatic nucleus (SCN)). Structures undergoing the smallest reduction in R2DGU between euthermia and hibernation include the SCN, pontine nuclei and a limbic structure, the lateral septal nucleus. The only structure of the brain to undergo a significant increase in R2DGU during hibernation is the paratrigeminal n., a poorly studied cell group of the medulla. The zone of activity in the autoradiographs is continuous between the paratrigeminal n. and the marginal zone of the spinal trigeminal nucleus. Because of the known thermosensitive function of the latter, a similar function is suggested for the paratrigeminal n.

An important general principle that has emerged is that the % reduction in R2DGU of a structure between euthermia and hibernation is directly proportional to the euthermic R2DGU of that structure. However, some structures undergo less of a reduction than would be predicted from this relationship and, hence, may have significant roles in hibernation. These include the paratrigeminal n., suprachiasmatic n. and cochlear nuclei. (Supported by NIH NS10367 to H.C.H. and Danforth and National Science Foundation Graduate Fellowships to T.S.K.)

82.7 REGIONAL BRAIN GLUCOSE UTILIZATION IS LOWER IN UNSTRESSED RATS. R. M. Bryan, R. A. Hawkins, A. M. Mans*, and R. B. Page*. Depts. of Surgery, Anesthesia, and Physiology, M.S. Hershey Medical Center of The Pennsylvania State University, Hershey, PA 17033.

The rate of cerebral glucose utilization (CMRglu) was measured in awake, freely moving, unstressed rats using (2-^{14}C)glucose autoradiography. CMRglu decreased as much as 40% and showed a different distribution between brain regions from that previously reported for conscious (paralyzed or restrained) rats, using either (2-^{14}C) glucose or ^{14}C-deoxyglucose. The jugular and epigastric veins were chronically catheterized and each rat was placed in a specially designed box for 7 days to recover from surgery. The rat could be viewed through a one way mirror. The catheters were accessible to the investigators by passing them through the top of the box. After recovery, plasma glucose was normal (7.5 mM), plasma ketones were low (0.13 mM), and body weight was maintained. Plasma catecholamines were lower than those ever reported for conscious rats; epinephrine (31 pg/ml) was approximately 1/100th of the level in restrained rats. Injections and blood withdrawals through the catheters did not produce changes in heart rate, blood pressure, plasma catecholamines, or blood metabolites. Visual observations demonstrated that the rats were unaware of (2-^{14}C)glucose injections and the subsequent blood sampling. This model thus provides a method for physiological studies in unstressed rats and has enabled the measurement, for the first time, of regional glucose utilization under basal conditions.

82.8 A NEW HIGH RESOLUTION POSITRON COMPUTED TOMOGRAPH (PCT) FOR MAPPING CEREBRAL GLUCOSE METABOLISM: STUDIES IN NORMAL AND SENSORY STIMULATED SUBJECTS. M.E. Phelps, E.J. Hoffman*, J.C. Mazziotta*, Div. of Biophysics and Dept. of Neurology, UCLA School of Medicine, Los Angeles, CA 90024.

A new neurological tomograph (NeuroECAT) consisting of 3 octagonal rings of 88 BGO detectors providing 5 simultaneous tomographic images has been developed. The high resolution mode provides an intrinsic resolution of 7.4 ± 0.5mm and a gantry tilt of ± 25° allows flexible image plane selection. This resolution is about a factor of two improvement over our previous tomograph. Images are reconstructed with an array processor in about 5 seconds per image plane and tomographic sections throughout the whole brain are rapidly viewed in a cinegraphic format (variable speed; gray scale or color) in transverse, coronal or saggital planes for convenient and rapid tracking of structures and extraction of local metabolic rates.

Studies of resting cerebral glucose metabolism with (F-18) fluorodeoxyglucose show the highest resolution brain studies performed to date. Images show clear delineation of the convolutions of the entire cortical ribbon, internal and external capsule, thalamus, basal ganglia separated by the anterior and posterior limbs of the internal capsule, separation between the putamen and insular cortex, brainstem, hippocampus and deep cerebellar substructures including the vermis and dentate.

The improved resolution of the NeuroECAT is also seen in the measured gray to white matter metabolic rate of 4 which is about a factor of two higher than with our previous tomograph (ave of 1.9) and is in agreement with the values found in monkeys and rats with autoradiography.

Auditory stimulation showed clear delineation of the known morphological asymmetries of Heschel's gyri and planum temporale (i.e., left larger and more posterior; right narrow, anteriorly angled and positioned). Requirements of memorizing the auditory input (evaluated by subsequent test) produced activation of the hippocampus. Metabolic patterns of visual and auditory stimulations of varying content will be presented to illustrate the potential of this technique.

We have also developed another approach for PCT: the signal amplification technique (SAT). Theoretical, computer simulation and an experimental study with a prototype tomograph illustrate that SAT provides a factor of two resolution improvement over the NeuroECAT. Examples of simulated brain sections with SAT will be shown.

PCT and physiologically active compounds labeled with positron emitting isotopes of F-18, C-11, N-13 and O-15 are setting a new standard for studying cerebral function in man.

82.9 POTENTIAL USE OF DIMETHYL SULFOXIDE TO OPEN THE BLOOD-BRAIN BARRIER. R. D. Broadwell, R. Kaplan* and M. Salcman. Laboratory of Experimental Neuropathology, Division of Neuropathology, Dept. of Pathol., Univ. of Maryland Sch. of Med., Baltimore, MD

In mammals the blood-brain barrier (BBB) prevents many blood-borne, non-fat soluble substances from gaining access to brain parenchyma. Ascertaining a non-injurious approach to reversibly opening the BBB would have important clinical implications in the polychemotherapeutic treatment of individuals harboring primary and metastatic brain tumors as well as in the delivery of psychotherapeutic drugs, antibiotics, and antidotes. One substance which offers clinical potential for temporarily opening the BBB is the membrane solvent dimethyl sulfoxide (DMSO). Forty-six mice were injected into the tail vein with 0.25-0.5 cc of 2-30% DMSO in combination with the enzymatic tracer horseradish peroxidase (HRP) (1 mg/gm body wt.). Within 2 hrs. HRP filled the extracellular clefts throughout the brain. HRP penetration was best with DMSO concentrations of 10% and above. Similar results were achieved in mice receiving 1 cc of 10-30% DMSO intraperitoneally and 0.25 cc of HRP intravenously. In the absence of DMSO, HRP failed to enter brain parenchyma except through the normally leaky circumventricular organs. The normal BBB is not absolute, however, for pericytes lying on the surfaces of cerebral microvasculature become labeled with HRP. Cerebral capillaries, arterioles, and pericytes associated with these vessels readily pinocytose HRP into vesicles which are channeled primarily to lysosomes, as identified by acid hydrolase cytochemistry. Pericytes perhaps serve as the first line of defense once the formal BBB has been breached. Pinocytosis of HRP and the lysosomal concentration in cerebral microvasculature appear to be increased with exposure to DMSO. Possible actions of DMSO in opening the BBB include stimulating vesicular transport through microvasculature, opening the tight junctions between contiguous endothelial cells, inducing the formation of transcellular endothelial channels, or rupturing the endothelial cell membrane. The latter possibility seems unlikely, since DMSO does not appear to adversely affect the ultrastructural morphology of brain parenchyma and vasculature. Whether or not vesicular transport occurs normally in cerebral microvasculature and is significantly increased by DMSO treatment require further investigation. Opening of the BBB with DMSO is transient. HRP entry into brains from mice intravenously injected with the protein 12 hrs. after DMSO treatment is similar to that seen in control mice. The search for a safe and effective means of reversibly opening the BBB may depend on an increased understanding of the mechanisms of barrier function as demonstrated in normal and DMSO-treated animals.

82.10 IN VIVO RELATIONSHIP OF INTRACELLULAR OXYGEN AVAILABILITY AND UTILIZATION DURING ANOXIA. K.Kariman*, F.F.Jöbsis, and S.R. Burns*. (SPON: P. Kaufmann), Departments of Medicine and Physiology, Duke University Medical Center, Durham, NC 27710.

To evaluate the relationship of oxygen availability and utilization within the cerebral cortex, we have examined, *in vivo*, the tissue PO_2 (P_tO_2) and the reduction-oxidation state of cytochrome *c* oxidase (*cyt a,a*$_3$) during anoxia. Thirteen cats were anesthetized, ventilated mechanically with room air, submitted to a limited craniotomy and then injected with 25 mg/kg of pyrene butyric acid (PBA) intravenously. One hour later P_tO_2, the redox state of *cyt a,a*$_3$ and the brain EcoG were measured simultaneously before, during, and after a one minute exposure to 100% nitrogen. These measurements were used to determine the range of responses from normoxia to values approximating anoxia (P_aO_2 ± 5 mmHg; no brain activity). Tissue PO_2 was measured from PBA generated fluorescence, emitted by monitored cerebral cortical cells. The *cyt a,a*$_3$ redox state was measured from differential absorption of monochromatic light at 605 vs 590 nm reflected from the same cortical cells. The following results were obtained: 1) in response to nitrogen ventilation, reduction of *cyt a,a*$_3$ occurred earlier and at a faster rate than decline in P_tO_2. At 50% of maximal reduction of *cyt a,a*$_3$ the tissue PO_2 decreased only 33 ± 4% (M ± SE) of total; when *cyt a,a*$_3$ reached its 100% reduction there was 78.9 ± 4% of total decline in P_tO_2. 2) In returning to the baseline, when animals were ventilated with room air, a similar lag between the increase in P_tO_2 and *cyt a,a*$_3$ reoxidation was observed. When *cyt a,a*$_3$ returned to 50% of baseline the P_tO_2 had returned only 20 ± 5% to the baseline P_tO_2. When *cyt a,a*$_3$ reached its baseline the P_tO_2 had returned only 66 ± 4% to its baseline. These observations indicate that hysteresis exists in the relation between P_tO_2 and *cyt a,a*$_3$ reduction level. This may be the result of the situation that 1) Low tissue oxygen concentration is partially compensated *in vivo* by accumulation of reduced *cyt a,a*$_3$. 2) Following brief periods of tissue anoxia the affinity of *cyt a,a*$_3$ to oxygen is increased.

Supported by a grant from the American Lung Association.

82.11 **DIFFERENTIAL RESISTANCE OF PERIPHERAL NERVE FIBERS TO ANOXIA.** B.R. Fink and A.M. Cairns*. Dept. of Anesthesiology, Univ. of Washington, School of Medicine, Seattle, Washington 98195

Ischemia involving peripheral nerve preponderantly tends to destroy myelinated fibers. We sought to evaluate whether these fibers are particularly susceptible to oxygen lack.

Desheathed cervical vagus nerve of rabbit was incubated at 37.5°C, pH 7.35 in Ringer solution containing (mM) $NaHCO_3$ 24 and glucose 5 or 20, bubbled with hypoxic or anoxic gas mixtures containing 5% CO_2 and N_2. Control nerves were treated with 5% CO_2 - 95% O_2. Stimuli supramaximal for A or for C fibers were applied once every 5-10 min. Compound action potentials were recorded on digital storage oscilloscope and magnetic disc. In other experiments NaCN 10 mM was present in the Ringer (pH 7.30), and the gas phase consisted of 10% CO_2 - 90% N_2. Solution P_{O_2}, pH and P_{CO_2} were monitored.

In controls the amplitude of the Aβ, Aδ and C components declined at average rates of 4 ± 6, 4 ± 3, and 2 ± 3 %/hr for 3 hrs, (n = 7). With 5.8% O_2 (n = 7) the decline rates were not statistically different from control. With 3.7-1.6% O_2 (n = 7) the decline of Aβ and Aδ averaged 13 ± 5 %/hr ($p < 0.02$) and 17 ± 3 %/hr ($p < 0.001$); the decline of C was the same as control.

In cyanide-Ringer with 5 mM glucose (n = 4) Aβ and Aδ were extinguished in 30 min; with 20 mM glucose Aδ survived at 50% of pre-cyanide amplitude in 2 of 4 nerves for over two hours. The C component was not extinguished by NaCN; with 5 mM glucose it declined by 50-70% and then partially recovered; with 20 mM glucose (n = 4) the total 3 hr decline averaged 23 ± 14%. Repetitive stimulation at 10/sec for 100 sec depressed C both before and during incubation with cyanide, and in both conditions was followed by recovery to the pre-stimulation amplitude within 15 min.

The results show that Aβ fibers are wholly dependent on oxidative phosphorylation for energy, the Aδ fibers somewhat less so. C fibers, in contrast, can maintain excitability under conditions of total anoxia, provided that enough ambient glucose is available to supply glycolytic energy. This result is surprising since C fibers have a smaller volume to surface ratio than A fibers, and on that basis should have a relatively lesser cytoplasmic capacity for glycolysis.

The observations extend existing data (Lundberg, Acta Physiol Scand 52:156, 1952) and suggest that unmyelinated fibers can survive functionally with little or no oxygen, but that myelinated fibers generally cannot. This may partly explain greater vulnerability of myelinated fibers in ischemic peripheral neuropathies.

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82.12 HIGH-ACCURACY MEASUREMENT OF CNS ZINC AND LEAD BY STABLE ISOTOPE DILUTION MASS SPECTROMETRY. C.J. Frederickson, M.A. Mallory*, M.H. Frederickson, G.A. Howell, and W.I. Manton*. Laboratory for Geochronology, Univ. of Texas at Dallas, Richardson, Tx.

Recent work has suggested that the hippocampus contains large amounts of lead and zinc, but has left unresolved the absolute amounts of those metals. For the rat, Danscher et al. (Brain Res., 1976, 112: 442) suggest a hippocampal zinc content of 500 Parts Per Million (PPM) (dry weight), and a lead content of 18 PPM, Kemp and Danscher (Histochem., 1979, 59: 167) report 120 PPM for zinc, and 3 PPM for lead, and data in Fjerdingstad et al. (Brain Res., 1974, 79: 338) indicate that hippocampal zinc varies from 46 to 585 PPM among rats.

In search of an accurate assay for zinc and lead in the hippocampus, we turned to the geochemists' technique of stable isotope dilution. Samples were removed fresh, trimmed, lyophilized, and weighed, and then a known amount of stable isotope "spike" (enriched ZN-64 and/or PB-206) was added to the sample. Next, the sample was decomposed, allowing the atoms of endogenous metal to mix freely with the atoms of the spike, and the metal (endogenous and spike alike) was separated from the matrix by ion exchange. All tissue processing was done in a clean room in which personnel wore lint-free overgarments, room air was filtered, and work stations were washed by laminar-flow air showers. Finally, the isotope ratio of the mixture (ZN-66/ZN-64 or PB-208/PB-206) was measured on an isotope-ratio mass spectrometer, and the amount of metal originally present in the sample was computed. For Bovine Liver (National Bureau of Standards Material #1577), our procedure yielded zinc and lead concentrations within 2% of the Bureau's values.

We used adult, Wistar albino rats and found that lead in a single hippocampus or cord was below the limit (2 ng) of accurate measurement; pooled tissue from two sets of five rats gave the following lead concentrations: hippocampus, .053 and .054 PPM, cord, .017 and .019 PPM. For zinc, the values for the same tissue were: hippocampus, 73.1 and 72.2 PPM, cord, 26.8 and 25.4 PPM. Hippocampal zinc concentrations for 10 individual rats ranged from 59 to 79 PPM, with left and right hippocampi differing by an average of 4 PPM within animals; cord zinc ranged from 19 to 34 PPM, with left and right halves differing an average of 3 PPM.

The results indicate: (1) The absolute amounts of zinc and lead in cord and hippocampus are from 2- to 100-fold lower than recently published estimates; (2) The amount of zinc in both tissues varies relatively little among rats.

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83.1 THE TIMING OF PROTEIN SYNTHESIS NECESSARY FOR THE ACQUISITION OF THE Na^+ ACTION POTENTIAL DURING DEVELOPMENT. L. Blair. Dept. of Biology, UCSD, La Jolla, CA 92093

The development of electrical excitability has been characterized in vivo for a population of developing spinal neurons, the Rohon-Beard cells of the clawed frog Xenopus laevis. When these cells first become excitable, their action potentials depend on an influx of Ca^{++}. They later acquire a Na^+ component to their action potentials and gradually lose the voltage-dependent Ca^{++} current (Baccaglini & Spitzer, 1977). Embryonic neurons in dissociated cell culture follow the same timetable (Spitzer & Lamborghini, 1976, Willard, 1980). Thus these cultures are a useful model system for studying the timing of the molecular events which underlie the development of the excitable membrane properties.

Protein synthesis inhibitors (cycloheximide, puromycin) have been used to find the time periods during which the proteins required for expression of these phenotypes are synthesized. Cultures of spinal neurons were made from neural plate stage embryos and plated in modified Steinberg's medium; no exogenous growth factors or other proteins were included. I have found that continuous exposure to inhibitors of protein synthesis, if started prior to 4.5-5 hr in culture (equivalent to early neural tube stage) prevents the appearance of morphologically differentiated neurons. Later addition of the inhibitor does not affect the normal outgrowth of neurites which occurs at 6-7 hr after plating.

These cultured neurons are normally capable of producing Ca^{++} action potentials as neurites are extended. A Na^+ component of the impulse is first detected at 10-15 hr after plating. Addition of inhibitor at 5 hr after plating did not affect the normal appearance of the Ca^{++} action potential, but blocked the acquisition of the Na^+ component. Later addition of the inhibitor, at about the time when the Na^+ current actually appears, did not affect the normal expression of this property. These results suggest that the protein synthetic events underlying the appearance of the Ca^{++} action potential occur very early in the development of these neurons. Thus, the period of RNA synthesis (D. K. O'Dowd, this volume) terminates shortly before the end of the period of protein synthesis required for the expression of the Na^+ component of the neuronal action potential.

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83.2 THE TIMING OF RNA SYNTHESIS NECESSARY FOR THE DEVELOPMENT OF THE Na^+-DEPENDENT ACTION POTENTIAL IN CULTURED NEURONS. Diane K. O'Dowd. Department of Biology, UCSD, La Jolla, CA 92093.

The ionic dependence of the overshooting action potential (AP) changes during early amphibian development, both in Rohon-Beard neurons in vivo and in a more diverse population of spinal neurons in vitro (Baccaglini and Spitzer,'77;Spitzer and Lamborghini,'76). At the time of onset of electrical excitability the voltage-dependent inward current is carried largely by Ca^{++}. The AP at slightly later stages acquires a Na^+ component, and the ionic dependence of the AP shifts gradually until Na^+ is the major charge carrier. Such a developmental sequence presumably involves changes in the metabolism of RNA or protein, or both. In this study, RNA synthesis of cultured spinal neurons was inhibited at different stages of development, to elucidate the timing of some of the molecular events underlying the observed phenotypic changes. Effects of protein synthesis inhibition in this system have also been examined (Blair, L., this volume).

Cultures were made from dissociated neural plates of Xenopus embryos, grown in minimal medium with no exogenous proteins, and exposed to an RNA synthesis inhibitor (actinomycin-D) at various times after plating. The concentration required to block 95% of RNA synthesis in the cultured neurons was determined by autoradiographic analysis of ^3H-uridine incorporation. Cultures plated directly into medium containing inhibitor and examined after 24 hrs. do not contain differentiated cells with neurites. However, when the inhibitor is added 3 or more hrs. after plating (when sibling embryos are at early neural tube stage), neurons with identifiable neurites appear at 6-7 hrs. and can be maintained up to 3 days, as in control cultures. This result suggests that RNA synthetic events during the first few hours of culture are necessary for the production of neurites.

Neurons from cultures inhibited at 3-4½ hrs. are morphologically similar to controls and have normal resting potentials and input resistances. The AP's of these cells depend on Ca^{++} when they first appear; however, the ionic dependence of these impulses does not change even after 2½ days in culture. In contrast, the ionic dependence of the AP in control neurons changes from Ca^{++} to Na^+ during this period. Application of the inhibitor after the appearance of the inward Na^+ current (10-15 hrs. in vitro) doesn't affect the normal developmental progression to a chiefly Na^+- dependent AP. These data suggest that the inhibitor prevents the appearance of the Na^+ component of the AP by blocking RNA synthesis, rather than by causing nonspecific cellular damage. Presentation of the inhibitor at different times in early development has revealed a temporal window during which the Na^+ component of the AP is sensitive to inhibition of RNA synthesis. One possibility is that the RNA coding for the voltage-dependent Na^+ channel is synthesized during this period. (NSF predoctoral fellowship, NIH(NS15918), ONR(N00014-79C-0798)).

83.3 APPEARANCE AND DEVELOPMENT OF CHEMOSENSITIVITY IN EMBRYONIC AMPHIBIAN SPINAL NEURONS IN VIVO. John L. Bixby* and Nicholas C. Spitzer, Dept. Biol., Univ. Calif. San Diego, La Jolla, CA 92093

The development of several neuronal membrane properties has been studied in Rohon-Beard neurons of the embryonic Xenopus spinal cord, with intracellular microelectrode recordings. Previous work has shown that these cells develop the capability to make Ca^{++} action potentials at the neural tube stage, and that the ionic dependence of these action potentials gradually shifts to Na^+ over a period of 3-4 days. These cells are electrically coupled, in a voltage-dependent manner, prior to the time of appearance of the Ca^{++} spike, but become permanently uncoupled around the time of first appearance of the Na^+ component of the impulse. At late stages of development, these neurons are sensitive to GABA, and insensitive to a variety of other neurotransmitter candidates. Here we report the time of onset and subsequent development of this membrane property.

In mature cells (tadpoles 5-9 days old), bath application of 50μm GABA produces a conductance increase and depolarization recorded in the cell body. Iontophoretic application of GABA to the soma yielded dose-response curves with slopes as high as 330 mV/nC. The reversal potential of the iontophoretic response was determined by injecting current to adjust the membrane potential to different levels. Voltage-dependent conductances were largely blocked pharmacologically, and measurements were made in the linear portion of the cells' I-V relation. Although the depolarizing response could be inverted, determinations of the reversal potential were routinely obtained by extrapolation. The reversal potential has a value of -30.2 ±1.6 mV (n=11). Ion substitution experiments indicate that GABA causes increases in Na^+ and K^+ conductance. The responses are blocked by 50 μM curare or picrotoxin.

Rohon-Beard neurons become sensitive to bath application of GABA at the early tail bud stage of the embryo (30 hrs old), about the time when the cells acquire the Na^+ component of their action potential and become electrically uncoupled from each other. The acquisition of GABA sensitivity is tightly correlated with the presence of detectable inward Na^+ current, but neither of these membrane properties is well correlated with the loss of electrical coupling. The sensitivity of cells with Na^+/Ca^{++} spikes to iontophoretically applied GABA was comparable to that obtained from mature cells. The reversal potential at this stage has a value of -28.5±2.9 mV (n=8); this value is indistinguishable from that obtained for mature cells. Thus, as previously found for vertebrate skeletal muscle and invertebrate nerve cells, the ionic basis of the response to a neurotransmitter appears to be constant during the development of these vertebrate neurons.

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83.4 ^3H-SAXITOXIN BINDING AND SCORPION TOXIN-STIMULATED $^{22}Na^+$ FLUXES SHOW DIFFERENT DEVELOPMENTAL KINETICS. Jesse Baumgold, J. Brian Parent* and Ilan Spector*. Lab. of Neurobiology, NIMH, Bethesda, Md 20205. Howard University Cancer Center, Washington, D.C., 20059 and Lab of Biochemical Genetics, NIH, Bethesda, Md 20205.

The kinetics of development of ^3H-Saxitoxin-binding and of scorpion-toxin-stimulated $^{22}Na^+$ fluxes were studied in chick skeletal muscle cultures. Myoblasts from 12 day old chick embryos were plated under tissue culture conditions which induced a very synchronous burst of fusion (myotube formation) 36-48 hours after plating. ^3H-Saxitoxin (^3H-STX) binding sites were not found in the unfused electrically inexcitable myoblasts. These binding sites were first observed around day 3 and were found to increase gradually until day 8, after which they remained constant at 6-7 fmole per mg protein. The dissociation constant for this binding was found to remain constant throughout this developmental process (Kd=1.6nM).

The developmental kinetics of the appearance of ^3H-saxitoxin binding sites correlates well with the previously published kinetics of the appearance of excitability (as measured by inward currents) in these same cells (Spector and Prives, P.N.A.S., 74: 5166, 1977).

The scorpion-toxin stimulated incorporation of $^{22}Na^+$ into cells in the presence of veratridine has recently been used in many labs as an assay for sodium channels. This toxin-stimulated incorporation of $^{22}Na^+$ can be inhibited in a dose-dependent manner, by the addition of tetrodotoxin (TTX). As an additional measure of the development of TTX-(or STX-) binding sites, we studied the dose-dependency of the TTX inhibition of the $^{22}Na^+$ flux into developing muscle cells. We found that, in contrast to the binding of ^3H-saxitoxin, the dissociation constant (half maximal effect) of the TTX inhibition of the $^{22}Na^+$ flux shifted considerably, during development, from 30-40 nM on day 3 to 3-6 nM on day 9. This finding indicated that the binding of ^3H-STX and the inhibition by TTX of the $^{22}Na^+$ flux measure different functional components of the "sodium channel" and that these components develop differently.

83.5 DEVELOPMENTAL CHANGES IN THE MEMBRANE PROPERTIES OF IDENTIFIED MOLLUSCAN NEURONS. Peter A. Pawson and Ronald Chase . Dept. Biol. McGill University, Montréal, Québec, H3A 1B1.

We have been studying the development of several identified neurons in the snail, Achatina fulica, from the last 1/3 of embryonic life to adulthood. In the course of an analysis of transmission at a developing synapse (Soc.Neurosci.Abstr.,Vol.6,p.677, 1980), we observed developmental changes in the waveforms of the spontaneous post-synaptic potentials (PSPs). Embryonic PSPs are slower rising and have much greater decay times than those recorded in the first month post-hatching; when the PSPs are more frequent, have faster rise-times and much faster decay times. In subsequent months; PSPs become less frequent, smaller in amplitude, have slower rise-times and are longer in duration.

To account for these observations, we examined some of the membrane properties of these neurons. The RMPs are similar during the period, as are the spike threshold values. The I-V curves are linear within ±15 mV of the RMP. Input resistances drop progressively from 97.42 ± 35.26 Mohms in embryos to 4.54 ± 1.73 Mohms in adults. There is however a striking change in the shape of the voltage transient in response to an intrasomatic current step. The time constant (Tm) of the neurons drops from 177.7 ± 51.54 msec. in embryos to 100.7 ± 53.83 msec. in 20-30 day-old animals. The Tm values then rise to an adult average of 312.54 ± 77.66 msec. The larger Tm found in embryos is not due to electrical coupling with other neurons, as a plot of ln (Vt/Vmax) vs. time is linear. Assuming a constant specific membrane capacitance during development, these changes in Tm values reflect changes in the neurons' specific membrane resistances. The apparent leakiness of the cell's membrane was also seen in that a cell with a short Tm also had a substantially higher value for rheobase current, although it possessed a normal threshold voltage value.

At the stage when the Tm values are the shortest, there is a large amount (both in terms of number of events and individual amplitudes) of spontaneous synaptic activity. It seemed possible that the large number of synaptic events might lead to a tonically higher resting membrane conductance, producing smaller Tm values. Using a 0 Ca/2xMg Ringer, we blocked all synaptic conductances and found that this had no effect on Tm values (103 ± 25%, N=10).

In conclusion, during the period of development when these neurons are presumably undergoing rapid morphological growth and increasing their number of synaptic inputs, their membranes exhibit a higher resting conductance level, unrelated to synaptic conductances. The aforementioned changes in PSP waveforms are therefore reflective of developmental variations in the resting conductances of the post-synaptic membrane.

83.6 ROLE OF CALCIUM CONDUCTANCE IN NEONATAL MOTONEURONS OF ISOLATED RAT SPINAL CORD. K.D.Walton and B.P.Fulton . Dept. of Biophysics, University College London, Gower Street, London, U.K.

We have previously reported the presence of Ca^{++} electroresponsiveness in spinal motoneurons (Mns) of neonatal rat (J.Physiol. 1981, in press). During this work it was noticed that spikes recorded from Mns of the youngest animals (3-6 d) differed from those recorded from older rats (to 11 d). This observation was investigated further in the light of the proposed role for Ca^{++} in neuronal growth (Llinas & Sugimori, Prog. Brain Res. 51: 323, 1979; Spitzer, Ann.Rev.Neurosci. 2: 363, 1979). The electrical properties of 3-6 d Mns are remarkable in three respects: 1. The antidromic spike is broad and has a prolonged afterdepolarization (ADP). 2. Spikes elicited by direct stimulation have a marked delayed depolarization (DD) and afterhyperpolarization (AHP). 3. The DD amplitude is increased by broad, small 'spikes', often accompanied by an increase in AHP amplitude.

In experiments where Mn^{++} or Mg^{++} replaced Ca^{++} in the saline, a Ca^{++} conductance was shown to be responsible for the increased width of the antidromic spike, and the DD and small 'spike' following the direct spike. Under these conditions the antidromic spike became narrower, its ADP was smaller and the direct spike was not followed by a DD or its 'spike'. Also, the AHP was absent suggesting that a Ca-dependent g_K underlies this potential. The input resistance was not significantly changed. Consistent with the interpretation that a Ca^{++} conductance underlies these phenomena is the finding that when g_K was progressively blocked by intracellular injection of Cs^+ the DD increased in amplitude and developed into a regenerative TTX-resistant spike.

The contribution of Ca^{++} entry to determining the repetitive firing of developing Mns (3-11 d) was studied. Firing during long current pulses (1.5 s) was examined in normal saline and saline in which Mg^{++}, Mn^{++} or Cd^{++} replaced Ca^{++}. Plots of firing frequency vs current in Mg^{++}, Mn^{++} and Cd^{++} salines were displaced upwards and had an increased gain with respect to controls. These results suggest that an increased intracellular Ca^{++} concentration, by activating a Ca-dependent g_K, limits the Mn firing rate for a given current level.

The presence of a marked Ca-dependent electroresponsiveness in spinal Mns during the first few postnatal days and its decrease with maturation is consistent with the suggestion that this early g_{Ca} is related to cell growth. In addition, the present results provide evidence that modulation of this Ca^{++} conductance serves to regulate Mn repetitive firing during development.

84.1 CORTICO-DCN PROJECTIONS FROM FUNCTIONAL SUBFIELDS OF SENSORI-MOTOR CORTEX IN THE CAT: AN ANTEROGRADE HRP STUDY. S.S. CHEEMA, A. RUSTIONI, B.L. WHITSEL. Depts. of Anatomy and Physiology, Univ. of North Carolina, Chapel Hill, N.C. 27514.

The organization of corticofugal projections upon the dorsal column nuclei (DCN) of adult cats were investigated by the use of anterograde transport of HRP. HRP injections were placed in various body representations involving areas 4,3a,3b,1 and 2. The availability of a detailed, "flattened" map indicating the somatotopic and functional arrangement in S_I of the cat (McKenna et al., 1981) allowed the injection of selected parts of this region without the need for electrophysiological monitoring. The terminal pattern of cortico-DCN fibers was reconstructed from 40µm thick, serial sections incubated in TMB. The results from different experiments were superimposed on a standardized,stereotaxic map of the DCN derived from serial series of Nissl stained sections, spaced one millimeter apart and normalized with reference to the obex. In our hands, the HRP-TMB method for anterograde tracing of pathways had greater sensitivity than the method of anterograde transport of tritiated aminoacids.

HRP injections that infiltrated the entire sensorimotor cortex labelled cortico-DCN fibers whose terminations were present at all levels of the DCN extending from 9 mm below to 4 mm above the obex. The terminal fields of the cortico-DCN fibers labelled by such large injections included the clusters region of both gracile and cuneate nuclei as well as the non-cluster regions. In both nuclei the maximal density of fibers labelled by large injections occupied terminal sites located ventral to the clusters region. When the enzyme injection was restricted to area 4, corticofugal afferents to the DCN were also distributed throughout the longitudinal extent of these nuclei but the clusters region was spared. On the other hand, after injections restricted to the hand representation of areas 3a and 3b, terminal labelling was negligible at the base of the cuneate nucleus, while patches of terminals were present in the clusters region.

Attempts are presently being made to combine TMB histochemistry with electron microscopy to identify the type of synapses established by corticofugal fibers reaching the clusters region of the DCN. Besides demonstrating a precise pattern of cortico-DCN connections which appear relatable to S-I topographic organization, the present results provide a map of DCN connectivity which shall be related to the nuclear terminations accessed by peripheral nerves as visualized by transganglionic transport of HRP and to maps of the body representation in the DCN obtained by the method of single unit analysis. (Supported by USPHS grants NS 16264).

84.2 VESTIBULAR INPUT TO MOTONEURONS OF THE CAT LATERAL FACIAL NUCLEUS. M. Shaw* and R. Baker (SPON: J. Gruner). Dept. Physiol. and Biophys., New York Univ. Med. Ctr., New York, N.Y. 10016

Previous studies have revealed that second order vestibular neurons which project to extraocular motor nuclei may also send axon collaterals to the lateral portion of the facial nucleus. To determine the synaptic effects following vestibular stimulation, intracellular recordings were obtained from antidromically identified facial motoneurons in the lateral subgroups.

Stimulating electrodes were placed on the superior buccal, zygomatic and nasal branches of the facial nerve to identify motoneurons innervating, respectively, the upper lip, orbicularis oculis (eyelid closure) and quadratus labii superioris muscles (whisker erection). Electrodes were also placed on both vestibular nerves and the facial nerve near the geniculate ganglion. Retrograde HRP injections and antidromic activation indicated that the motoneurons of orbicularis oculis and quadratus labii superioris lie intermingled in the dorso-lateral subgroup of the facial nucleus. Neither population received synaptic input from contralateral vestibular stimulation. Both populations contained some cells which received disynaptic input from ipsilateral vestibular stimulation, either an EPSP (latencies 1.1-2.2 msec) or an IPSP (1.1-2.2 msec). Stimulation of trigeminal fibers often induced EPSPs with latencies of 1.5-3.0 msec. Motoneurons activated antidromically by superior buccal nerve stimulation, located in the ventro-lateral subgroup, never displayed synaptic responses to vestibular stimulation. Another subgroup of motoneurons, on the medial edge of the dorso-lateral subgroup, could only be antidromically activated by stimulation of the whole facial nerve. Based on their location, these neurons probably project to the corrugator supercilii muscle, which pulls the ear forward. They received no ipsilateral vestibular input, but always had a disynaptic EPSP response to contralateral vestibular stimulation with latencies of 1.1-2.0 msec. These neurons also received longer latency EPSPs from trigeminal pathways.

We have demonstrated, with electrophysiological techniques, excitatory and inhibitory monosynaptic projections from second order vestibular neurons upon facial motoneurons. The input is segregated by nuclear subgroup. Since the activity of second order vestibular neurons projecting to both the extraocular motor nuclei and the facial nucleus relates to eye movement, the vestibular projection to the lateral facial nucleus must, in part, aid in the coordination of eyelid, whisker, ear and ocular movements. Supported by NIH #EY0504, Fight-For-Sight, NYC #F-346 and USPHS #NS13742.

84.3 SIMPLE REACTION TIME IS UNCHANGED BY COOLING MONKEYS' VENTRAL LATERAL THALAMUS[1]. A. D. Miller and V. B. Brooks. Department of Physiology, The University of Western Ontario, London, Ontario N6A 5C1, Canada.

The pathway through ventral lateral (VL) thalamus from cerebellum to motor cortex was tested for participation in movement initiation. Three Cebus monkeys performed a simple reaction time (RT) task involving prompt elbow flexion or extension in response to an audiovisual GO! signal. Mean RTs were on the order of 300 ms as detected by a velocity threshold. RTs were not changed by reversible cooling of the VL arm area (which includes rostral VPL) contralateral to the operant arm (20 trials). The caudal-lateral portion of n. centralis lateralis (CL) was also included in the region cooled in one of these monkeys. In contrast, cooling the ipsilateral cerebellar dentate nucleus prolonged RTs by about 75 ms, as tested in 2 of the same monkeys (18 trials). These delays of movement onset were not due to lower initial velocities during cooling. The results confirm cerebellar participation in prompt generation of well-learned intended arm movements (J. Neurophysiol. 1977, 40: 1038-1050), but they also indicate that the route through the VL arm area to motor cortex is not essential for initiation of the same movements. The supposition is made that at least 3 sets of parallel pathways normally sustain each other: cerebello-thalamic to motor cortex and cerebello-thalamic to association cortex, e.g. supplementary motor area (SMA), and hence to motor cortex. In addition, spinal projections from motor cortex can converge with cerebello-reticulospinal projections.

1 Supported by the Medical Research Council of Canada. Present address for ADM: The Rockefeller University, New York, NY 10021

84.4 COORDINATION BETWEEN GRIP FORCE AND VERTICAL LIFTING FORCE WHEN LIFTING OBJECTS BETWEEN INDEX AND THUMB. R. S. Johansson* and G. Westling* (SPON: R. H. LaMotte). Dept. of Physiology, Univ. of Umea, S-901 87, Umea, Sweden.

Precision manipulation of a small object requires a refined coordination of the forces exerted on the object by the tips of the fingers and thumb. The present paper deals quantitatively with this coordination.

A small object, standing on a table, was gripped between the index and thumb, lifted and held stationary about an inch above the table. The weight and the surface structure of the object could be changed between consecutive liftings without changing its visual appearance. Three sets of surface structures were used (sandpaper, suede, silk), all of which had different coefficients of static friction in relation to the skin.

The grip force (GF) and the vertical lifting force (LF) acting on the object were measured, as well as the vertical position of the object. When the object was gripped, there was a small, short lasting increase in GF. Thereafter, the GF and LF increased in parallel until the LF overcame the force of gravity acting on the object and the object began to move. In contrast to the rate of LF increase, the rate of GF increase was dependent on the surface structure of the object. The lower the friction between the skin and the object, the higher the GF/LF ratio.

After a transitional phase in which the object was elevated, the GF reached a static value and the LF counterbalanced the force of gravity acting on the stationary object. The static GF force was greater, by a small safety margin, than the minimal grip force required to prevent slippage. This minimal force, in turn, was dependent on the friction and the weight of the object. The safety margin varied between subjects and could be influenced, to some degree, by the surface structure of the object in the previous trial. Local anaesthesia of the fingers abolished the appropriate adjustments of the GF with changes in friction.

We conclude that when a small object is lifted, the friction between the gripped object and the skin of the finger tips profoundly influences the coordination between the muscle units accounting for the GF and the LF. This was the case during the dynamic as well as the static phases of the liftings. The findings probably apply to fine manipulation in general in the sense that friction unconsciously influences the relationship between the GF and the forces applied to overcome forces counteracting intended manipulation. The CNS most likely obtains information about friction via cutaneous mechanoreceptive afferents.

84.5 ACTIVATION MECHANISMS AND RECOVERY OF FUNCTION AFTER UNILATERAL DORSAL RHIZOTOMY IN THE MONKEY. A.J. Berman, D.E. Teodoru, T.A. Tran.* Dept. of Neurosurgery, V.A. Hospital, Bronx, N.Y. 10468.

The sequence of recovery of function in monkeys after both unilateral and bilateral dorsal rhizotomy (DR) of the forelimbs is such that movements which bring the deafferented limb into contact with intact body parts occur long before movements which extend the limb into extrapersonal space. This can be attributed to substitution of sensation from intact body regions for sensory feedback from the responding limb. To examine the relationship between sensory substitution and disuse of a DR limb, five monkeys with unilateral DR, two of which had undergone bilateral dorsal column nuclei (DCN) lesion six months previously, were tested. A grape was brought into contact with intact body areas (face, torso, or arm) or was placed into the non-DR hand which was restrained. In all cases, the animals used their DR limb to reach smoothly and accurately, grasp and bring the grape to the mouth. Blindfolding did not degrade the movement with the exception that, if the grape was placed in the restrained non-DR hand, the deafferented hand of the three DR monkeys failed to grasp when the non-DR hand opened to transfer the grape. This was true even though thumb-finger apposition on the DR side was occuring regularly in other situations. Testing was continued for a total of over 500 trials, but the grape was always dropped. On the other hand, the two with DCN lesion as well as DR were able to transfer the grape from the DCN to the DCN-DR hand when blindfolded.

The inability of animals to make simultaneous movements of their non-DR and DR limbs in response to a sensory cue from the non-DR side may be based on a biasing of lateralized attentional mechanisms. Sensory input is believed to activate subcortical attentional mechanisms which control cortical responsiveness to ascending input (Wright, et.al. 1978). Sensory guidance of movements, on the other hand, functions by means of cortical circuits (Evarts, 1973). When sensory input from the non-DR side of the body is used to guide ipsilateral movements, subcortical attentional mechanisms may block interhemispheric transmission of the information to the contralateral side.

Since monkeys with bilateral DCN lesion and unilateral DR are capable of using residual input from the non-deafferented limb to guide both limbs, it can be inferred that they can transmit sensory information transcortically. Thus, DCN must be a part of the lateralized attentional mechanisms as proposed by Wall (1970).

84.6 DYNAMIC ROLE OF RUBRAL NEURONS IN CONTACT PLACING IN CATS. Deirdre Batson*, Vahe Amassian and Larry Eberle*. Dept. of Physiology, SUNY Downstate Medical Center, Brooklyn, New York, 11203.

Destruction of the feline red N abolishes contact placing (CP) in the contralateral forelimb (Amassian in: Integration in the Nervous System, 279-304 pp, ed: H. Asanuma and V. J. Wilson, Igaku-Shoin,1979). To test for a possible dynamic role of red N in CP, we recorded from individual rubral neurons with our standard techniques. Parameters of placing movements were monitored with photoelectric or force transducers supplemented by TV-Computer measurements at 60 Hz of shoulder, elbow and wrist joint angles. After being brought into contact at various positions on the placer, the forepaw (unguided by vision) had to ascend different distances before reaching the top of the apparatus.

Antidromic invasion following stimulation of contralateral caudal medulla was demonstrated in 52% of the 84 neurons recorded ; antidromic latencies ranged from 0.5-2.5 msec. In 54 neurons responding during the first phase of CP (lifting-withdrawal), the firing rate initially increased in 93%. The variable interval between contact and the onset of movement aided the differentiation of four types of response: 1) locked to initial contact regardless of whether CP subsequently occurred; 2) locked to initial contact, but markedly reduced or even reversed if CP subsequently failed to occur; 3) locked to and preceding movement by eg. 40-180 msec; 4) locked to, but starting either with or during , the movement. In types 1) and 2), the contact locked activity had a short latency eg, 20-40 msec and usually persisted during the contactual ascent. In all types, the increased discharge rate usually fell (sometimes transiently) when the forepaw broke contact at the top of the placer. Rarely, neurons began responding at this transition. A precontact response (presumably vestibular related) was identified in some neurons if the animal was rapidly moved towards the placer.

Types 2) and 3) rubral neurons increase in discharge rate in time to contribute to activation of the flexors that cause the lifting-withdrawal phase of CP. However, contactual ascent results in further cutaneous stimulation, presumably accounting in part for the maintained discharge of some rubral neurons, which in turn may contribute to continued contactual ascent of the forepaw. Such a positive feedback arrangement presumably involves a coordinated contraction of agonists acting at several joints, but not the muscle directly related to the cutaneous area stimulated, extensor digitorum communis being strongly inhibited following contact with the dorsum of the forepaw.

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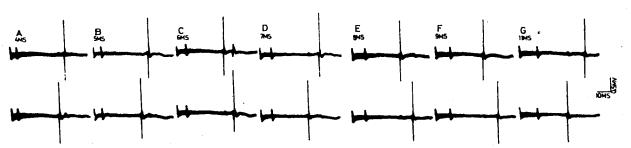
84.7 H-REFLEX RECOVERY STUDIES WITH SINGLE FIBER EMG. M.A. Sabbahi and E.M. Sedgwick*, Liberty Mutual Research Center, Hopkinton, MA., U.S.A., and Wessex Neurological Center, Southampton, U.K.

The primary facilitation and inhibition periods of the H-reflex recovery curve with paired stimuli are not well understood. Peripheral as well as central factors are involved in these recovery periods.

In this study a total of 30 motor units (MU) were studied by single fiber EMG while recording the H-reflex. The H-reflex was elicited by stimulating the posterior tibial nerve unifocally at threshold strength. The soleus muscle action potential was recorded using unipolar single fiber needle as well as surface electrodes. Single muscle fiber recording followed previously reported criteria (Sabbahi, 1976). Identical paired 1 msec pulses with varying interstimulus intervals or a conditioning train of 17 pulses for 30 msec followed by a test pulse at intervals were used.

Results show that with paired stimuli at threshold level the MU fired at H_1 only while with subthreshold stimuli it fired at H_2. It never fired simultaneously at H_1 and H_2. This dual firing occurred at 2-12 msec interstimulus interval with maximum firing occurrence at H_2 at 5-6 msec. H_2 was never recorded between 12-80 msec. When the subthreshold pulse train preceded the test stimulus with 1-10 msec they summated to fire the MU at H_2. From 10-15 msec, firing occurrence decreased with firing failure between 15-50 msec. Recovery was noticed after 50 msec interval when threshold testing pulses were used.

These results indicate no primary facilitation period in the recovery curve. The H_2 reflex during early periods is possibly due to temporal summation. Primary inhibition period lasted up to 50 msec due to strong inhibition mechanisms.



The motoneuron fired during either H_1 or H_2 but never during both.

84.8 THE HOFFMANN REFLEX IS ENHANCED BY SENSORY STIMULI. A. P. Rudell. Dept. of Physiol., Downstate Medical Center, SUNY, Brooklyn, N. Y. 11203.

A sudden sensory stimulus, such as a tone pip or a light flash produces a general arousal reaction. In cats it has been demonstrated that such stimuli enhance electrically evoked responses recorded from the visual cortex and from skeletal muscles.[1] A similar effect has now also been demonstrated in human subjects, using the Hoffmann reflex (H-reflex) as the response indicator.

The H-reflex is elicited by electrical stimulation of the tibial nerve, the response being recorded from gastrocnemius and soleus muscles. It is the electrical analog of the stretch reflex that is usually elicited by tapping the Achilles tendon. The response is monosynaptic. Fluctuations in the amplitude of the H-reflex may be due in part to fluctuations in the excitability of the alpha motoneurons.

Preliminary results indicate that a portion of the auditory visual, or somatosensory afferent signal is transmitted to the spinal cord, where it increases the excitability of the gastrocnemius alpha motoneurons.

A question of primary importance is the time course of the change in excitability following a sensory stimulus. An estimate of the time course was made by varying the interval between the onset of the sensory stimulus and the presentation of the electrical stimulus to the tibial nerve. For tone pips the minimum interval for enhancement of the response was 30 to 60 msec. Greatest enhancement occurred at about 150 msec, at which interval the H-reflex could be as much as ten times greater than the control level. A period of suppression below control level was observed at about 200 msec. The effects of light flashes and tone pips were similar, but the minimum time interval and the interval for greatest enhancement were about 30 msec longer for light flashes than for tone pips. Vibratory stimuli showed an intermediate time course.

The short minimum latency required for enhancement, taking into account the substantial conduction time from the sensory afferents to the spinal cord, indicates that a relatively direct connection exists between sensory afferents and alpha motoneurons.

[1]Rudell, A. P. 1980, Physiology & Behavior 25: 901-909.
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84.9 EFFECT OF COOLING ON STRETCH REFLEXES. P. Bawa and I. Mekjavic* Kinesiology Dept., Simon Fraser University, Burnaby, B.C., V5A 1S6.

Torque motor imposed angular displacements of upper limb joints result in two main reflex components in the EMG recorded from stretched muscles. Similar two components have been observed in wrist flexors during tendon taps. These two components are distinguished primarily by their latencies. Experiments were conducted on cooled human subjects to test whether there were differential effects on the latencies of the two components. Also, whether the observed impaired motor control during hypothermia is reflected in abnormal reflexes.

The subject was immersed in 10°C or 15°C cold water monitoring their heart rate, esophageal, rectal and tympanic temperatures. He was taken out after an hour or when his esophageal temperature dropped to 35°C. He sat in a sleeping bag for rewarming while his reflex data were collected every ten minutes. EMG was recorded from wrist extensors and flexors. Reflexes were elicited both with a torque motor and with tendon taps of the wrist flexors. Rectified and unrectified records were averaged on a PDP8 computer.

No differential effect of temperature was apparent on the shorter or longer latency (SL or LL) components when the stretches were imposed by torque motor. However, when the tendons were tapped there was an additional delay of 4-5 msec in the SL component and 7-8 msec delay in the LL component at the lowest temperature as compared to the precooling values. This would suggest that the two reflex pathways for the SL and LL components are separate. The additional delay in the LL component above that of the SL component is perhaps due to slowing down of the synaptic transmission and/or slowing down in the conduction velocity in the 'long-loop' pathway. The lack of any apparent effect with torque motors is attributed to the slower rates of stretches and lesser degree of synchronization of afferent volleys resulting in broad overlapping reflex peaks.

The impaired motor performance during hypothermia is not related to large abnormalities in reflexes. It may perhaps be localised to the muscle itself.

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84.10 REGULATION OF HUMAN TRICEPS SURAE MUSCLE STIFFNESS BY SHORT LATENCY REFLEXES. K.-H. Mauritz*, J.H.J. Allum and H. Vögele*. Brain Research Institute, University of Zurich, CH-8029 Zürich, Switzerland and Dept. of Neurology, University of Freiburg, D-78 Freiburg, Germany.

The incremental stiffness of human triceps surae (TS) muscles was tested by rotating the foot about the ankle joint. Subjects were asked to maintain a constant force prior to each rotation by contracting TS. For the force range used, 6 to 20 Nm plantar flexion, the antagonist tibialis anterior (TA) muscles were inactive. The rotations, 1 to 8 degrees amplitude, were applied by a servo-controlled torque motor which otherwise held the foot isometrically. To observe separately the two mechanims contributing to incremental stiffness (the intrinsic visco-elastic stiffness of active muscle and the force recruited via the stretch reflex) the duration of foot rotations was limited to 60 msec.

Muscle force in response to rotations stretching TS exhibited a step increase followed by rapid yielding as stretch velocity decreased. At 55 ms TS reflex action reversed this yielding. Force then continued to increase and then levelled off just prior to medium latency TS activity at 120 ms. The incremental force just prior to yielding (a measure of intrinsic visco-elasticity) increased with the prior level of tonic TS contraction, whereas the total force increment at 120 ms remained roughly constant. Muscle force in response to release of TS showed only a slow yielding once release terminated. Weak reflex activity at 70 ms in TA and TS arrested this yielding.

Our results indicate that short latency reflexes compensate for muscle non-linearities and regulate muscle stiffness. Similar results have been reported for cat soleus muscle (Nichols, T.R. and Houk, J.C., J. Neurophysiol. 39: 119, 1976; Hoffer, J.A. and Andreassen. S., J. Neurophysiol. 45: 267, 1981).

84.11 ADAPTIVE PLASTICITY OF PRIMATE M1 RESPONSE. J. R. Wolpaw, V. A. Kieffer*, D. J. Braitman and M. G. Sanders*. Div Labs Res, NYS Dpt Hlth, Albany NY 12201; AF Radbiol Res Inst, Bethesda MD 20014.

To learn if the earliest, largely monosynaptic, M1 component of the muscle stretch reflex can adapt, we trained rhesus monkeys to maintain elbow angle at 90°(±1.5°) against steady extension force. If correct angle was held for a randomly selected 1-2s period, and if the average absolute value of biceps EMG (from chronic i.m. electrodes) for the final 0.5s was 1.0-1.5 X a preset value, a 20ms pulse of extra extension force occurred. This stimulus (stim) extended the elbow 3-4°. M1 amplitude (amp) was measured as the average absolute value of biceps EMG 12.5-21.5ms after stim onset minus pre-stim EMG amp. Under the Control condition, reward was given 70ms after stim onset. Under the M1↑ or M1↓ condition, reward was given only if M1 was greater (M1↑) or less (M1↓) than a criterion value. Control and M1↑ or M1↓ data were obtained from each of 6 monkeys over 2-5 mos. Each usually completed 2500-6000 trials daily. Electrodes, pre-stim EMG, steady extension force, stim amp, and stim-induced movement were stable throughout.

Under the impetus of the M1↑ or M1↓ condition, 5 of the 6 monkeys markedly and appropriately changed M1 amp (Fig), while one showed no change. Changes became evident in 3-5 days, could continue to develop for at least 4 wks, and did not regress during the 2-4 day rest periods (Fig.). M1↑ could increase M1 to nearly twice control M1, while M1↓ could decrease it to less than 1/3 control.

Monkeys can markedly change M1 amplitude over days, without change in alpha motorneuron tone as measured by pre-stim EMG amp. Possible mechanisms include change in gamma motorneuron tone, in alpha motorneuron recruitment, or in Ia synaptic function.

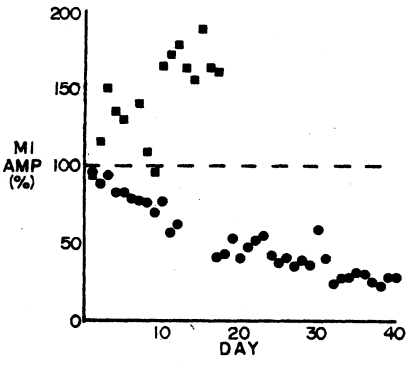

Fig. Squares are daily M1 amplitudes of one monkey after onset of M1↑ training; circles are daily M1 amplitudes of another monkey after onset of M1↓ training. M1 amplitudes are given as % of the monkey's average control M1 amplitude for the preceding 6 days. Each point represents 2500-6000 trials. Breaks in data are rest periods.

84.12 LONG LATENCY REFLEXES IN PATIENTS WITH COMPLETE SPINAL CORD TRANSECTION. K.C. Hayes*, K.L. Robinson* and J.D. Spencer* (SPON: J.D. Cooke) Fac. of Med., Univ. of Western Ontario, London, Ontario N6A 5C1.

The current interest in long latency stretch reflexes in spinal animals prompted our investigation designed to test whether similar long latency autogenic facilitation could be found in spinal man. H-reflex recovery curves were recorded from four patients with verified complete transection of the spinal cord. The recovery curves were obtained following conditioning with subthreshold and suprathreshold H-reflex stimuli. The mean subthreshold conditioned recovery curve exhibited periods of pronounced facilitation at interpulse intervals of 10 msec, 50-75 msec and 150-175 msec that were superimposed upon a long lasting (>1000 msec) depression of alpha motoneuron excitability. The time course of these periods of facilitation and inhibition showed a close correspondence to H-reflex recovery curves recorded from normal subjects. Differences were noted, however, in the amplitude and duration of the various phases of facilitation. The early phase, which peaked at 10 msec, and corresponded to EPSPs, was extended in one patient who had been a spastic quadriplegic for 12 years. This observation may reflect compensatory axonal sprouting of Ia afferent fibres. Facilitation at IPI= 50-75 msec occurred slightly earlier in all four patients than in normal subjects. Although supressed by a profound inhibition, the same three periods of facilitation were identifiable in the recovery curves following suprathreshold conditioning stimuli. These results indicate the presence of mechanisms intrinsic to the spinal cord in humans that are capable of yielding rhythmic autogenic facilitatory effects in response to a unitary discharge from Ia and cutaneous afferents. Identification of this spinal mechanism in humans appears to provide further insight into the pathophysiology of myoclonus and the mechanisms of load compensation.

85.1 SENSORY TRANSDUCTION IN THE COCKROACH FEMORAL TACTILE SPINE. Andrew S. French and Janice E. Kuster*. Department of Physiology, University of Alberta, Canada.

The femoral tactile spine of the cockroach has been used in a number of previous quantitative studies of sensory transduction because of the relative ease with which it can be stimulated and recorded from. Recent work in this laboratory has shown that the transduction mechanism may be characterised as a linear dynamic system with memory followed by a nonlinear system without memory. The linear component behaves as a fractional differentiator over the frequency range which has been studied.

Although the receptor has been used in a number of functional studies, we have only recently described its ultrastructure. The morphological findings place the receptor in the group of cuticular arthropod mechanoreceptors, characterised by a dense dendritic sheath and well defined tubular body, which is typical of trichoid sensilla, campaniform sensilla and chordotonal organs. However, the mechanical linkage between movement of the tactile spine and deformation of the sensory ending is not directly comparable with any of these three groups.

There are currently few theories to explain the dynamic properties of transduction in cuticular sensilla in terms of their known morphological components. The partial differentiator behaviour which has now been described in several of these receptors is difficult to relate to simple mechanical components or to membrane electrochemistry. The present work is aimed at discovering ways in which the morphological components of the sensilla could give rise to such behaviour and is particularly concentrated on possible roles of the visco-elastic behaviour of the tubular body in transduction.

The experiments consist of stimulating receptors with pseudo-random band-limited modulation of position of the spine within the socket. The resultant sensory action potentials and the position information are both fed to a digital computer and are analysed by both linear and nonlinear systems analysis techniques in the frequency domain. By redesigning the stimulating apparatus, we have now managed to extend the description of the receptor behaviour over a much wider bandwidth than has previously been possible and we have also measured the system parameters as a function of the strength of the stimulating position signal. Both of these additional sets of results support the description of the behaviour as a fractional differentiator and have reinforced our suggestions that the fractional power is very close to one half, which is relevant to discriminating between a number of possible models of mechanotransduction.

85.2 MORPHOLOGY AND PHYSIOLOGY OF CHEMORECEPTORS IN THE WALKING LEGS OF THE LOBSTER HOMARUS AMERICANUS. Charles D. Derby. Boston Univ. Marine Program, Marine Biological Lab., Woods Hole, MA 02543.

The chemoreceptor neurons in the legs of lobsters, which function in localization and handling of food, innervate several different types of sensilla: simple and squamous setae, located in rows and tufts on all five pairs of pereiopods; serrate setae, found on the last two pairs of walking legs; and squat sensilla, situated along the inner cutting edges of the chelate walking legs and on apposable cuticular regions of the last two segments of the non-chelate fourth pair of walking legs.

Multi-unit neurophysiological recordings, used to describe the response properties of the population of chemoreceptors as a whole, reveal that certain amino acids and amines are highly excitatory stimuli, some peptides and proteins are moderately excitatory, whereas carbohydrates, alcohols, nucleosides, and nucleotides are in general only slightly excitatory. Single-unit extracellular recording techniques were then employed to describe the specificity and threshold of single chemoreceptive cells.

Several different types of narrow-spectrum chemoreceptors were found, each type responding with maximal sensitivity to only one of the following compounds: L-glutamate, L-glutamine, L-arginine, taurine, betaine, and ammonium chloride. The most extensively studied type of receptor - the L-glutamate sensitive cell group - responded with less than 8% of the L-glutamate response to 25 other compounds at equimolar concentrations. The mean threshold of the population of L-glutamate sensitive cells was near 10^{-8} M; the lowest threshold recorded for any L-glutamate cell was 3.5×10^{-9} M. Ammonium chloride sensitive cells are also highly specific and have low thresholds: one ammonium chloride sensitive cell had a threshold of 3.5×10^{-14} M, while several had thresholds near 10^{-9} or 10^{-10} M. The other groups of narrow-spectrum cells - L-arginine, L-glutamine, taurine, and betaine sensitive chemoreceptors - showed equally strong specificity as well as thresholds of 3.5×10^{-8} to 3.5×10^{-9} M. There is also evidence for a protein-best cell: this cell responded to hemoglobin but not to its enzymatically-digested components. There was one cell that did not fit the narrow-specificity response pattern: it responded to at least 7 of 15 compounds tested at 3.5×10^{-4} M, 4 of 15 at 3.5×10^{-5} M, and 3 of 15 at 3.5×10^{-6} M. These results indicate that the peripheral coding system in the legs of lobsters is based largely but perhaps not exclusively on narrow-spectrum chemoreceptors.

85.3 THE FINE STRUCTURE OF LEECH SENSILLA. C. E. Phillips and W. O. Friesen. Dept. of Biology, University of Virginia, Charlottesville, VA 22901.

The leech Hirudo medicinalis responds to the water movements associated with surface waves by initiating crawling or swimming movements which are directed with a high probability towards the wave source (Young, Dedwylder & Friesen, J. Comp. Physiol., in press). Stimulation of a body wall flap in a dissected preparation with water waves evokes afferent impulses in peripheral segmental nerves. Transduction of this type of stimulation occurs only at the sensilla, specialized sensory structures found on the middle annulus of each body segment (Friesen, J. Exp. Biol., in press). The sensilla are papillar structures 100-200 μm in diameter which may protrude up to 50 μm out from the body wall. SEM studies of the Hirudo body wall have revealed that two types of filiform projections occur at the sensilla: single, 3-10 μm long processes and grouped, 1-2 μm long processes (DeRosa & Friesen, Biol. Bull., in press). One of these projections evidently is responsible for the detection of water movements.

We describe here the ultrastructure of Hirudo sensilla. Three types of ciliated sensory cells were observed at the sensilla. Two types, uniciliated and multiciliated sensory cells, with elongated cilia projecting through the body wall cuticle, correspond, respectively, to the single and grouped filiform processes observed in the SEM. The third cell type is multiciliated as well, but the cilia do not penetrate the cuticle, rather they lie between it and the epithelial cell layer. This receptor type was not detectable with the SEM. The single cilia have the characteristic 9 x 2 + 2 microtubular structure, but the arrangement in the multiciliated cells is somewhat variable. All the cilia arise from cup-like depressions in the apical surface of the sensory cells. In the uniciliated receptors, the cilium is surrounded by 10 pyriform stereocilia which are interconnected by a fine tubular feltwork of electron dense material extending to the level of the basal body. The sensory cells are narrow (1-2 μm) and very elongated, extending 40-50 μm below the cuticle. A U-shaped nucleus lies at the base of the cell body. In this region, bundles of microtubules are usually present and the cytoplasm undergoes a transition, taking on the appearance of axoplasm. These ciliated cells appear, therefore, to be primary sensory neurons. The uniciliated cells appear to be the sensory neurons transducing low amplitude water movements. Supported by NIH grant #NS 14965 to W.O.F.

85.4 INTACT AND REGENERATED SNAIL TENTACLES STUDIED WITH HRP STAINING AND OLFACTOMETRY. Ronald Chase and Rifa'at Kamil*. Department of Biology, McGill University, Montreal, Quebec, H3A 1B1.

The posterior tentacles of pulmonate molluscs possess a specialized epithelial pad that is sensitive to stimulation by wind and odors (Chase & Croll, J. Comp. Physiol., 1981; Chase, Comp. Biochem. Physiol., 1981). The sensory pad is innervated by the digitate extensions of a large peripheral ganglion. In the terrestrial snail Achatina fulica these structures regenerate following excision of the tentacular tip.

Backfills of the tentacular nerve with horseradish peroxidase (HRP) reveal that the intact tentacle contains at least five cell types with axons projecting to the CNS: 1) Small multipolar neurons (mean diameter, 7 μm) with cell bodies and dendritic processes limited to the ganglion; 2) Monopolar neurons (15 μm) arrayed along the digitate extensions; 3) Bipolar neurons (20 μm) with cell bodies and dendrites limited to the digits; 4) Bipolar neurons (20 μm) with cell bodies scattered between the digits, and dendrites extending to the cuticular surface; 5) Large multipolar neurons ("collar cells"; 35 μm) clustered in the digits near to the ganglion.

Each of the enumerated cell types is also seen in regenerated tentacles. The largest neurons reappear first, the smallest last. In intact tentacles the fiber pathways are neatly bundled to form sterotyped patterns. In regenerated tentacles the fibers are initially chaotic, but by six months their organization approaches that of intact tentacles. The regenerated fibers are thicker than the intact fibers.

Chemical sensitivity was quantitatively evaluated using a two-chambered tentacular olfactometer. The measure of sensitivity was the number of ipsilateral turns elicited by unilateral presentations of the diluted vapor of amyl acetate. Ten weeks after the lesion the regenerated tentacle shows definite olfactory sensitivity, but the threshold is higher than for the intact tentacle in the same animal. HRP backfills at this time demonstrate a complete absence of ganglion cells (Type 1). By seven months after the lesion, the regenerated tentacle is hypersensitive compared to the intact tentacle. The anatomy of regenerated tentacles at this time reveals the presence of superfluous, misplaced ganglion cells, as well as the repeated branching of processes directed toward the cuticular surface. Either or both of these features could account for the hypersensitivity of regenerated tentacles.

85.5 PRESYNAPTIC INHIBITION TO AFFERENTS OF THE CRAYFISH BRAIN. R.M. Glantz, Dept. of Biology, Rice University, Houston, Tx. 77001 Multimodal, descending sensory interneurons in crayfish brain exhibit EPSPs in response to shocks of up to eight afferent sensory roots and/or central nerve tracts. If pairs of afferent pathways are stimulated simultaneously the resultant compound excitatory synaptic potential is an approximately linear sum of the individually evoked synaptic events. If stimulation of one of the input pathways (CS-conditioning stimulus) precedes stimulation of a second pathway (test stimulus) by 20 to 40ms the monosynaptic response to the test stimulus is depressed by 64% $\pm$ 3.4% (S.E.). The following results suggest that the depression is due to presynaptic inhibition: a) The postsynaptic action of the conditioning stimulus is an EPSP capable of eliciting postsynaptic action potentials and with a reversal potential at least 30 mV more depolarized than the peak of the test EPSP; b) If CS are presented in pairs separated by intervals of 20 to 40 ms the homosynaptic depression of the CS pathway is generally substantially smaller (15.7$\pm$10.3%, S.E.) than the heterosynaptic depression. This result indicates that it is unlikely that CS elicits a remote IPSP. A notable feature of the heterosynaptic interaction is its selectivity. Although a given cell may receive monosynaptic input from up to eight afferent pathways, only a single pair will exhibit a heterosynaptic interaction and the interaction occurs only in one direction. Furthermore, if the same pair of inputs are examined in a different postsynaptic cell they may show no presynaptic interaction or possibly interact in the opposite direction. The presynaptic interaction cannot be initiated by the electrical activity of the postsynaptic cell as is the case for the lateral giant interneuron (Krasne and Bryan Science 182:590-592, 1973). The phenomena may be related to the findings of Kennedy et al (Science 186:451-454, 1974) which document primary afferent depolarizations contingent upon sensory activation of interneurons in crayfish abdominal ganglia. In agreement with the findings of Kennedy et al terminals entering the crayfish brain are subject to synaptic input with reversal potentials near the membrane resting potential. The reversal potential can be shifted to more positive levels by the injection of chloride ions into the afferent neuron terminal. The terminal synaptic potentials are elicited polysynaptically from selected afferent pathways and their latency and time course are strikingly similar to the latency and time course of the heterosynaptic interactions described above. The results imply that afferents entering the brain excite interneurons which presynaptically modulate the output of a select group of other afferent pathways.
Supported by N.S.F. Grant No. BNS79-10335.

85.6 LATERAL INHIBITION IN THE CRAYFISH VISUAL SYSTEM. B.Waldrop, M.D.Kirk and R.M.Glantz. Biology Department, Rice University, Houston, TX 77001

Sustaining fibers (SF), tonic light-ON cells in the crayfish optic tract, have well defined excitatory corneal receptive fields and whole-eye inhibitory surrounds. Lateral inhibition is postulated to be the basis of stimulus-dependent oscillations of SF output. (Glantz and Nudelman, J.Neurophysiol.39:1257-1271 (1976))

We have impaled SF's near their dendrites in the medulla externa. Large, light-evoked, depolarizing compound synaptic potentials can be seen giving rise to spikes. Extrinsicly injected current can affect both the size of these potentials and the SF firing rate. Small light spots placed in the inhibitory surround lead to a decrease of the depolarization and firing rate evoked by excitatory light stimuli, but do not cause hyperpolarizations when given alone, nor do they decrease the firing rate of a cell depolarized with extrinsic current.

Inhibitory light pulses can, depending on their position, give rise to oscillations in SF membrane potential and firing rate when added to steady state light-evoked depolarization. This begins with a decrease in depolarization which never brings the potential lower than rest level in the dark. Extrinsic current alone produces only tonic firing. No evidence for direct SF-SF synaptic interactions has been seen.

We conclude from these observations that only excitation, and not inhibition, is synaptically expressed on the SF's by visual input, and that the inhibitory interactions are contained entirely in cells interposed between photoreceptors and SF's.

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85.7 Morphological Representation of Crayfish Sustaining Fiber Visual Receptive Fields. M. D. Kirk and R. M. Glantz, Dept. of Biology, Rice University, Houston, Texas 77001.

Crayfish (Pacifastacus leniusculus) sustaining fibers (SF) were impaled near their dendritic arborizations in the medulla externa using a semiintact eye-cup preparation. Subsequent to a careful mapping of the corneal receptive field with a fibre optic light guide the individual cells were filled with the dye lucifer yellow.

Each SF possesses an expanded region of axon at the proximal edge of the medulla externa which gives rise to a major transverse process within the neuropil (Waldrop and Glantz, 1980, Neurosci. Abstr. 6:221). Secondary dendrites arise from this process to form a uniplanar sheet of branches parallel to the proximal and distal faces of the externa. The SFs all have an efferent process within the medulla terminalis as well as a major termination within the brain.

Most importantly each SF's dendritic tertiary arborization is restricted to an area of the externa corresponding to its corneal receptive field (assuming a precise retinotopic projection through the first optic neuropile and chiasmata). Therefore, it is proposed that every SF has a well defined anatomical representation of its visual receptive field within the medulla externa.

Presently, experiments are being conducted to determine the variability in the weighting of SF receptive fields and whether they are accompanied by corresponding differences within the secondary or tertiary dendritic branching patterns.

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85.8 THE CERCUS-TO-GIANT INTERNEURON SYSTEM OF THE PRAYING MANTIS, ARCHIMANTIS BRUNNERIANA. E.E. Ball, G.S. Boyan*, R.C. Stone*. Dept. of Neurobiology, Research School of Biological Sciences, Australian National Univ., Canberra City, ACT 2601, Australia.

Wind and sound-sensitive giant interneurons, which are driven by the cercal sensilla, have been studied intensively in crickets and cockroaches and the input-output relations of some of them are now understood in considerable detail. Mantids are phylogenetically related to crickets and cockroaches but have a very different mode of life. We therefore wondered whether they would have giant interneurons driven by cercal receptors, and, if so, how similar these would be to those of crickets and cockroaches.

The major receptor types on the cerci of mantids are filiform and trichoid sensilla. Filiform sensilla are thinner, frequently longer than the trichoid sensilla surrounding them, and are innervated by a single neuron. Trichoid sensilla show variable patterns of innervation with three to five neurons per sensillum. Neither external nor internal features, however, allow us to divide these sensilla into distinct classes. Both number of cercal segments and total number of cercal sensilla vary considerably between animals. Cobalt backfills of the cercal nerve show a projection that is mainly ipsilateral and near the midline, with some fibers extending forward into the connectives. Groups of fibers extend to or across the midline particularly at the anterior end of the ganglion, but three areas without such fibers reflect the ontogenetically fused nature of the terminal ganglion. On entering the terminal ganglion some cercal fibers run first ventral and then dorsal to surround two glomeruli. Intracellular recordings from unidentified wind-sensitive cercal receptors have been obtained from the terminal ganglion and their projections have been filled with Lucifer Yellow.

Lucifer Yellow or cobalt-filled electrodes have been used to record from and fill wind-sensitive interneurons in the terminal ganglion. On the basis of cell body position, pattern of dendritic branching and physiological responses at least eight classes of wind-sensitive cells are present. The responses of these interneurons to pulses of wind of different velocities and durations have been examined. Bilateral cercal inputs to the wind-sensitive cells were found in some cases. In terms of cell body position and neurite shape there are morphological similarities between some of the wind-sensitive mantid interneurons and the giant interneurons of cockroach and, to a lesser extent, cricket. However, detailed patterns of dendritic branching are quite different in mantid, with greater bilateral dendritic development. There are approximately nine "giant" neurons in each ventral cord.

85.9 CENTRAL MODULATION OF REFLEX EFFECTS OF THE LOCUST METATHORACIC FEMORAL CHORDOTONAL ORGAN. Sasha N. Zill* (SPON: G. Hoyle). Dept. of Biol., Univ. of Oregon, Eugene, OR 97403.

In many animals, functional connections between proprioceptive sense organs and motoneurons can be modulated by the central nervous system. The neuronal mechanisms underlying changes in gain of reflex effects of an insect leg mechanoreceptor, the locust metathoracic femoral chordotonal organ, are examined in this study.

The chordotonal organ consists of groups of bipolar neurons whose dendrites insert into ligaments that are coupled to the tibial segment of the leg. Extracellular recordings from the nerve of the chordotonal organ show that it monitors the angle of the femoro-tibial joint. Intracellular recordings from individual sensory neurons show that the organ exhibits range fractionation.

Reflex effects of the chordotonal organ were studied by mechanically pulling or releasing the main ligament of the organ, mimicking flexion or extension of the femoro-tibial joint. Abrupt displacements of the ligament elicit resistance reflex discharges in slow excitatory motoneurons of the extensor or flexor tibiae muscles. Intracellular recordings from motoneurons show that resistance discharges occur as 1) a constant phasic excitatory post-synaptic response and 2) a subsequent sustained excitatory response that is variable both in magnitude and duration and is subject to central modulation.

Intracellular recordings from non-spiking interneurons in the metathoracic ganglion have shown that: 1) some interneurons exhibit maintained shifts in membrane potential of an amplitude proportional to the magnitude of ligament displacement; 2) others show only a phasic response; 3) some interneurons receive tonic excitatory input from the chordotonal organ and also excite appropriate leg motoneurons upon depolarization and thus could mediate the resistance reflex; 4) depolarization or hyperpolarization of other interneurons can modulate the resistance reflex.

It is concluded that non-spiking interneurons are able to set the gain of reflex effects of proprioceptive sense organs.

Supported by NSF Grant SPI-7914916.

85.10 NEURAL BASIS OF THE DIRECTIONAL SELECTIVITY OF WIND-RECEPTIVE FIELDS OF COCKROACH GIANT INTERNEURONS. Darryl L. Daley and Jeffrey M. Camhi. Sect. Neurobiology and Behavior, Cornell Univ., Ithaca, NY 14850.

The giant interneurons (GI's) of the cockroach (Periplaneta americana) show different directional selectivity in response to controlled puffs of wind. While the wind-receptive fields of some GI's are quite restricted, others are much broader and some are even omnidirectional. The wind-sensitive receptors, filiform hairs located on the ventral surface of each of the two cerci (peg-like abdominal appendages), also show directional selectivity. The hairs are arranged on the cerci in longitudinal columns. All hairs of a particular column have the same best excitatory direction. When a single column of sensory hairs is left intact (usually 6 hairs), controlled puffs of wind elicit synaptic potentials and often action potentials over approximately 180° of wind angle (Daley and Camhi, Neurosci. Abst., 1980). We report here on the pattern of connectivity (presence or absence of connection, sign, and relative strength) between identified columns of sensory hairs and GI's 1, 2, and 3.

GI 1, a somewhat directionally selective interneuron receives excitatory synaptic input from all hair columns of the ipsilateral cercus. GI 2, an omnidirectional cell, also receives excitatory synaptic input from ipsilateral hair columns. The difference in directional selectivity of GI's 1 and 2 appears to be due to differences in the strength of connection of two of the nine hair columns. The strength of connection, for both hair columns, is greater to GI 2 than to GI 1. Contralateral inputs to these two GI's are weaker than ipsilateral inputs and do not show the large difference in connection strength.

The sharply restricted wind-receptive field of GI 3 appears to result from both selectivity of afferent connection and inhibition. GI 3 receives strong excitatory input from ipsilateral hair columns whose best excitatory directions fall well within the receptive field of the cell. Hair columns with best excitatory directions near the edges of the receptive field of GI 3 provide no detectable input, while those hair columns with best excitatory directions outside the wind-receptive field provide inhibition. Contralateral inputs to GI 3 are similar to ipsilateral inputs though weaker.

(This work supported by NIH grant #NS09083.)

85.11 ANTERIOR-MOST PROJECTIONS OF GIANT INTERNEURONS IN ACHETA DOMESTICUS TERMINATE IN MECHANO-RECEPTOR NEUROPILE OF THE BRAIN.
John S. Edwards and Les Williams. Max-Planck-Institut fuer Verhaltensphysiologie, D-8131 Seewiesen, Federal Republic of Germany.

Anterior projections of the lateral and median giant interneurons of Acheta were determined from intensified cobalt fills of the ventral nerve cord. Anterograde fills prepared by cutting the connectives in an anterior abdominal segment filled large numbers of axons. Prolonged filling of the cercal sensory nerve (up to 4 days at 4-9°C) led to selective transneuronal filling of giant interneurons. We assume that cobalt is taken up from an extracellular pool delivered to the cercal glomerulus in which a large number of cercal afferents terminate. Comparison of the two filling techniques allows detailed reconstruction of distant projections.

Aborization in each of the abdominal ganglia appear to be similar in form and reflect metameric organisation. This is also evident in the two abdominal neuromeres of the condensed metathoracic ganglion. The more extensive lateral aborizations in each of the thoracic ganglia reflects the greater lateral extension of neuropile in these segments, and also show serial homology.

It is possible that median projections from each of the paired giant interneurons communicate by mutual synapses within each segment.

The anterior terminations lie within the brain in a specific region of the lateral protocerebrum which is known also to contain other ascending mechanoreceptive inputs such as high and low frequency acoustic units. In common with these mechanoreceptor units the median and lateral giant interneurons traverse the ventral nerve cord in the ventral intermediate tract.

This work was supported by the Alexander von Humboldt Stiftung and NIH Grant NB 07778 (JSE), the Max Planck Society and Deutsche Forschungsgemeinschaft OT 62/4 (L.W.).

85.12 SENSORY CHARACTERISTICS OF LOCAL NEURONS IN THE LAST ABDOMINAL GANGLION OF THE CRAYFISH. Heinrich Reichert, Mark R. Plummer, Grace Hagiwara*, Richard L. Roth* and Jeffrey J. Wine. Dept. of Psychology, Stanford Univ., Stanford, CA 94305.

We have begun a systematic description of the properties of local circuit neurons (LCNs) in the sixth abdominal ganglion of the crayfish, Procambarus clarkii. Estimates of the number of LCNs reveals that as many as one-half of the cells in the ganglion may be LCNs. This was determined by repeatedly backfilling with cobalt the thirteen roots and two connectives that leave the ganglion, and comparing the resulting soma counts with the total soma counts obtained from Azure B stained 7 µm cross sections. The largest count for backfills was 322 somata. The total number of somata in the ganglion was 620. Thus approximately 300 neurons in the ganglion were not stained by cobalt backfills and hence may not have axons which leave the ganglion.

To determine the sensory properties of such LCNs we have been recording intracellularly with Lucifer-filled electrodes from the neuropilar processes of these cells in semi-intact, viable animals while presenting various forms of natural stimuli. We find sixth ganglion LCN cell types with sensory characteristics which correspond to those of each major class of sixth ganglion projecting interneuron. LCN cell types which respond to either 'headward' or 'tailward' water movements (directionally selective cells) as well as cell types which respond to tactile stimulation, to pinch or to movement of uropods have been identified. One modality we have not seen represented is photosensitivity. Although represented in the population of projecting neurons, it is rare and therefore sampling considerations do not preclude there being photosensitive LCNs.

We have also begun to examine the functional role of sensory LCNs within the ganglion. For example, experiments of Wiese, Wollnik and Schultz (pers. commun.) indicate that directionally sensitive projecting neurons of similar directional preference inhibit their contralateral counterparts. We have preliminary evidence that large, non-spiking LCNs with bilateral processes mediate this type of lateral inhibition, since these cells respond to sinusoidal water movements with directionally selective depolarizations and injection of depolarizing current into these cells causes inhibition of specific projecting sensory interneurons of the same directional preference.

Supported by NSF Grants BNS 78-14179, BNS 80-15583, a DFG Postdoctoral Fellowship (H.R.), and an NSF Graduate Fellowship (M.R.P.).

86.1 AN INSECT CARDIOACTIVE PEPTIDE MODULATES HEART RATE DURING DEVELOPMENT. N. Tublitz and J.W. Truman. Department of Zoology, University of Washington, Seattle, WA 98195.

Cardioactive factors have been isolated from the CNS of many invertebrates. In the insects, these factors have been pharmacologically identified in many orders, however, none to date have been implicated in any cardioregulatory function. We report on the identification, isolation, and partial characterization of a cardioactive peptide(CAP) from the tobacco hawkmoth, Manduca sexta, which is released into the haemolymph to cause a significant increase in heart rate.

To quantify the activity of various cardioactive factors, we have developed a highly sensitive(threshold=0.1 picomole serotonin) in vitro Manduca heart bioassay. Crude extracts from the ventral nerve cords of pharate adults cause a dose dependent increase in heart rate when applied to the isolated heart. This cardioactive factor is pronase sensitive, resistant to boiling, and has an apparent molecular weight of less than 1000 daltons, suggesting that this factor is a peptide. CAP has been primarily localized to the abdominal transverse nerves, an unpaired segmental nerve that is a classical neurohaemal site. CAP appears not to be proctolin, an insect neuropeptide that is pharmacologically cardioactive in some insects. Proctolin is ineffective on the isolated Manduca heart at concentrations up to 10^{-4} M, and does not coelute with CAP on gel filtration columns.

To investigate a possible physiological role for CAP, heart rate was recorded in vivo during adult eclosion and wing spreading behaviors, a 75 minute developmental period during which the animal sheds its pupal cuticle and inflates its wings to their proper adult length. A significant rise(50%) in heart rate is seen immediately following eclosion and remains elevated throughout wing spreading. This increase returns to pre-eclosion levels only after the wings are fully inflated. We next measured blood CAP titers to ascertain whether CAP was responsible for the increase in heart rate. Significant levels of CAP were detected in the blood immediately after eclosion and remained elevated throughout wing spreading with the peak CAP concentration at 5 minutes after the initiation of eclosion. Furthermore, an 80% decrease in the amount of CAP stored in the ventral nerve cord was measured during this period. These data suggest that CAP, released from the abdominal transverse nerves, acts as a neurohormone to increase heart rate during eclosion and wing spreading behaviors in Manduca sexta.

(Supported by a NSF predoctoral fellowship to N.T. and a NSF grant #PCM 80-20975 to J.W.T.)

86.2 PEPTIDERGIC NEURONS: A BIOCHEMICAL, ANATOMICAL, IMMUNOLOGICAL AND PHYSIOLOGICAL ANALYSIS. M. O'Shea, C.A. Bishop and M. Adams*. Dept. Pharmacol. Physiol. Sci., The University of Chicago, Chicago, IL 60637 and Abt. Huber, M.P.I.V., Seewiesen, W. Germany.

We are interested in the neurobiology of individually characterized peptidergic neurons. In the central nervous systems of invertebrates, uniquely identifiable neurons may provide model systems to study peptide action on the cellular level. A prerequisite for such a study is the individual identification of neurons containing a sequenced bioactive peptide. Proctolin (H-Arg-Tyr-Leu-Pro-Thr-OH) is among the few sequenced bioactive invertebrate peptides. It was first extracted from cockroaches. We have therefore developed techniques which combine intracellular electrophysiological, intracellular dye injection, immunocytochemical and biochemical techniques in an attempt to identify individual proctolin-containing neurons in cockroaches and other animals.

We have produced a proctolin antiserum which can be employed in the immunocytochemical visualization of neuronal cell bodies in whole, unsectioned central ganglia. Immunoreactive neurons include bilaterally paired cell bodies in cerebral, thoracic and abdominal ganglia. Bilateral symmetry at the single cell level suggests the neurons are individually identifiable and this has now been confirmed for some of the larger immunoreactive neurons by combining intracellular Lucifer yellow injection with immunocytochemistry. Anti-proctolin sera may crossreact with peptides other than proctolin and immunocytochemistry cannot therefore identify proctolin in immunoreactive neurons. Reverse phase high performance liquid chromatography (HPLC) applied to single identified neurons provides more direct evidence and we are currently studying individual proctolinergic neurons which were biochemically characterized in this way. These studies focus on biochemical and physiological aspects of identified neurons. For example, we are attempting to describe the biosynthesis of proctolin and to identify the bioactive compounds other than proctolin in proctolin-containing neurons. Our functional studies are concerned with the physiological action of proctolin neurons on identified targets. Recent anatomical evidence on the projections of immunoreactive neurons and pharmacological experiments suggest identified octopaminergic neurons may be postsynaptic to proctolinergic neurons.

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86.3 EFFECT OF PROCTOLIN ON THE LOBSTER CARDIAC GANGLION. R. E. Sullivan, K. Tazaki*, and M. W. Miller*. Bekesy Laboratory of Neurobiology, University of Hawaii, Honolulu, HI 96822.

We have examined the actions of proctolin (PROC) on the isolated cardiac ganglion of Homarus americanus. PROC has concentration-dependent excitatory effects on this 9-cell motor pattern generator. Intra- and extracellular recording reveals that bath applied PROC (10^{-8} - 10^{-5} M) produces distinct changes in motor output. These include: 1) increase in burst duration, 2) increase in burst frequency, 3) increase in the number of impulses per burst, 4) alteration of intraburst impulse patterning. In addition, PROC effects a long-lasting depolarization of the anterior motor neurons. The PROC-induced, ganglionic excitation is mediated by both small (pacemaker) and large (motoneuron) cell effects. The predominant effect on small cells, as recorded extracellularly from the trunk, is an immediate (<10 sec) increase in the duration of small cell bursting and the number of spikes per burst. This effect reverses within 5 minutes of washout. In contrast, selective application of PROC to the anterior large cell region effects a depolarization (up to 10 mV) of the somatic membrane and enhances interburst pacemaker potentials. Anterior cell effects are maximal within 60-80 seconds and require 20-30 minutes of washout for complete reversal. Prolonged application of PROC to either anterior or posterior regions results in increases in duration and frequency of bursting. The distinctive time courses for each effect give rise to a "characteristic" temporal sequence of altered motor output when whole ganglia are exposed to PROC. The motor output of ganglia exposed to a pulse of ($5X10^{-7}$ - $5X10^{-6}$ M) PROC frequently exhibit a "doublet" burst pattern within 30 sec of exposure which is associated with the prolonged small cell burst. Shortly thereafter (1 min), the ganglion returns to a singlet burst mode of high frequency, and prolonged duration characteristic of large cell effects. These effects were not observed with crab ganglia (J. Benson, pers. comm.). In ganglia silenced with TTX ($3X10^{-7}$ M), application of a low concentration (<$5X10^{-7}$ M) of proctolin produces a long-lasting depolarization which is accompanied by an increase in the large cell apparent input resistance. Higher concentrations also elicit trains of driver potentials (DP), i.e. slow (200-300 msec), regenerative, Ca^{++}-dependent potentials which have been postulated to participate in burst formation. Preliminary voltage clamp experiments on TTX-treated, ligatured, large cells suggests that PROC (10^{-6} M) may delay a voltage-dependent K^{+}-conductance which underlies DP repolarization. Proctolin induced, repetitive DP have also been observed in ligatured anterior large cells in normal saline. Although out of synchrony with the remaining network, the frequency of these repetitive DP is comparable to the burst frequency of the PROC-treated ganglion. These results suggest that neurohormones may direct temporal sequences of patterned motor output from simple networks through differential interactions with pacemaker and motor elements. Supported by NIH grant NS06191 to RES, NIH NS11808 to I. M. Cooke, and the University of Hawaii Foundation.

86.4 PROCTOLIN-LIKE IMMUNOREACTIVITY IN THE NERVOUS SYSTEM OF THE LOBSTER HOMARUS AMERICANUS. T. L. Schwarz*, G. Lee* and E. A. Kravitz. Dept. of Neurobiology, Harvard Medical School, Boston, MA 02115.

The ability of proctolin (Arg-Tyr-Leu-Pro-Thr) to cause a contracture of the dactyl opener muscle of the lobster walking leg (Schwarz et al., J.Neurobiol., 11:623, 1980) has motivated us to seek endogenous proctolin-like substances in this organism and to study their distribution. To this end a radioimmunoassay has been developed for the peptide.

To obtain a rabbit antibody to proctolin, several immunization protocols were tried. An antiserum suitable for use in an RIA was obtained from a rabbit that had been immunized with a carbodiimide cross-linked proctolin-BSA complex. At a 1:1000 dilution this antiserum can detect 10fmoles of proctolin. (70 fmoles gives a 50% displacement of the maximum binding of iodinated tracer). The specificity of this serum was checked with 0.5-20 nmoles of 11 other peptides. No cross-reactivity was detected.

Lobster tissues were extracted and examined with this RIA. Proctolin-like immunoreactivity was found in nearly every portion of the nervous system. Substantial amounts were detected in each of the thoracic ganglia (2-5pmoles), the abdominal ganglia (1-2.5pmoles), the subesophageal (8pmoles) and circumesophageal (1pmole) ganglia, brain (5 pmoles) and the optic nerve and eyestalk (5pmoles). In contrast, no proctolin-like material was detected in exoskeletal muscles.

The highest amounts of proctolin-like immunoreactivity (up to 20pmoles) were found in the pericardial organs (PCO's) of the thoracic segments. The concentration per mg protein in these structures is 400 times that in a larger organ such as the brain. Another region that is greatly enriched in proctolin-like material is the proximal region of each of the second thoracic roots. Both the PCO's and the proximal regions of the roots are known to synthesize and release two amine hormones (serotonin and octopamine) into the haemolymph. The highest concentrations of proctolin-like material are thus associated with neurosecretory structures. A proctolin-like peptide in crustacean PCO's has also been reported by Sullivan (J.Exp.Zool. 210:543).

In a preliminary experiment, haemolymph from a lobster was found to contain $4x10^{-10}$ m/l of a proctolin-like material. This is 4 times the threshold for the action of the peptide on the opener muscle. These results and earlier studies of amine levels in the haemolymph raise the possibility that basal levels of tension in lobster muscles are continuously adjusted by circulating hormones.

Supported by the NIH.

86.5 LIMULUS CARDIOACTIVE PEPTIDES. W. Watson, K. Neill*, E. Tillinghast*, Zoology Dept., U.N.H., Durham, N.H. 03824 R. M. Dores*, U. of Colorado Med. Center, Dept. Physiol., Denver, CO, G. Augustine, Biol. Dept., UCLA, Los Angeles, CA. T. O'Donohue*, NIH, Bethesda, MD.

The nervous system of Limulus contains two peptides which modulate the activity of the isolated neurogenic heart. One appears to be a proctolin-like peptide which increases the strength of heart contractions without influencing the heart rate. The other is similar in its biochemical properties to Limulus chromatophorotropic factor (LCF, Dores and Herman, CBP 67:459, 1980), and it has a potent inhibitory effect on the rate and strength of heart contractions.

The initial separation of both peptides was achieved via gel filtration chromatography using a modification of a procedure described previously by Pezalla et al. (Biol. Bull. 154: 148, 1978). This protocol resulted in two peaks with biological activity when assayed on the Limulus heart. Both peaks fell between standards with mol. wts. of 400 and 1400, and both were insensitive to trypsin.

Both peptides were assayed further to determine whether they were acting on the cardiac ganglion neurons or the myocardium. Simultaneous intracellular recordings from cardiac ganglion follower neurons and tension recordings from the cardiac muscle revealed that the inotropic peptide, like synthetic proctolin, was acting directly on the myocardium, while the inhibitory peptide hyperpolarized follower cells and abolished antidromic spikes. A small fraction (0.4%) of one Limulus brain yielded enough proctolin-like material to increase the strength of heart contractions by 100%. A 50% reduction in heart rate was observed following addition of 10% of the inhibitory factor present in 1 brain.

In order to test whether the inhibitory peptide was equivalent to LCF we performed chromatophorotropic assays on Uca pugilator. LCF activity was present in brain extracts but peak LCF activity did not correspond exactly with peak inhibition. Further purification of extracts with HPLC demonstrated that the peptides were indeed distinct.

Further studies are presently underway to purify both peptides, map their distribution in the CNS, and determine their mechanism of action.

86.6 PROCTOLIN INDUCES MYOGENICITY IN THE DEGANGLIONATED LIMULUS HEART. T. Hoshi* and W. Watson (SPON: E. Hagstrom). Zoology Dept., Univ. of New Hampshire, Durham, NH 03824.

Proctolin is a pentapeptide (Arg-Tyr-Leu-Pro-Thr) originally isolated from the viscera of the cockroach (Starratt, A. N., Brown, B. E., Life Sci. 17: 1253, 1975). This peptide or its analogue is present in a variety of insects, several crustaceans, and Limulus (Brown, B. E., J. Insect Physiol., 23: 861, 1977; Sullivan, R. E., J. Exp. Zool., 210: 543, 1979; Benson et al., Brain Res., in press). Its primary physiological role appears to be modulation of muscle fiber contraction/contracture. In Limulus bath application of 10^{-8} M proctolin results in a 200% increase in the amplitude of heart contractions. This inotropic effect is the result of proctolin acting directly on the muscle fibers, rather than on the neurons which comprise the cardiac ganglion (Benson et al., Brain Res., in press; Watson et al., in prep.).

At high concentrations (> 10^{-7} M) proctolin induced slight contracture and strong rhythmic (but not synchronous) contractions of the deganglionated Limulus heart. The frequency and amplitude of the proctolin-induced contractions in the deganglionated heart were equivalent to normal heart contractions with the cardiac ganglion intact. At a concentration of 10^{-6} M proctolin the myogenicity usually required 2 - 10 minutes to develop and 1 hour to reverse following perfusion with normal saline solution.

The proctolin-induced myogenicity persisted in Na^{+}-free choline Cl, and TTX + normal saline solutions, which were sufficient to block all the cardiac ganglion activity and muscle EJP's. However, Mn^{++} (15 mM) and Co^{++} (25 mM) blocked the myogenicity. One cAMP analogue, caffeine (10 mM), and procaine (25 mM) had no effect.

Proctolin could induce myogenic contractions in the neurogenic deganglionated Limulus heart by either affecting the electrical properties of the muscle membrane or internal contractile mechanisms. Preliminary intracellular recordings from cardiac muscle fibers indicate that no significant potential or resistance changes are associated with the proctolin-induced contractions. However, the obvious hypothesis that high concentrations of proctolin induce Ca^{++}-dependent spikes cannot be rejected without more rigorous experiments.

87.1 INTRACELLULAR RESPONSES OF IDENTIFIED HRP-FILLED DORSAL RAPHE NEURONS TO VMT STIMULATION. M.R. Park, H.Imai* and S.T. Kitai. Dept. of Anatomy, Michigan State University, E. Lansing, MI. 48824.

Intracellular recordings were made from neurons of the dorsal raphe nucleus of the rat. In order to localize the nucleus, the overlying cerebellum was removed exposing the floor of the fourth ventricle. Recordings were made with beveled microelectrodes filled with 4% horseradish peroxidase (HRP) in 0.1 M tris buffer, pH 7.6 and 0.5 M potassium methylsulphate. Bipolar stimulating electrodes were placed in midline ventral tegmentum (VMT) just dorsal to the interpeduncular nucleus. The two types of responses to VMT stimulation observed were correlated with axonal trajectory. Intracellularly labeled cells with dorsally or laterally directed axons showed only an inhibitory postsynaptic potential (IPSP). In addition to IPSPs, antidromic action potentials were observed in neurons with axons projecting from the nucleus in a ventral and rostral direction. Increasing stimulus strength produced a reduction in the latency of the IPSP but not the action potential. The onset of the IPSP could preceed the antidromic action potential. There was also a latency shift in the IPSP of neurons not antidromically activated. This decrease in latency with increasing stimulus strength is in itself a failure to demonstrate monosynaptic inhibition. Two possible explanations are as follows: 1) Multiple inhibitory inputs to dorsal raphe are being activated, any one of which could be monosynaptic, but with the faster conducting system having higher threshold. 2) The inhibition is not monosynaptic and therefore involves an interneuron interposed between antidromically activated dorsal raphe axons and the somata of dorsal raphe neurons being recorded from. IPSPs were seen to be reduced in amplitude with the injection of hyperpolarizing current. They are therefore due to an increase in membrane conductance. Examination of action potentials (>65mV amplitude and with 15 mV overshoot) shows a repolarization which declines exponentially toward an asymptote, the equilibrium potential for outward current (E_{I_o}),and which is roughly equal to the resting membrane potential. The IPSP following the action potential reaches a potential 13 mV more hyperpolarized than E_{I_o}. If repolarization is principally dependent on potassium then E_{I_o} represents the potassium equilibrium potential (E_K). The IPSP passes below E_K and therefore cannot be K^+ mediated. By elimination it must be due to a conductance increase to Cl^- ions. Action potentials show a clear inflection in their rising phase, suggestive of separate initial segment (IS) and soma-dendritic (SD) components. Hyperpolarization caused failure of the SD spike. (Supported by USPHS Grant 14866 to S.T.K. and NIH BRSG Grant RR 05772-04 to M.R.P.).

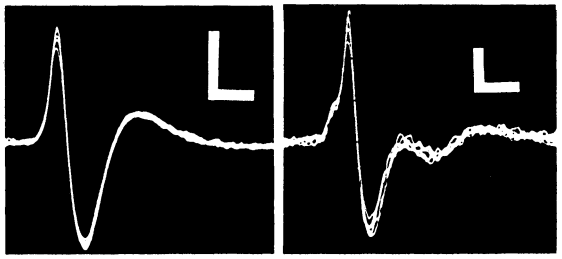
87.2 ONE TYPE OF SEROTONERGIC UNIT IN THE NUCLEUS RAPHE MAGNUS (NRM) HAS A DISTINCTIVE SPIKE SHAPE. E.G. Anderson and M.W. Wessendorf. Dept. of Pharmacology, Univ. of Ill. Med. Ctr., Chicago, IL 60680.

We have reported that, in the NRM, 2 groups of neurons conducting between 0.5 and 1.1 m/sec and between 2.5 and 6.0 m/sec are destroyed by 5,7-dihydroxytryptamine and can be identified as serotonergic. We now report that some 5-HT units also exhibit a characteristic spike shape. Using single barrel glass microelectrodes pulled to a tip diameter of about 0.75 µm for extracellular recording, 5-HT neurons in the NRM were observed for their spike shape and their responsiveness to i.v. LSD. NRM units were also observed for their responsiveness to 5-HT using a 7-barrel iontophoretic electrode glued to the recording electrode.

5-HT units conducting at less than 1.1 m/sec consistently exhibited a unique spike shape characterized by a negative inflection on the afterpotential (right frame). In contrast, the 5-HT units conducting between 2.5 and 6.0 m/sec never showed this inflection (left frame). Their spike shapes were indistinguishable from faster-conducting, non-5-HT units.

Some quantitative, but no qualitative differences were observed in the responses of the cell groups to LSD or 5-HT. The response to 5-HT was predominantly inhibitory in both groups of 5-HT cells. (Some excitatory responses to 5-HT were observed in non-5-HT units). LSD produced both inhibitory and excitatory effects on 5-HT neurons.

We conclude that the spike shape of the 0.5-1.1 m/sec 5-HT units is an identifying characteristic. However, pharmacological responsiveness to LSD is not uniform within either group of 5-HT units.



Left: Spike shape of unit conducting at 4.5 m/sec. Positivity is upwards. Right: Spike shape of unit conducting at 0.8 m/sec. Note the negative inflection superimposed on the after potential. Scale: 0.5 mV, 0.5 msec.
(Supported by PHS NS 14985 and PHS NS 12649)

87.3 NEURONAL INTERACTIONS IN THE DORSAL RAPHE NUCLEUS. R.Y. Wang and G.K. Aghajanian, Dept. Pharmacol., St. Louis Univ., Sch. Med., St. Louis, MO 63104 and Depts. Pharmacol. and Psychiat., Yale Univ., Sch. Med., New Haven, CT 06508.

In previous studies (Brain Res. 132:186-193, 1977; Neuropharmacol. 17:819-825, 1978), we have shown that in the rat dorsal raphe nucleus (DRN) there is a powerful serotonin (5-hydroxytryptamine; 5-HT) autoregulatory system, which exerts its action onto 5-HT cells presumably via 5-HT axon collaterals and/or dendrodendritic junctions. The aim of the present study was to further elucidate the nature of 5-HT autoinhibition and to analyze local interactions of cells in the DRN by use of autocorrelation and crosscorrelation histograms.

Chloral hydrate anesthetized rats were used. 5-HT cells in the DRN were tentatively identified by their wave form and by their slow, regular and rhythmic firing pattern (0.5-3Hz). This firing pattern has been demonstrated previously by use of combined single cell recording and fluorescence histochemical methods and by antidromic stimulation of the ascending 5-HT pathway and collision tests to be characteristic for 5-HT-containing neurons but not for non-5-HT cells in the DRN.

The autocorrelation histograms of 5-HT cells showed an initial prolonged quiescent period followed by periodic fluctuation with equally spaced peaks; the periodic density peaks slowly flattened out. This silhouette of 5-HT neuron's autocorrelogram bears a striking resemblance to the shape of autocorrelograms generated from computer-simulated pacemaker cells with inhibitory synaptic input. Crosscorrelograms revealed that all adjacent 5-HT neuronal pairs (N=21) recorded simultaneously by a single-barreled micropipette displayed complicated patterns of interactions. The interactions could be grouped into the following categories: 1) synchronization, 2) synchronization and direct inhibition and 3) synchronization and mutual inhibition. In contrast, only 1 out of 9 non-adjacent 5-HT neuronal pairs (recorded by double-barreled micropipettes with 100 µm tip separation) showed positive functional interaction; no interaction was found between 5-HT and non-5-HT cells. The synchronous firing among adjacent 5-HT neurons could be the result of direct activation from one cell to another or the result of sharing a direct activation from a third neuron. However, based upon available evidence, it is most likely that the synchronous firing was due to simultaneous disinhibition from a shared inhibitory input from a common source. These results support our previous finding that in the DRN 5-HT neurons contain a powerful auto and mutual regulatory system. (Supported by USPHS Grants MH-34424 to R.Y.W., MH-17871 and MH-14459 to G.K.A. and by the State of Connecticut.)

87.4 SEROTONIN-INDUCED DEPOLARIZATION IN RAT FACIAL MOTONEURONS: EVIDENCE FOR DECREASED POTASSIUM CONDUCTANCE. C.P. VanderMaelen and G.K. Aghajanian, Depts. Psychiatry and Pharmacology, Yale University School of Medicine, New Haven, CT 06508.

Previous work has shown that microiontophoretic application of serotonin onto rat facial motoneurons causes a depolarization of the membrane, accompanied by an increase in neuronal excitability, and an increase in input resistance (i.e., a decrease in net membrane conductance; Nature, 1980, 287, 346-347). The present experiments were undertaken to investigate the ionic conductance changes which occur during these serotonin-induced responses. Intracellular recordings were made in vivo from facial motoneurons of rats anesthetized with chloral hydrate. Intracellular recording electrodes were filled with K-acetate, K-citrate, or KCl, and glued to 5-barrel micropipettes used for microiontophoresis, with tip separations of 40-60 µm. Responses to iontophoretically applied serotonin were compared to those of other putative neurotransmitters and their analogues.

The serotonin-induced depolarization could conceivably be caused by a decrease in resting potassium conductance (g_K) or resting chloride conductance (g_{Cl}). The latter was ruled out because estimates of reversal potentials for responses to GABA, glycine, or the GABA analog, muscimol, which presumably are chloride mediated, were always more positive than for the serotonin response. When intracellular chloride concentrations were increased by recording with KCl electrodes, GABA, glycine, or muscimol caused large (20 mV) depolarizations along with profound decreases in membrane resistance, clearly indicating that E_{Cl} was displaced in the depolarizing direction. On these same neurons serotonin still produced its typical depolarizing response with increased resistance. Thus, serotonin does not produce depolarization by decreasing g_{Cl}, nor does it do so presynaptically by disinhibition of a tonic GABA or glycine input to these neurons.

Subthreshold depolarization by current injection causes an increase in input resistance (anomolous rectification) in these neurons. Perhaps because of this, depolarizations produced by glutamate or its analog DL-homocysteic acid (which presumably increases g_{Na}) were also accompanied by increased input resistance. However, the glutamate response was shown to be different from that of serotonin since hyperpolarization of the membrane by current injection to approximately E_K greatly attenuated or slightly reversed the serotonin response, but not the glutamate or DL-homocysteic acid responses.

These experiments suggest that decreased g_K accounts for the serotonin-induced depolarization and is not secondary to a sodium-driven depolarization. Supported by USPHS Grants MH-17871 and MH-14459 and the State of Connecticut.

87.5 A COMPARISON OF CORTICAL NEURONE RESPONSES TO IONTOPHORETICALLY APPLIED TRYPTAMINE (T) AND 5-HYDROXYTRYPTAMINE (5HT). R.S.G. Jones* (SPON: A.A. Boulton) Psychiatric Research Div.; Univ. Hosp; Saskatoon, Sask. Canada S7N 0X0

Previous iontophoresis studies in various brain regions have shown that T evokes similar neuronal responses to 5HT and it has been assumed that T acts simply as an agonist at 5HT receptors. The present experiments have made a detailed comparison of the actions of T and 5HT on single spontaneously active neurones in the somatosensory cortex of urethane anaesthetized rats. In addition, the release of ^{14}C-labelled T and 5HT has been determined *in vitro* and used to calculate the ionic transport number of the indoleamines in four microelectrodes.

For the *in vivo* experiments, recording and extracellular drug application techniques were entirely conventional. When a suitable neurone was located its spontaneous firing rate was recorded and responses to identical applications of T and 5HT determined. Of 123 neurones tested 115 were depressed by iontophoretically applied T, 4 excited and 4 unaffected. Sixty-four of the neurones depressed by T were also depressed by 5HT but on 46 cells 5HT was excitatory and on 5 had no effect. In addition two of the 4 cells excited by tryptamine were depressed by 5HT. Thus on 42% of the neurones responsive to both amines opposite effects were observed. A comparison of depressant responses to the two amines on individual cells was made with reference to the 5HT responses. This revealed that the T responses had a mean (± SEM) duration of 185±15% of the 5HT response. The maximum decrease of firing from baseline was 115±6% compared to 5HT and the time taken to reach the maximum was 80±8%. Thus the tryptamine responses were quicker in onset and had a much greater duration than 5HT. The mean transport numbers for the two amines were 0.303±0.04 for 5HT and 0.283±0.04 for T indicating that the differences in responses were genuinely biological. Neurones in animals which had received prior (10 days) lesions in ascending 5HT pathways showed similar differences in depressant responses indicating that the difference is probably postsynaptic in origin. Finally, the effects of the putative 5HT antagonist metergoline (0-25nA) on T and 5HT depressant responses of 10 neurones was tested. On five cells, responses to T were antagonized by metergoline in the absence of any effect on the 5HT responses. This occurred with the weak ejecting currents 0-5nA). At higher currents responses to both indoleamines were blocked. On 5 other cells no differential antagonism was noted. These results may suggest that T has postsynaptic neuronal effects independent of 5HT. It is possible that specific tryptaminergic receptors may exist in the cortex. Supported by Sask. Health and M.R.C.

88.1 COLUMNAR ORGANIZATION OF PRIMATE PREFRONTAL CORTEX: ARRANGEMENT OF CALLOSAL AND ASSOCIATIONAL TERMINAL FIELDS. P.S. Goldman-Rakic and M.L. Schwartz, Sect. of Neuroanatomy, Yale Univ. Sch. of Med., New Haven, CT. 06510.

Recent studies using autoradiographic tracing methods have revealed that callosal fibers in the prefrontal association cortex of rhesus monkey are distributed in the form of well-defined columns alternating with roughly equal sized areas that remain free of callosal input (Goldman and Nauta, Brain Res.,122: 393, 1977). In order to determine the source and pattern of distribution of axons that terminate in the acallosal spaces, we employed two distinctive anterograde tracers to label converging cortico-cortical systems in the same animal. Acrylamide-bis gel HRP pellets were implanted in the intraparietal sulcus (Brodmann's area 7) of the parietal lobe in one hemisphere; at the same time, a mixture of ^{3}H-proline and ^{3}H-leucine was injected into the dorsal bank of the principal sulcus (Brodmann's area 9) in the frontal lobe of the other hemisphere. Serial sections were alternately processed for HRP activity and autoradiography. The distribution of terminal labeling was analyzed in the dorsal bank of the principal sulcus that was ipsilateral to the implant in parietal cortex and contralateral to the injected principal sulcus.

In harmony with earlier studies, both the associational and callosal projections to the prefrontal cortex were distributed in a spatially periodic pattern in which bands of labeled axons alternated with irregular spaces lacking label. In general, both HRP and radioactively labeled columns extended across the full thickness of the cortex. The major new findings exposed by serial reconstruction of adjacent autoradiograms and HRP-reacted sections was that in areas of convergence, the two sets of cortico-cortical afferents tended to occupy mutually exclusive columnar territories with some variable amount of overlap along the borders of adjoining columns. This evidence suggests homotopic callosal fiber columns in prefrontal cortex interdigitate with associational fibers from the same side to provide a unique set of ipsilateral-contralateral hypercolumns that could provide a modular basis of interhemispheric integration. Whether this relationship of callosal and associational fiber systems represents a general principle of organization for callosally recipient cortex or is restricted to an area of frontal association cortex remains to be demonstrated.

(Supported by Grants NS16666 and MH 00298 and Fellowship Award MH 08308).

88.2 CARBONIC ANHYDRASE HISTOCHEMISTRY REVEALS SUBPOPULATIONS OF MYELINATED AXONS IN THE DORSAL AND VENTRAL ROOTS OF RAT SPINAL NERVES. D.A. Riley, S. Ellis* and J. Bain*. Dept. of Anatomy, UCSF, San Francisco, CA 94143.

Carbonic anhydrase (CA) functions in acid-base balance, secretory processes and transport of water and ions across membranes. It occurs both as soluble and membrane bound forms in central and peripheral nervous tissues, localized predominantly in neuroglia and Schwann cells. Neurons of the dorsal root and celiac ganglia are the only nerve cells demonstrated histochemically to contain CA activity. The cell bodies of the dorsal root neurons were reactive, but their axons exhibited no activity.

While characterizing the distribution of anhydrase activity histochemically in rat skeletal muscles, we noted that some myelinated axons within the intramuscular nerve bundles possessed intense axoplasmic staining. The present study sought to identify the nerve type of the reactive axons. Rats were anesthetized and perfused with 2.5% glutaraldehyde. Perfusion proved essential for retaining enzymatic activity. Tissues were frozen and cryostat sections were reacted for CA activity using Hanson's medium either with or without 10^{-6}M acetazolamide, a specific inhibitor of neural CA.

The common peroneal, tibial and sural cutaneous branches of the sciatic nerves all contained mixtures of reactive and nonreactive axons. Within lumbar dorsal roots, about 49% of the myelinated axons of all sizes (1.5 - 16μm in diameter) were stained. A subpopulation of dorsal root ganglion cells and their axons exhibited dark cytoplasmic staining. In contrast, none of the large axons in the companion ventral roots were reactive. However, axons ranging in size from 3 to 8μm were stained and they constituted approximately 24% of the myelinated axons. Cross sections of lumbar spinal cord contained many darkly stained axons in the dorsal columns. Nerve cell bodies were not stained in the ventral horn. In all cases, histochemical staining was inhibited by acetazolamide.

The present findings indicate that a subpopulation of sensory neurons contains cytoplasmic carbonic anhydrase in their axons and cell bodies. The reactive axons in the ventral roots appear not to originate from ventral motoneurons because motoneurons are nonreactive. Some of the ventral root axons may be sensory fibers entering the spinal cord by an aberrant pathway. Supported by NASA Ames grant NAC2-OR665-903.

88.3 COMMISSURAL CONNECTIONS OF THE HIPPOCAMPAL FORMATION IN THE RHESUS MONKEY. D.L. Rosene and G.W. Van Hoesen, Dept. of Anatomy, Boston Univ. Sch. of Med., Boston, MA, and Depts. of Anatomy and Neurology, Univ. of Iowa Coll. of Med., Iowa City, IA.

The hippocampal formation and entorhinal area in the rat have an extensive set of commissural connections. In the rhesus monkey we can now report both anatomical and physiological data indicating the surprising absence and or topographic restriction of these commissural connections.

The commissural system in the monkey that is most consistent with the organization observed in rats is the crossed projection from the presubiculum to the contralateral entorhinal cortex. In the monkey, both injections of 3H-labeled amino acids (HAA) into the presubiculum and horseradish peroxidase (HRP) injections into the entorhinal area demonstrate this pathway from all levels of the presubiculum to layer III of the entorhinal area. In contrast, two crossed projections originating from the entorhinal cortex in the rat, the homotopic connection to the opposite entorhinal area and the crossed projection to the opposite hippocampus, were, respectively, exceedingly weak and totally absent when assessed with injections of HAA and HRP into the monkey entorhinal area or hippocampus.

Two other commissural systems that are prominent in rats are the crossed Schaffer collateral system and the crossed dentate gyrus commissural system. In the monkey, both HAA and HRP injections located anywhere in the rostrocaudal extent of the main body of the hippocampus fail to demonstrate either of these commissural systems. To confirm this anatomical data evoked potential experiments were conducted. Electrical stimulation of the body of the hippocampus produced only weak and long latency (> 50 msec) contralateral evoked responses suggesting multisynaptic conduction (e.g. through the septum). However, when the stimulating and recording electrodes were placed homotopically in the uncal hippocampus, a strong response was evoked at a 20 msec latency. This response was used to guide HAA and HRP injections into the uncal hippocampus. In the contralateral uncus anterograde termination was observed in the inner third of the dentate gyrus molecular layer and the stratum radiatum of CA3 and CA1 as well as retrograde labeling of CA3 and hilar (CA4) neurons. These data indicate that in the monkey only the uncal hippocampus retains hippocampal commissural connections while the body of the hippocampus lacks these connections and the entire hippocampus lacks commissural input from the entorhinal area. These data suggest that the functional lateralization of the primate cerebral cortex and related restriction of commissural connections demonstrated in motor and sensory systems may also occur in the hippocampal formation.
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88.4 QUANTITATIVE AND QUALITATIVE DIFFERENCES IN UPTAKE OF PEROXIDASE (HRP) AND PEROXIDASE-LECTIN (HRP-WGA) CONJUGATE. W.W. Pugh* and M. Kalia. Laboratories of Anatomy, School of Vet. Med., Inst. of Neurological Sciences, Univ. of PA., Phila., PA., 19104 and Dept. of Physiology, Hahnemann Med. Col., Phila., PA., 19102.

The affinity of lectins (WGA in this case) for neuronal surface membranes has been shown to be mediated through specific binding to the n-acetyl glucosamine receptor at cell surfaces of perikaria and terminals of neurons as well as glia and vasculature. Due to this affinity, there is a disproportionate distribution of applied WGA or HRP coupled to WGA in the extracellular space of the injection site as lectins accumulate around the surface membranes. The resultant uptake of HRP-WGA occuring during endocytosis will be much greater compared to the uptake of free HRP distributed randomly in the extracellular space. HRP-WGA was prepared according to the method of Gonatas et al. with the following modifications: after initial conjugation, all procedures were carried out at 4^{0}C; separation of HRP-WGA from free HRP and free WGA was accomplished by several passes through a Sephadex G-150 column.

The nodose ganglion in the cat, containing cell bodies of sensory vagal afferents and passing efferent fibers of vagal motor neurons, was injected unilaterally with 3-30 uL of either HRP or HRP-WGA and subsequently examined at 24-48 hrs. using TMB histochemistry. Injections of 33% HRP labeled sensory afferent fibers in tractus solitarius and terminal fields in nucleus solitarius (anterograde) as well as vagal motor neurons (passing retrograde) in the brainstem (Kalia and Mesulam). Injections of 0.167% (200:1 dilution) HRP-WGA intensely labeled the sensory projections but did not retrogradely label any passing fiber motor neurons. Similar injections of 0.167% HRP failed to label any sensory terminals but did label motor neurons. A third injection of 0.167% HRP-WGA mixed with 100X excess free WGA (16.7%) did not label either sensory terminals or motor neurons, demonstrating a competitive inhibition of HRP-WGA uptake in the presence of excess free WGA.

In conclusion: 1) high concentrations of HRP result in uptake by perikaria and passing fibers; 2) low concentrations of HRP showed uptake by passing fibers with no uptake by cell bodies or consequent anterograde transport; 3) HRP-WGA in low concentrations intensely labeled anterograde projections but did not significantly label passing fibers; 4) selective uptake by cell bodies and not passing fibers of HRP-WGA can be inhibited by excess WGA demonstrating a surface membrane affinity and resultant great increase in uptake by endocytosis in the presence of WGA either free or coupled to HRP.

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88.5 PATHWAYS RESOLVED BY TRANSNEURONAL UPTAKE OF HORSERADISH PEROXIDASE IN C.N.S. OF FLIES. D.R. Nässel*. European Molecular Biology Laboratory, D-6900 Heidelberg, W. Germany. (SPON: B. Mulloney).

It was recently shown that horseradish peroxidase (HRP) in addition to labeling injured neurons passes transneuronally into contiguous nerve cells (Nässel, D.R., Brain Res., 206: 431, 1981). This transneuronal uptake of the enzyme resulted in detailed secondary, tertiary and quarternary labeling. This technique has now been used to resolve neuronal pathways connecting the optic lobes (lamina, medulla, lobula and lobula plate) with thoracic ganglia in the flies Calliphora, Musca and Drosophila.

After HRP uptake through severed thoracic ganglia four consecutive sets of neurons could be resolved in the brain and optic lobes. The tertiary and quarternary labeling was achieved after long uptake (18 hrs) or with the addition of 3% lysolecithin to the enzyme solution. One pathway consisted of (from periphery to center): columnar small field centripetal neurons of the lamina, columnar neurons of the medulla, columnar lobula neurons and a cluster of large descending neurons connecting the brain to the thoracic ganglia. Among these descending neurons is the giant descending neuron which is known to be part of an important giant pathway to tergotrochanter muscles and dorsal longitudinal flight muscles (King, D.G., Wyman, R.J., J. Neurocyt., 9: 753, 1980). A second pathway includes large horizontal and vertical motion sensitive neurons of the lobula plate (Hausen,K., Z. Naturforsch., 31c: 629, 1976) and descending neurons with more posterior dendrites.

The first pathway is especially interesting since it includes several identified neurons whose inputs are known and/or electrophysiologically studied (all but the lobula units have been recorded from). The three giant components of this pathway are under study in several laboratories and future work hopefully will further characterize the remaining units of the entire polyneural chain from the retina to muscles.

88.6 A BASAL AMYGDALOID PROJECTION TO THE CENTRAL AMYGDALOID AREA IN THE MONKEY. K.C. Kosel, G.W. Van Hoesen and D.L. Rosene Dept. of Anat. and Neurol. Univ. of Iowa, Iowa City, IA. 52242 and Dept. of Anat., Boston Univ., Boston, MA. 02118.

In axon stains like the Cajal or Vogt methods numerous pathways can be discerned coursing in the amygdala. In primates, these are sizeable, and course between nuclear groupings in the amygdala. In the monkey, the ventral amygdalofugal system, a prominent pathway, exits the posterior amygdala as a dorsal continuation of intrinsic amygdaloid white matter. However, we have observed that before leaving the amygdala a sizable contingent of axons that arise from the basal complex terminate within the amygdala, and in particular, in the central amygdaloid area.

In 5 monkeys ablations were made in the piriform lobe that damaged the entorhinal cortex and the dorsally adjacent basal amygdaloid complex (basomedial and basolateral amygdaloid nuclei). The brains were processed with the Fink and Heimer method. An additional 7 monkeys received labeled amino acid injections in the entorhinal cortex or in one or more of the basal amygdaloid nuclei (basomedial, basolateral and accessory basal). An additional 2 monkeys had injections in the medial and the lateral nuclei.

The results reveal that injections of the medial and lateral entorhinal cortex or the medial and lateral amygdaloid nuclei do not give rise to projections to the central amygdaloid area. However, all ablation cases and all autoradiographic cases involving the basal nuclei give rise to either terminal degeneration in, or terminal labeling over, the central amygdaloid area throughout its rostrocaudal extent, and these projections are sizable. Ventrally, they sweep through the basolateral nucleus. Dorsally, they course between the medial edge of the lateral nucleus and the lateral edge of the basolateral nucleus. Terminal degeneration and terminal labeling is most intense over the lateral part of the central amygdaloid area.

These findings are of interest as recent reports have shown that the central amygdaloid area projects to visceral and autonomic centers in the hypothalamus and brainstem. In view of the fact that the basal complex receives afferents from temporal cortical areas, cingulate gyrus and the subiculum, this intrinsic amygdaloid pathway may play a role in mediating telencephalic influences on visceral and autonomic reactions (Supported by grants NS 14944 to G.W.VH and NSF BNS 7924099 to D.L.R.).

88.7 SOME CORTICAL CONNECTIONS OF THE FRONTO-CINGULATE CORTEX IN THE RAT. S.L. Wertheim*. (SPON: W.J.H. Nauta). Dept. of Psychology, Mass. Institute of Technology, Cambridge, MA 02139.

In the rat, the cingulate and prefrontal cortices overlap on the medial wall of the hemisphere. This area has at least four cytoarchitectonic subdivisions. HRP has been used to trace the afferents and efferents of two of these zones: the anterior cingulate area and the precentral medial field (PrCm) of Krettek and Price (J. Comp. Neurol., 171:157, 1977). The enzyme was placed either iontophoretically or as pellets of HRP contained in a matrix of polyacrylamide gel.

HRP deposits that encompass both fields show two main types of ipsilateral cortical connections; connections with limbic cortex that include orbital, retrosplenial, presubicular, perirhinal and entorhinal cortices and connections with sensory cortex that include areas 17, 18a and 18b. Labeled cells in the orbital cortex are found primarily in the ventral orbital area. There is terminal labeling in layer I of the ventral and medial orbital areas. Both granular and agranular retrosplenial cortex contain many labeled cells in layer V. Layer II-III in agranular retrosplenial cortex contains an occasional labeled cell. Terminal labeling appears in layers V and I. There is a projection to layers I and III of the presubiculum. Labeled cells in the entorhinal area are in layer IV. There are many heavily labeled cells in areas 18a and 18b mainly in layer V. Area 17 contains fewer, more lightly labeled neurons, also mainly in layer V. The fronto-cingulate projection to these areas is to all layers of area 18b, to layers I, V, and VI of area 18a and to layer I of area 17.

Small pieces of HRP-gel placed in PrCm result in distinctive labeling of the thalamus and midbrain but show essentially the same pattern of cortical connections as deposits encompassing both fields. However, far fewer labeled cells are seen in granular retrosplenial cortex in these cases.

The pattern of distribution of visual cortical terminals in the fronto-cingulate cortex remains to be determined. In one animal, placement of HRP-gel in the region of area 18a resulted in cell and terminal labeling predominantly in PrCm. This relationship between visual cortex and PrCm is especially interesting because of cytoarchitectonic (Caviness, J. Comp. Neurol., 164: 247, 1975) and electrophysiological (Hall and Lindholm, Brain Res., 66:23, 1974) evidence that it is homologous to area 8 of the primate. There are some labeled cells in the sensory cortex outside the visual areas in these animals. Serial section reconstructions are being undertaken to determine whether what appear as isolated cells in transverse sections form an overall pattern.

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89.1 REGENERATION FOLLOWING SELECTIVE AXOTOMY OF AN IDENTIFIED VERTEBRATE CENTRAL NEURON. S.J. Zottoli. Dept. of Biology, Williams College, Williamstown, MA 01267.

Axonal regeneration is known to occur across the site of spinal cord lesions in adult goldfish and this growth may result in return of function (i.e., swimming). However, the apparent failure of the Mauthner cell (M-cell) to regenerate its axon may indicate an intrinsic inability of this cell to sustain regrowth (Kiernan, Biol. Rev. 54, 1979). In order to investigate the M-cell response to axotomy, the M-axon has been lesioned exclusive of all other axons.

Goldfish, 9-12cm in body length were anesthetized and the skull overlying the caudal portion of the brain was removed. On separation of the vagal lobes both M-axons were usually visible and easily penetrated with glass microelectrodes filled with 2.7 M KCl (3-5 M Ω). Selective axotomy resulted when the electrode was vibrated by gently tapping the manipulator until the resting potential was reduced to at least 1/3 its initial value (i.e., 28mV) and held at or below this level for ten minutes. After removal of the electrode, the brain was covered with a vaseline-paraffin oil mixture and the hole sealed with epoxy. At post-operative intervals ranging from 5 to 79 days, four goldfish were anesthetized and the medulla was exposed as described above in order to fill their M-axons with Lucifer Yellow. After fixation, the brains were prepared for wholemount observation utilizing fluorescent microscopy. Then the brains were sectioned at 15μm, observed again with fluorescence and subsequently stained with Cresyl Violet. The brains of eleven other fish were prepared for histological examination exclusively.

An axon reaction characterized by partial chromatolysis and/or a perinuclear ring of basophilic material was visible as early as 9 days after axotomy 2mm caudal to the M-cell soma. This reaction persisted for at least 79 days. A total of 6 M-axons were filled with Lucifer Yellow. Retraction bulbs were apparent at 7 days after axotomy but no growth was seen. At 21 days sprouts were seen emanating at or slightly rostral to the tip. Finally, at 79 days the M-axon had undergone extensive regrowth, one process projecting rostrally and the other in a caudal direction well past the lesion site. These results clearly indicate the ability of the adult M-cell to regenerate its axon and the possible correlation of this regeneration to morphological changes in the cell soma. Furthermore, long-term changes in membrane excitability of the M-cell which parallel this cell's axon reaction (Faber and Zottoli, in press) emphasize its potential as a model for further studies on neuronal regeneration. (supported in part by NSF Grant No. BNS-7924655; special thanks is extended to W. Stewart for supplying the Lucifer Yellow.)

89.2 GLUCOSE UTILIZATION BY THE AXOTOMIZED RED NUCLEUS OF RAT. L.R. Rodichok, K.D. Barron and A.J. Popp. Depts. of Neurology and Neurosurgery, Albany Medical College, Albany, NY 12208.

The earliest biochemical responses required for successful regeneration after axon injury are essentially unknown. Increases in ^{14}C-2-deoxyglucose (^{14}C-2-DG) utilization have been demonstrated in rat facial and hypoglossal nuclei within 24 hours of neurotomy and persist for 14-28 days (Agranoff, Smith and Sokoloff, 1980; Kreutzberg and Emmert, 1980; Singer and Mehler, 1980). The facial and hypoglossal nuclei are aggregations of extrinsic neurons. We have examined ^{14}C-2-DG uptake after axotomy in the intrinsic neuronal population represented by the rat red nucleus. The right rubrospinal tract was knife sectioned at high cervical (C3) and mid-thoracic (T6) levels. The tract is completely decussated. Two lesioned and a control animal were sacrificed after 3, 6 and 9 days. ^{14}C-2-DG utilization was measured by the method of Sokoloff et al (1977). Under methoxyflurane anesthesia, male Sprague-Dawley rats weighing 300-400 gm were injected intravenously with ^{14}C-2-DG, 50-60 uCi/kg and sacrificed after 45 minutes by pentobarbital overdose. 20 um frozen sections of whole brain were cut serially at -22°C and mounted on coverslips. One series, comprising every 3rd section, was applied to Kodak SB5 X-ray film for two weeks. Another series was stained with 0.1% cresylviolet, pH 3.5. The third set of sections was held in reserve. Densitometric measurements were made with a Zeiss microspectrophotometer using a .07 mm aperture and monochromatic 585 mu light. The spinal cord was fixed in formalin and serially sectioned through the lesion.

Despite clear chromatolytic changes in the left red nucleus and the absence of detectable cytological alterations in the right red nucleus, there were no differences between the optical densities of the two red nuclei as measured on autoradiographs 3, 6 and 9 days postoperatively. This was true for both cervical and thoracic lesions. Animals surviving 1, 14, 30, 60 and 100 days are in preparation or under examination. Our data indicate an absence of an early increase in ^{14}C-2-deoxyglucose utilization by an axotomized central neuronal population. This negative finding seems to strengthen the proposition that the responses of mammalian central (intrinsic) and peripheral (extrinsic) neurons to axotomy can be fundamentally different. This difference may well be relevant to the failure of regeneration in mammalian CNS.

89.3 INTERACTIONS BETWEEN ASTROCYTES AND THE SCHWANN CELLS THAT INVADE AND SUBSEQUENTLY MYELINATE SPINAL CORD AXONS. T. J. Sims and S. A. Gilmore, Departments of Pathology and Anatomy, University of Arkansas for Medical Sciences, Little Rock, Arkansas 72205.

Myelination of spinal cord axons by Schwann cells has been shown to occur following irradiation of the lumbosacral spinal cord in 3-day-old rats (Gilmore and Duncan, '68, Sims and Gilmore '81). In the present study, further light and electron microscopic observations were conducted on rats 5 to 25 days after irradiation (4000R at 3 days of age), for the purpose of determining what factors were responsible for the invasion and free intermingling of Schwann cells in CNS tissue. On post-irradiation days 5 and 10 the dorsal funiculi (DF) were composed of naked axons and were strikingly acellular as compared to controls. At these times, astrocyte processes forming the glia limitans showed a variety of changes due to the irradiation. In some cases the foot-like, subpial processes showed signs of retraction, whereas in others these processes formed large, variegated protrusions extending beyond the surface of the cord. The basal lamina was intact, covering the entire surface of the irradiated cord, although taking a rather tortuous course over the astrocyte processes. By post-irradiation day 25 Schwann cells invaded and myelinated large-caliber axons in the superficial regions of the DF. The deeper regions of the DF remained unmeylinated and acellular as Schwall cells had not yet invaded to this depth. Subpial astrocyte processes were present but did not form a continuous glia limitans as they did in control subjects. The large variegated protrusions seen at earlier times were no longer present. In subpial regions where Schwann cells had invaded the DF, the basal lamina was interrupted by Schwann cells or their cytoplasmic extensions. From these observations it is our view that the initial disruption of the glia limitans and the substantial reduction in the astrocyte population following irradiation are the factors that allow Schwann cells to invade the spinal cord. We observed no evidence that Schwann cells have a toxic or inhibitory effect on astrocytes as has been suggested by other investigators. Furthermore, the basal lamina in itself is not a barrier to migrating Schwann cells.
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89.4 RESTORATION OF SYNAPTIC AND BEHAVIORAL FUNCTION WITH EMBRYONIC TRANSPLANTS OF CHOLINERGIC NEURONS. Walter C. Low, Steven B. Dunnett, S. Terri Bunch, Steven R. Thomas, Peter R. Lewis, Susan D. Iversen, Anders Bjorklund and Ulf Stenevi, Physiological Laboratory and Experimental Psychology, Univ. of Cambridge, Cambridge, U.K. and Dept. of Histology, Univ. of Lund, Lund, Sweden.

Recent anatomical studies of embryonic transplants containing cholinergic neurons have demonstrated a reinnervation of the hippocampal formation in adult host SD rats. The pattern or reinnervation into the hippocampal formation is quite specific and appears to be restricted to laminae which are normally innervated by cholinergic fibers. We now provide electrophysiological evidence that embryonic transplants of cholinergic neurons form functional excitatory synaptic connections. Furthermore, these newly formed synapses enhance the spatial memory performance of rats in comparison to those without transplants.

Three groups of SD rats received either sham operations, bilateral lesions of the fimbria-fornix to eliminate the cholinergic innervation of the hippocampal formation, or bilateral lesions of the fimbria-fornix followed by bilateral transplants of cholinergic septal nuclei from 16-17 day old rat embryos. Five months after surgery the spatial memory performance of each animal was tested using an 8-arm radial maze task. Sham operated animals and animals with transplants exhibited a gradual improvement in maze performance with time ($p<0.001$ and $p<0.05$ respectively) whereas animals with bilateral lesions exhibited no improvement with repeated trials. The overall performance of the transplant group, however, did not significantly differ from the bilaterally lesioned animals. After 30 days of testing, animals were then tested for an additional 8 days in which they were alternately injected (i.p.) with 0.05 mg/kg of physostigmine, an AChE inhibitor, or with saline. Both sham operated animals and animals with cholinergic transplants exhibited enhanced maze performance ($p< 0.05$ and $p<0.001$ respectively) with physostigmine while bilaterally lesioned animals exhibited no change.

After behavioral testing, animals with transplants were used for electrophysiological analysis of synaptic function. Electrical stimulation of the transplants evoked field potentials in the hippocampus and dentate gyrus, reflecting functional synaptic connections. Furthermore, it was found that dentate field potentials evoked by the electrical stimulation of intact perforant path fibers could be potentiated when preceded by the electrical stimulation of the transplant. These results indicate that neural transplants from embryonic septal nuclei are capable of forming viable homosynaptic and heterosynaptic connections with the hippocampal formation of adult hosts. These functional synapses in turn have the apparent capacity to partially restore cognitive behavioral function.

89.5 REGENERATION OF CATECHOLAMINE FIBERS INTO A COLLAGEN BIOIMPLANT AFTER CORTICAL ABLATION. J. C. de la Torre. Univ. of Miami School of Medicine, Miami, FL 33101

A bioimplant composed of cell-free solubilized bovine collagen which polymerizes (hardens) at body temperature was used to study the regenerative capacity of central catecholamine axons and blood vessels in rat brain. Two groups of male Long-Evans hooded rats were anesthetized with sodium pentobarbital-ketamine and a boneflap was removed from the right side of the skull using a drill. The craniectomy extended 5 mm posteriorly from the bregma and 3 mm laterally from the midline. The dura was retracted and using a soft suction catheter, the cortex and an area 2 mm subcortically was ablated by aspiration. The ablated area measured 3.5 mm in the antero-posterior plane and 5 mm deep. After hemostasis, the bioimplant was inserted to fill the lesion. Control rats underwent the same surgery but gelfoam was inserted into the lesion. The boneflap was returned and held in place by suturing the periosteum over it. All rats were given 500 mg/kg nialamide i.p. 2 hours before sacrifice to enhance aminergic fluorescence. Rats were sacrificed 10, 21, and 31 days postoperatively (dpo). Brains were removed at each time point and processed for the SPG histofluorescence method. All rats showed a denser distribution of catecholamine-containing varicosities (CCV) in the caudal and medial aspect of the lesion with less CCV in rostral or lateral brain regions. These "reactive" CCV were larger and more intensely fluorescent than contralateral intact CCV. At 10 dpo, moderate CCV density were seen around the lesion in rats with the bioimplant. At this time, CCV were also seen entering the bioimplant at its interface with the brain tissue. A moderate number of blood vessels were noted within the bioimplant. At 21 and 31 days dpo, CCV within the bioimplant were seen rostrally with greater frequency than at 10 dpo. In control rats, CCV density was light to moderate near the necrotic tissue zone and a few blood vessels were seen at the scar:brain tissue interface. By contrast, these control rats showed no CCV or blood vessels within the scar tissue at any time point. Despite a necrotic zone of autofluorescing cellular debris around the lesion, reactive CCV were often seen traversing this zone in both groups of rats as early as 10 dpo. These preliminary findings indicate that the experimental bioimplant is capable of sustaining a blood supply as well as CCV that enter it, presumably from central regenerating catecholaminergic processes. No CCV or blood vessels were seen within the scar tissue in control rats. It is further suggested that following a cerebral lesion of this type, the scar tissue but not the necrotic tissue zone, poses a greater barrier to regenerating catecholaminergic processes.

89.6 SUBSTANCE P HAS A SPARING EFFECT ON LESIONED NORADRENALINE NEURONS IN THE RAT BRAIN DURING ONTOGENY. G. Jonsson* and H. Hallman* (SPON: G. Grant). Dept. of Histology, Karolinska Institutet, S-10401 Stockholm, Sweden

Systemic administration of the catecholamine neurotoxin 6-hydroxydopamine (6-OH-DA) in the neonatal stage causes a marked alteration of the postnatal development of the central noradrenaline (NA) neurons, in particular of the locus coeruleus NA system. The 6-OH-DA treatment results in pronounced and permanent denervations of distant NA nerve terminal projections (e.g. in the cerebral cortex and the spinal cord), whereas the cell-body near projections are increased leading to a hyperinnervation (e.g. in the cerebellum and the pons-medulla). The consequences following the 6-OH-DA treatment are mainly related to a 'pruning effect'. The purpose of the present study was to investigate whether or not substance P (SP) can modify the postnatal development of the central NA neuron and their alteration induced by 6-OH-DA, as monitored by chemical NA assay, ^{3}H-NA uptake in vitro and fluorescence histochemistry. Intracisternal injection of SP (2x50 µg in 10 µl; 20 hr interval) 4-6 hr (the first dose) after the administration of 6-OH-DA (100 mg/kg, s.c.) to newborn rats was found to cause a clear-cut counteraction of the 6-OH-DA induced NA denervation (analysis in the adult stage). The counteracting action of SP was significant in all regions of the cerebral cortex and the spinal cord, being most pronounced in the frontal cortex. When testing the effect of various doses of SP on the reduced NA levels produced by 6-OH-DA in the frontal cortex a clear dose-response was observed. SP was also found to counteract the 6-OH-DA induced hyperinnervation, both in the cerebellum and the pons-medulla. Neonatal treatment with SP alone had no effect on the NA neurons in most regions analysed. The present study has shown that SP can counteract the alterations of the postnatal development of central NA neurons produced by 6-OH-DA. Both the NA denervations and hyperinnervations are counteracted, the latter probably of consequence of the sparing effect SP has on distant NA nerve terminal projections. It is unlikely that this effect of SP is related to an interference with the degenerative actions of 6-OH-DA, since SP was administered at a time when the neurotoxic actions of 6-OH-DA are largely completed. The exact mode of action of the observed effect SP is at present unknown. It is suggested that it may be related to a growth stimulatory effect of SP associated with the known excitatory effect SP has on locus coeruleus neurons.

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89.7 ACCURATE AXON AND SYNAPSE REGENERATION IN THE ABSENCE OF GLIA. Ellen J. Elliott and Kenneth J. Muller. Carnegie Institution of Washington, Baltimore, MD. 21210.

Glia are thought to play several different roles, some constructive and some obstructive, in nerve regeneration. In the leech Hirudo medicinalis, two large glial cells ensheath all the axons in the three connectives linking adjacent ganglia. To investigate the role of glia in nerve regeneration, individual connective glial cells were destroyed by intracellular injection of protease. A week later, the axons in the connectives were severed by cutting or crushing, and the regeneration of particular axons, those of the S interneuron and mechano-sensory neurons, was followed by physiological and morphological means.

Each sensory neuron studied sends its axon down one of the two paired lateral connectives, each of which is ensheathed by a single large glial cell. In lateral connectives in which the glial cell had been killed, sensory cell axons were able to regenerate to form chemical synapses in the next ganglion with normal motor neuron targets. The success rate of regeneration was similar to that of regeneration in the presence of the glial cell, i.e.,~20% in preparations examined from 3 to 16 weeks after injury. About 60% of those sensory cells that did not regenerate, whether glial-ensheathed or not, did grow across the crush toward the next ganglion. Desheathed axons, while able to make correct synaptic connections, generally sprouted more profusely than glial-ensheathed axons.

The axon of the S interneuron lies in the medial connective, which is ensheathed by one or both connective glial cells. The axon makes an electrical synapse at its tip with the next S cell midway between ganglia in the connective. After glial destruction, the injured axon regenerated as normally to synapse with the tip of the adjacent S axon, which did not grow. By 4 or more weeks after axons were severed, they reconnected selectively with their normal targets in all but 2 of 14 preparations. One difference in the course of regeneration without glia was that some distal stumps of severed S axons, which survive for months in the presence of glia, degenerated during the first month. Thus the glial cell may help the distal stump survive.

These experiments show that glia may influence the growth of axons in the leech, since sensory axons sprout more profusely in the absence of glia, and that glia may contribute to axon stump survival, as seen for S cell stumps. However, successful regeneration of sensory axons and S cell axons can occur in the absence of the connective glial cell.

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90.1 PHENCYCLIDINE DISRUPTS SHORT-TERM SPATIAL MEMORY BY RATS IN A T-MAZE. Lynn S. Arenella* (SPON: G.J. Thomas), Center for Brain Research, Univ. of Rochester Sch. of Med., Rochester, NY 14642.

Phencyclidine (PCP) has different behavioral effects in different species. In rats, i.p. doses above 3 mg/kg produce increased motor activity, while doses between 5 and 10 mg/kg produce stereotyped behaviors and ataxia. The neurochemical bases for PCP's effects have been attributed to cholinergic, dopaminergic, serotonergic, and opiate neurons and to "PCP receptors". Glick et al. (Eur. J. Pharmacol. 59:103, 1979) concluded that PCP's impairment of spatial alternation had an anticholinergic basis because of various classes of drugs tested, only the muscarinic anticholinergics caused a similar impairment and only the concurrent administration of muscarinic agonists was able, partially, to block the effects of PCP. Because the task, lever pressing, used in the latter study involved an operant conditioning paradigm where many unknown performance factors could confound the results, the specificity of PCP's action in rats was tested on a more "truly" spatial task: contingently reinforced spatial alternation in a T-maze.

Young adult male Long-Evans rats were used as subjects. The T-maze and the behavioral procedures used in adaptation and acquisition trials were similar to those of Thomas (J. Comp. Physiol. Psych. 92:1128, 1978). All drug and saline control solutions were administered i.p. and the rats were tested in the T-maze 20 min later. In the first experiment, PCP was administered alone at the following doses: 0.25, 0.5, 1.0, 2.5, and 5.0 mg/kg. In the second experiment, PCP (5.0 mg/kg) and oxotremorine (0.2 mg/kg) were administered concurrently.

PCP administered alone disrupted contingently reinforced spatial alternation in the T-maze in a dose-dependent manner. At doses greater than 1.0 mg/kg, PCP caused a decrease in the median percent alternation. AT 5.0 mg/kg, PCP resulted in all rats (save one) performing at chance levels. Following concurrent administration of PCP and oxotremorine, the rats were not protected from the deleterious effects of PCP. For all drug sessions, the median response times were not significantly different from controls.

While this experiment does not disprove the proposed anticholinergic mechanism of action of PCP, it does show that the effects of PCP are task specific. Most experiments on the effects of PCP have used stereotyped responses. This is the first report of PCP's effect on the rat's ability to perform a complex task which presumably utilizes "higher cognitive abilities". While other explanations are possible, the results are consistent with the interpretation that the failure to perform a previously learned spatial alternation task is due to PCP's disruption of short-term spatial memory.

90.2 PHENCYCLIDINE INCREASES THE INTENSITY AND SPONTANEITY OF FIGHTING IN ISOLATED MICE. Carole Boyko-Wilmot*and Christina Vander Wende, Dept. Pharmacology, Rutgers Univ., College of Pharmacy, Piscataway, N.J. 08854.

There are numerous clinical references to a psychotic, often violent, state associated with acute and more frequently chronic phencyclidine (PCP) abuse, with a limited understanding of the neurochemical basis of this behavior. Whether the direct neurochemical effects of PCP increase the predisposition to violent behavior or indirectly release from inhibitions an inherent predisposition has not been established.

Using a pathological model of aggression, isolation-induced fighting in mice, we have examined the manner in which PCP alters the development and expression of fighting behavior. Male CF-1 mice were housed individually for 10,20, or 30 days, after which they were assigned to 4 treatment groups: no treatment, vehicle, PCP 1.25, or PCP 2.5 mg./kg.,i.p. Each mouse was tested individually for fighting with a standard stimulus mouse (olfactory bulbectomized), which does not initiate a fight or fight in return, but is an effective eliciter of aggression. Each mouse was tested only once to avoid the effects of repeated experience. The latency (LAT) in sec. to the first attack and the total fighting time (TFT) in sec. in a 10-min. interval were recorded. An acute treatment of PCP-1.25 did not alter the mean LAT in the 10-d. isolates, but did lower the mean LAT in the 20-d. and 30-d. isolates, and also significantly increased the TFT compared to the control groups. The effects of PCP-2.5 were more variable and attributed to ataxic effects impairing fighting performance. This is consistent with the increased neural sensitivity of isolates to central stimulants.

The quality of fighting under the influence of PCP showed a greater spontaneity; 33% and 50% of the fighters in the 20-d. and 30-d. isolation groups attacked the stimulus mouse in less than 10 sec., whereas only 5-10% of the control groups showed highly spontaneous fighting.

The aggression-eliciting effects of PCP were also compared between 2 groups of mice of different ages at the onset of a 30-35 d. isolation period, young sexually immature mice 35-40 d. old, and sexually mature mice 70 d. old. Again PCP-1.25 decreased the LAT and increased the TFT in both age groups compared to their respective controls. The younger isolates showed a higher spontaneity with a significantly lower LAT than the older isolates.

These results indicate that an acute PCP treatment appears to affect the spontaneity and intensity of fighting when a fighting predisposition is present.

90.3 BEHAVIORAL PHARMACOLOGY OF PHENCYCLIDINE. B.D. Greenberg* and D.S. Segal. Psychiatry Dept., UCSD School of Medicine, La Jolla, CA 92093.

The behavioral effects of phencyclidine (PCP) in rats were investigated in a series of studies. Rats were habituated individually to activity chambers for 3 days prior to s.c. administration of PCP (0.25 - 5.0 mg/kg). Food and water were available ad libitum, and injections were given during the light phase of the 12-hr light/dark cycle. A relatively complete behavioral inventory was obtained, which included continuous computer-monitored measures of locomotion (crossovers and rearings) and of duration of contact with the food grid and sipper tube. In addition, individual components of the response spectrum, including stereotyped behaviors were rated at regular intervals throughout the acute response. Phencyclidine (0.25 - 5.0 mg/kg) produced a dose-related increase in crossovers, rearings (at 5.0 mg/kg), sniffing, repetitive head and mouth movements, shuffling, ataxia, and in latency to onset and duration of eating and drinking. Phencyclidine caused dose-related decreases in crossovers and rearings during the dark phase following its acute administration. A low dose of PCP (0.25 mg/kg) given to rats not habituated to the activity chambers produced an initial decrease in behavioral measures (e.g., sniffing), followed by an increase relative to control values. Chronic administration of PCP (1.0 - 10.0 mg/kg) for 4 days caused differential changes in the response as a function of dose (e.g., crossovers progressively increased at 1.0 and 5.0 mg/kg, whereas repetitive head movements decreased at 10.0 mg/kg). The hypothesis that PCP exerts some of its effects through opioid mechanisms was examined by co-administration of PCP (1.0 and 2.5 mg/kg) and naloxone (0.5 and 10.0 mg/kg). An interaction between PCP and naloxone was found with respect to some components of the response (e.g., a delay in onset of PCP-induced eating).

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90.4 COCAINE/FIGHTING INTERACTIONS IN MICE. M. G. Hadfield, D. E. W. Mott* and E. A. Nugent*. Neuropathology Section, Dept. of Pathology, Med. Coll. Va./Va. Commonwealth U., Richmond, Va. 23298.

Last year, we reported that cocaine escalates fighting behavior in isolated male ICR mice (SN 110.8, 1980). Fight duration was increased by over 50% at 10 mg./kg. and was more than doubled at 35 mg./kg.

This year, we wish to report on follow-up studies concerned with cocaine/fighting interactions in the following situations: (i) group-housed male mice; (ii) isolated female mice; (iii) short-term or incompletely isolated male mice; (iv) Full-term isolated male mice fighting one-on-one instead of in groups of four as in last year's study; (v) A lower dose regimen in full-term isolated male mice and (vi) a high dose temporal course in full-term isolated male mice to correlate injection time with peak fighting effect. These extensive controls were instituted to help define the specificity of our drug effect on isolation-induced fighting behavior.

Drug-free group-housed males and isolated females did not fight. Moreover, cocaine did not produce fighting in these animals. It produced equivocal increases in fight duration in animals isolated for only two weeks. In fully isolated male mice fight duration was increased using just two animals as shown before with multiple animals. At doses of 5 mg./kg. or less, cocaine did not significantly alter fighting behavior in fully isolated male mice. Its peak effect after injection is noted at 1/2 hour in the latter animals.

The results indicate that cocaine enhancement of isolation-induced fighting is a specific one which occurs only at higher doses. This effect is important because most drugs inhibit fighting in this model, often by non-specific means such as sedation, etc. However, cocaine may enhance fighting by increasing general activity levels. Once fighting is precipitated, hyperactivity, coupled with cocaine's anti-fatigue properties may accelerate and sustain the fight. On the other hand, cocaine may specifically stimulate agonistic neurotransmitter systems already aroused by isolation.

Since (i) the catecholamines (CA's), in particular, have been implicated in aggressive behavior, since (ii) CA activity is increased in the brains of isolated animals and since (iii) we have demonstrated for the first time that in vivo cocaine competitively inhibits the uptake of the major central CA, norepinephrine, in cortical nerve endings (Biochem. Pharmacol. 29: 1861-1863, 1980), cocaine may increase fighting behavior in isolated animals by prolonging CA activity at the synapse.

90.5 SOME EFFECTS OF PHENOBARBITAL ON PREGNANT AND NONPREGNANT MICE. L.D. Middaugh, W.O. Boggan, T.N. Thomas and J.W. Zemp. Med. Univ. of So. Car., Charleston, SC 29425.

Perinatal exposure to phenobarbital appears to be deterimental to development. Offspring of mice and rats injected with the drug during pregnancy reportedly have neurochemical, hormonal and behavioral abnormalities. We are interested in the particular factors contributing to these abnormalities and in the present study have determined effects of phenobarbital on pregnant mice, in some cases using nonpregnant females as a control. C57BL/6J mice were used and phenobarbital was injected at doses and stages of pregnancy previously determined to be detrimental to offspring. Peak plasma concentrations produced by initial injections of 20 mg and 80 mg/kg doses 12 days after detection of a vaginal sperm plug were 4.6 µg/ml and 14.5 µg/ml, values 16% and 29% lower than for nonpregnant females. In spite of having lower plasma concentrations of phenobarbital, mice 12 days pregnant had greater drug-induced reductions in locomotor activity than noted for non-pregnant controls; and did not have the elevated activity exhibited by nonpregnant females at low drug doses. Pregnant mice also differed from nonpregnant females by having a greater increase in plasma concentration of corticosterone at one hour following the stress of saline injections. Phenobarbital prevented the stress induced increase of the steroid in nonpregnant but not pregnant mice; however, the drug did not extensively alter the normal increase of corticosterone observed during pregnancy in this species. Continued daily injections of phenobarbital (20 mg, 40 mg, 80 mg/kg) during pregnancy did not alter weight gain of dams, litter size or birth weight of pups. The results of the present study reduce the probability that growth retardation during gestation contributes extensively to the effect of maternal phenobarbital on offspring; and indicate that the reaction to stress and to phenobarbital is increased in pregnant mice, presumably due to accompanying hormonal changes. Grants DA0041 & AA03532.

90.6 PENTOBARBITAL INDUCES A NALOXONE-REVERSIBLE DECREASE IN MESOLIMBIC SELF-STIMULATION THRESHOLDS. Jules M. Nazzaro, Eliot L. Gardner, Wagner H. Bridger, Kristin R. Carlson, and Thomas F. Seeger*. Depts. of Psychiatry and Neuroscience, Albert Einstein College of Medicine, Bronx, N.Y. and Dept. of Pharmacology, University of Massachusetts Medical School, Worcester, Mass.

Site-specific increases in response rate for intracranial self-stimulation (ICSS) or decreases in the current level required to sustain ICSS have been demonstrated in rats for a variety of commonly abused drugs, including opiates, stimulants, chlordiazepoxide, alcohol, and phencyclidine. We now report the effects of sodium pentobarbital, another agent of common abuse potential, on ICSS thresholds in rats with electrodes aimed at the ventral tegmentum (A-10 DA cell body area). The testing paradigm was a two-lever rate independent threshold titration system, used in order to partially control for non-selective alterations in motor activity, arousal level, or coordination.

A low dose of pentobarbital (5 mg/kg i.p., 15 min. prior to test) did not significantly change ICSS thresholds, while a high dose (20 mg/kg) rendered the subjects too ataxic to respond reliably in the operant task. However, with a separate group of rats (N=17), a medium dose level (10 mg/kg) induced a highly significant decrease in thresholds in 16 of the subjects (average 17% below baseline saline levels, $p<0.001$), without apparent deterioration in their response capability. The concurrent administration of naloxone (2 mg/kg i.p.) with this dose of pentobarbital reversed the current decrease back towards baseline in 13 rats.

Histological examination of 16 electrode placements showed that the majority were within or just anterior to the target nucleus. Those rats which did not show naloxone-reversibility of their threshold changes demonstrated more lateral placements, primarily in the substantia nigra, pars compacta. We have previously demonstrated a similar relationship for the effects of naloxone on morphine-induced changes in ventral tegmental and substantia nigra ICSS (Pharmacology, Biochemistry and Behavior, 14:325, 1981).

The present results suggest that barbituates, in common with other drugs of abuse, induce a dose-dependent enhancement of brain reinforcement sensitivity and that these effects are mediated, in part, by endogenous opiate peptide systems.

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90.7 DRUGS OF ABUSE AND BRAIN STIMULATION REWARD: EFFECT OF NUCLEUS ACCUMBENS LESIONS. C. Muñoz* and S. Lorens, Dept. Pharmacology, Loyola University Medical Center, Maywood, IL 60153

Several compounds with abuse liability enhance responding for lateral hypothalamic (LH) self-stimulation (SS). This suggests that brain stimulation reward (BSR) can be used as a model to study the neural substrate mediating the positively reinforcing effects of abused substances. Recently, Roberts et al (Pharm. Biochem. Behav., 12: 781, 1980) reported that 6-hydroxydopamine (6-DA) lesions of the nucleus accumbens (NAS) blocks intravenous cocaine self-administration in rats. Our work was directed at determining whether 6-DA lesions of the NAS would block the enhancing effect of amphetamine (AMP) as well as morphine (MOR) and chlordiazepoxide (CDP) on responding for BSR.

Responding for LH SS was stabilized according to published procedures (Lorens, Psychopharm., 48: 217, 1976) then surgery performed. Experimental animals (n=8) received bilateral injections of 6-DA (8µg base in 2µl vehicle) into the NAS, whereas control rats (n=12) received either vehicle injections or sham operations. Beginning 6 days post-op, the animals were tested daily in 10 min sessions. D-amphetamine sulfate was administered once every 3-4 days between post-op days 14 and 29 in doses of 0, 0.125, 0.25, 0.5, 1.0 and 2.0 mg/kg, i.p. as the base. CDP (0,4 and 8 mg/kg) was administered similarly between post-op days 32 and 38. On drug days, the rats received a 10 min predrug control session then were tested 10 min at 30 min post-injection. Finally the rats received saline then daily injections of morphine sulfate (5mg/kg, s.c. as the base) and their rates of responding 1, 3 and 5 h post-injection compared to predrug control. The animals were sacrificed 48-50 days post-op.

The 6-DA lesions significantly reduced (64%) dopamine and DOPAC concentrations in the NAS and olfactory tubercle. Responding for BSR post-op did not differ between the lesion and control group. The dose-response curve for AMP was significantly attenuated in the lesion animals. However, the enhanced responding produced by CDP and MOR was unaffected.

These data suggest that the mesolimbic dopaminergic projections to the NAS and surrounding structures may mediate the rewarding property of amphetamine, but not the rewarding properties of CDP and MOR.

90.8 SELF-INJECTION OF NICOTINE IN RATS. W. T. Nelson,Jr. and Brian Cox. Addiction Research Foundation, Palo Alto, CA 94304.

Female Wistar rats (250-300 g) with jugular cannulae were tested 23 hours a day in a paradigm of one week pre-drug saline, two weeks nicotine, and two weeks post-drug saline. Two response bars were available. Each press on the active bar delivered either saline or nicotine (3, 10, or 30 ug/kg) in an infusion of 100 ul/kg. Bar-presses on the control bar had no consequence and served as a control for nicotine's locomotor stimulant effects at these doses. In the 30 ug/kg group of seven rats, response rates on both bars were elevated in the nicotine tests and returned to pre-drug saline levels in the post-drug saline tests. A dim cue light over the drug bar failed to improve the rats' discrimination between the bars. Rats tested in operant chambers twice the standard size also showed no better discrimination than those tested in the standard size chambers.

Three of seven rats self-administered the 10 ug/kg dose above saline levels. Two of these rats selectively responded at higher rates on the drug bar, with unchanged rates on the dummy bar. Preliminary data on three rats indicate that 3 ug/kg has no effect on baseline responding in drug-naive animals. A group of six rats tested for five continuous weeks on saline showed no changes in response rates over time.

Another group of seven rats was tested in a paradigm of one week pre-drug saline, one or two weeks nicotine (30 ug/kg), one week nicotine (10 ug/kg), and two weeks post-drug saline. When the dose of nicotine was lowered, response rates increased on the drug bar and decreased on the dummy bar, but neither rate change was statistically significant compared to the prior week of nicotine 30 ug/kg. Experiments are now in progress in which the dose of nicotine is lowered from 30 to 3 ug/kg in the same paradigm as above. Preliminary data in three rats indicate that when the dose of nicotine was lowered, response rates significantly increased on the drug bar, with no change in rates on the dummy bar.

These studies demonstrate that rats will self-administer nicotine in the absence of any other coupled appetitive drive, such as hunger induced by food deprivation. The results also emphasize the importance of controlling for motor activity effects of the drug in studies of nicotine self-administration.

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90.9 OPIATE AND STIMULANT INTRAVENOUS SELF-ADMINISTRATION IN RATS: MEDIATION BY SEPARATE NEURAL SYSTEMS. A. Ettenberg, G.F. Koob, H.O. Pettit*, and F.E. Bloom. Arthur Vining Davis Center for Behavioral Neurobiology, The Salk Institute, San Diego, California, 92138.

Recent reports have suggested that the reward produced during self administration of different psychoactive drugs might be mediated by a single common pathway, such as a dopamine or an endogenous opioid peptide system. The present study was devised to examine these possibilities by observing the effects of both the dopamine receptor antagonist drug alpha-flupenthixol and the opiate antagonist drug naltrexone on the self administration of either cocaine or heroin in rats.

Male albino rats implanted with jugular catheters were permitted to lever press for either cocaine (0.75 mg/kg/infusion) or heroin (60 ug/kg/infusion) during daily 3h sessions. On test days, animals were pretreated with i.p. injections of either naltrexone (0.01, 0.05, 0.1, 0.2, 1.0, or 10.0 mg/kg), alpha-flupenthixol (0.01, 0.5, 0.1, 0.2 or 0.4 mg/kg) or saline. For some animals the effects of these treatments were subsequently observed during drug reinforced responding while others were tested during a single extinction (i.e. no reinforcement) session. The dopamine antagonist (at doses below 0.2 mg/kg) produced dose-dependent increases in lever-press rates for cocaine but had no effect on the response rates (or pattern) of animals self administering heroin. Similarly, opiate receptor antagonism produced dose-dependent increases in responding for heroin without affecting responding for cocaine (even at the highest dose). The high doses of alpha-flupenthixol (0.2 and 0.4 mg/kg) either markedly reduced or completely eliminated all responding in both the cocaine and heroin conditions. There was, however, no evidence that the pattern of response disruption at these doses produced behaviors resembling the pattern of behavior observed during extinction. In addition, alpha-flupenthixol but not naltrexone, markedly attenuated the response rates of animals that were tested under conditions of extinction where the drug-reinforcement was withheld.

These data suggest that separate neural systems mediate the rewarding consequences of cocaine and heroin self administration. Dopamine receptors appear to be involved in cocaine but not opiate reinforcement with the opposite being the case for opiate receptors. These data also support the contention that high doses of neuroleptics, in addition to their effects on reward, may also produce a generalized or nonspecific disruption of response performance.

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91.1 HORSERADISH PEROXIDASE DEMONSTRATION OF THE AFFERENT AND EFFERENT CONNECTIONS OF THE SUBFORNICAL ORGAN. D.A. Wheeler* and J.B. Simpson (SPON: R.H. Lovely). Department of Psychology, University of Washington, Seattle, WA 98195.

The subfornical organ (SFO), located on the rostral wall of the third ventricle at the level of the intraventricular foramen, has been shown to be involved in the central control of body fluid homeostasis. Knowledge of the connections of the SFO is a prerequisite to understanding its involvement in body fluid regulation. However, the neural connections of the SFO are still incompletely known (Miselis et al., Sci. 205: 1022, 1979).

Retrograde and anterograde transport of horseradish peroxidase (HRP) was used to locate the afferent and efferent connections of the SFO. Injections of 40-60 nl of 200 μg/μl HRP (Sigma Type VI) were made through a 31 ga cannula stereotaxically placed in or adjacent to the SFO. Injections often included portions of the ventricle fornical commissure adjacent to the SFO. The animals were sacrificed at times ranging from 19 h to 42 h post-injection. The perfusion procedure of Rosene and Mesulam (J. Histochem. Cytochem. 26: 28, 1978) and the tetramethyl benzidine procedure of Mesulam (J. Histochem. Cytochem. 28: 1255, 1980) were used to visualize the transport of the HRP.

Afferents to the SFO from the preoptic area were shown from: the organum vasculosum of the laminae terminalis; nucleus medianus; and the lateral, medial, and paraventricular preoptic nuclei. Afferents to the SFO from the septum were shown from: the medial, lateral, fimbrial, and triangular septal nuclei; the nucleus of the diagonal band; and the bed nucleus of the stria terminalis. Hypothalamic afferents to the SFO were shown from: the periventricular, paraventricular, anterior, and lateral hypothalamic nuclei. The anterior dorsal, anterior ventral, anterior medial, medial, ventral, lateral, and reticular thalamic nuclei were shown to have afferent connections to the SFO. Additionally, the anterior continuation of the hippocampus and the indusium gresium had afferents to the SFO.

Efferent connections from the SFO were shown to: the organum vasculosum of the laminae terminalis; nucleus medianus; the medial, lateral, fimbrial, and triangular septal nuclei; the periventricular and supraoptic hypothalamic nuclei; and the anterior dorsal thalamic nucleus.

The demonstrated connections may be parts of the neural substrate for the control of body fluid homeostasis subserved by the SFO and the other nuclei connected with it.

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91.2 HYPERTONIC SALINE- AND ANGIOTENSIN-INDUCED DRINKING IS BLOCKED BY DESTRUCTION OF DORSAL NUCLEUS MEDIANUS. M.L. Mangiapane, T. Gardiner* and J.B. Simpson. Dept. of Physiol., U.C. San Francisco, CA 94143.

Several structures bordering the third ventricle have been implicated in the regulation of drinking, including the organum vasculosum (OVLT), subfornical organ (SFO), and anteroventral third ventricle (AV3V). Also in this region is the nucleus medianus (NM), a midline structure wrapped vise-like about the anterior commissure (AC), roughly equidistant from OVLT and SFO. NM receives input from SFO, and is one of several structures included in the AV3V lesion. In view of its central location in a region so strongly implicated in the control of fluid balance, we made lesions of the NM and assessed their effect on hypertonic saline- and angiotensin II (AII)- induced drinking. With 127μ tungsten electrodes, small electrolytic lesions were made in male Long-Evans rats. Using 7 millicoulombs x 3 penetrations, we destroyed only that portion of NM dorsal and anterior to AC. Ventral nucleus medianus and periventricular AV3V tissue (including OVLT) were undamaged. Control lesions were placed in lateral septum and diagonal band. One week later, animals were tested for drinking to s.c. injections of hypertonic saline or AII. The hypertonic saline dose (3.33 ml/kg, 2M) raised plasma osmolality by 3%; the AII dose (1.5 mg/kg) was chosen to replicate studies by others on the AV3V lesion syndrome of adipsia and deficits in drinking to AII.

Destruction of the dorsal-anterior NM (DNM) almost totally blocked drinking to hypertonic saline (1.2 ± 0.6 ml vs. 5.6 ± 0.7 ml for control-lesion rats, $p < .001$). Drinking to AII was significantly reduced (2.6 ± 1.7 ml vs. 7.8 ± 1.2 ml for control-lesion animals, $p < .05$). In addition, latencies to drinking were significantly longer in DNM-ablated rats ($p < .05$) given either dipsogen. With ad-lib drinking intact, the observed blockade of drinking to AII and hypertonic saline in DNM-ablated rats distinguishes them from SFO-ablated animals (no drinking to AII) and from AV3V-ablated animals (adipsia + deficits to both dipsogens). While the DNM region appears necessary for drinking induced by these two dipsogens, fibers of passage from the actual receptors involved may have been destroyed in this lesion. These are clearly not mutually exclusive explanations for the results.

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91.3 DIPSOGENIC ADDITIVITY IN RODENT SPECIES WITH DIFFERING CENTRAL ANGIOTENSIN II BINDING CAPACITY. J. W. Wright, S. L. Morseth* and J. W. Harding. Departments of Psychology and Veterinary Comparative Anatomy, Physiology and Pharmacology, Washington State University, Pullman, WA 99164.

Cellular dehydration and hypovolemia are currently believed to activate separate receptor mechanisms responsible for the control of drinking. This dual detection hypothesis predicts that elevated effective osmotic pressure of body fluids and decreased intravascular volume, which stimulates renin release which in turn converts angiotensinogen into angiotensin in the circulation, exert additive effects upon water consumption. In the present series of experiments Sprague-Dawley rats, Mongolian gerbils, Golden Syrian hamsters and the South American rodent Octodon degus were initially tested with two treatments known to facilitate the release of renin in rats, viz. polyethylene glycol (PG) and isoproterenol (ISOP), plus the osmotic stimulus, hypertonic saline (NaCl). All species were responsive to NaCl. Rats were dipsogenically responsive to PG and ISOP treatments while degus and hamsters were maximally responsive to PG. Gerbils were unresponsive to PG but did drink following ISOP injection.

On the bases of these results either ISOP or PG, which ever was maximally effective, was paired with NaCl in a test of dipsogenic additivity in additional members of each of the four species. Rats revealed additivity when PG and NaCl were paired and also when ISOP and NaCl were paired. Gerbils failed to show additivity with ISOP + NaCl. Hamsters also failed to demonstrate additivity with the pairing of PG and NaCl. Degus did show additivity with PG + NaCl.

These results were not predicted on the bases of central angiotensin II (AII) receptor binding. The gerbil revealed binding only in the olfactory bulb while the degu indicated no binding anywhere in the brain. The rat and hamster indicated high levels of binding throughout many brain structures (Harding, J. W., Stone, L. P. and Wright, J. W. Brain Research, 205:265, 1981). In order to explain these results we hypothesized that gerbils and degus may possess angiotensin III (AIII) brain receptors. If this is the case these species would be expected to respond to the intracerebroventricular infusion of AIII. Gerbils prepared with chronic cannulas were found to drink vigorously in response to 5 and 50 ng AIII. We are presently extending this evaluation to the other three species.

91.4 LACK OF PENETRATION OF ANGIOTENSIN II INTO THE CEREBROSPINAL FLUID FOLLOWING INTRAVENOUS INFUSION. S.P. Frankmann*, I.A. Reid, and J.B. Simpson. (SPON: J.S. Lockard). Dept. Psychol., Univ. Washington, Seattle, WA 98195 & Dept. Physiol., UCSF, San Francisco, CA 94143.

Angiotensin II (AII) in the circulation is normally excluded from the cerebrospinal fluid (CSF) by the blood-CSF barrier (BCB). A question then arrises: do intraventricular injections of AII, which provoke various effects, mimic the central effects of circulating AII? Acute hypertension causes a transient disruption of the BCB, possibly allowing passage of normally excluded molecules from the blood into the CSF. Under certain pathophysiological states, such a systemic administration of large quantities of AII or renal hypertension, AII may cross the BCB. We measured quantities of AII reaching the CSF during acute hypertension induced by high circulating levels of AII.

Saline vehicle or AII doses of 1, 10, 100, or 1,000 ng/min were infused via jugular vein in anesthetized 300 gm rats. CSF from the cisterna magna and blood from the descending aorta were collected after a 30 min infusion (which continued during collection). The collection procedures took 8 min or less. Plasma and CSF AII concentration was measured by radioimmunoassay. Mean arterial pressure was monitored.

Blood pressure exhibited a dose-dependent increase up to the asymptotic AII dose of 100 ng/min. Out of 36 animals infused with AII, including 20 cases with plasma values greater than 1200 pg/ml, only 5 had values greater than the limit of detection of the assay of 100 pg/ml. In no case was the CSF value greater than 140 pg/ml. In contrast, a 10 ng injection of AII into the lateral ventricle (n=10) produced cisternal CSF concentrations of 2,652 pg/ml, 2 to 10 min later.

Thus, in some instances small quantities of AII may cross the BCB and appear in the CSF. These data suggest, however, that circulating AII does not normally enter the CSF in appreciable quantities, and question the physiological significance of intraventricular injections of AII.

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91.5 ALTERATION OF DRINKING BEHAVIOR BY TRANSPLANTATION OF KIDNEY CORTEX TO THE RAT BRAIN. J.M. Morihisa,* H.E. Cannon-Spoor,* R.J. Wyatt, and W.J. Freed (SPON: A.P. Oliver). Adult Psychiatry Branch, National Institute of Mental Health, Washington, D.C. 20032.

Because of its special immunological characteristics, grafts placed in the brain are unlikely to be rejected. These favorable properties of the brain as a site for transplantation have recently been employed to produce grafts which retain some of their original function. In the present experiment we investigated the possibility that drinking behavior could be altered by increasing the production of the proteolytic enzyme renin, through transplantation of kidney cortex to the rat brain. Intracranial injections of renin stimulate drinking in the rat (Epstein, A.N., et al., J. Physiol. 210:457, 1970) and the cat (Brophy, P.D. and Levitt, R.A., Pharmacol. Biochem. Behav. 2:509, 1974). Thus, if grafts of kidney cortex survive and continue to produce renin, they would be expected to cause increases in drinking behavior.

Forty-four Sprague-Dawley male rats were housed individually in wire-mesh cages with free access to food and water. Some of these animals received grafts of pieces of kidney cortex from one-month old donors into both lateral ventricles. Control animals received bilateral injections of lactated Ringer's or no operation. Water intake was then recorded for a period of one to two months.

The rats with grafts of kidney cortex showed a transient two-fold increase in drinking, which was apparent immediately after grafting but returned to control levels within two weeks after the operation. Histological studies revealed surviving grafts in all of the animals, with cells and structures similar to those of typical kidney cortex.

Intracerebral grafts of renin-producing tissue cause elevated drinking behavior as do intracerebral injections of renin and angiotensin. Thus, cortical kidney grafts survive in the brain and transiently retain some of their functional characteristics. We are now attempting to determine whether the return to baseline drinking levels is due to adaptation of the brain to continuously elevated renin levels or to a loss of ability of the grafts to produce renin. The possibility of using the brain as a site for enzyme producing homografts to effect physiological changes is partially supported by these data.

91.6 STUDIES OF ANGIOTENSIN IN PRIMATES. E.P. Petersen*, J.W. Wright, and J.W. Harding. Department of Veterinary and Comparative Anatomy, Pharmacology and Physiology, and Psychology, Washington State University, Pullman, WA 99164.

Angiotensin II (AII) is an octapeptide that has a major role in maintaining body water hemeostasis and insuring adequate tissue perfusion in times of vascular volume loss. The mechanisms by which AII accomplishes its function include both peripheral and central nervous system mediated events. Present concepts concerning the action of AII in primates have been extrapolated primarily from rodent studies. Recent studies in our laboratory have highlighted many interspecies differences in AII systems and mechanisms of body water maintenance. This has stimulated a more rigorous direct examination of primates. African green monkeys were found to respond to peripherally injected AII or AII-dependent stimuli by increasing water intake. In addition to this central effect, the peripheral release of aldosterone from the adrenal cortex was also noted. These results support the notion that AII in primates exerts both central and peripheral effects. Classically it is possible to envision these effects as being mediated through the interaction of AII and cell surface receptors. Because of the diverse actions initiated by AII, biochemical examination of AII receptors at different sites seemed warranted. Such a study, which measured ^{125}I-II binding, indicated the presence of different receptor types in brain, uterus, and adrenals. These receptors exhibited differences in metal ion requirements as well as differences in ligand specificity. In contrast to previous studies on rats, it was of particular interest to note the superior binding potency of angiotensin III (AIII), a naturally occurring AII derivative. This finding provides the initial suggestion that AIII, not AII, is the active peptide in primates. A second important finding that casts doubt on the use of rodents to predict AII characteristics in primates was a difference in brain region distribution of AII binding in the monkey as compared to rat.

92.1 EFFECTS OF CHRONIC AMPHETAMINE ON BLOOD, BRAIN AND ADRENAL CONCENTRATIONS OF TRYPTAMINE AND TETRAHYDRO-β-CARBOLINE: BEHAVIORAL IMPLICATIONS. R.E. Harrison, S.T. Christian, P. Hyde and G.V. Pegram, Neurosciences Program, Univ. of Alabama in Birmingham, Birmingham, Al. 35294.

Recent investigations from this laboratory have indicated that psychological stress will alter tryptamine (TA), N-methylated indole, and tetrahydro-β-carboline (THBC) content in rat brain and adrenal glands. Continuous administration of amphetamine is known to alter brain indole metabolism and gives rise to a behavioral syndrome similar to that produced by hallucinogens. Stress or amphetamine will cause a progressive enhancement (sensitization) of a similar behavioral syndrome. These phenomena might suggest that stress- and amphetamine-associated behaviors act through a common mechanism involving TA and its metabolites. To examine this question further, rats were subjected to a regimen of either saline or 3 mg/kg q.i.d. d-amphetamine x 8 days. Animals from both the control and amphetamine groups were sacrificed every two days. The blood, brain and adrenal TA, dimethyltryptamine (DMT), 5-methoxydimethyltryptamine (5-MeOT), 6-methoxytetrahydro-β-carboline (6-MeOTHBC) and THBC levels from these animals were measured with isotope-dilution gas chromatography/mass fragmentography. Additional assessments were made of heart rate, blood pressure, stereotypy and open-field behavior. In these experiments it was shown that 1) d-amphetamine caused increases in blood, brain and adrenal TA and THBC, but the increase in blood and adrenal TA and THBC concentration was greater than in the brain; 2) the increases in TA and THBC content of any tissue in rats given amphetamine were not dosage dependent; 3) the blood pressure was indirectly proportional ($r=-.911$) to the concentration of THBC in blood, but only in animals treated with d-amphetamine; 4) multiple injections of d-amphetamine produce sensitization phenomena during the initial portion of the amphetamine response. It is proposed that TA and THBC are involved in behavioral and physiological modifications resulting from amphetamine administration.

92.2 SHIFT FROM AMPHETAMINE-INDUCED INHIBITION TO EXCITATION IN THE NEOSTRIATUM, BUT NOT IN THE NUCLEUS ACCUMBENS, FOLLOWING LONG-TERM TREATMENT. Kevin D. Alloway and George V. Rebec. Dept. Psychol., Indiana Univ., Bloomington, IN 47405.

Acute administration of amphetamine produces a dose-dependent shift in neuronal activity in the neostriatum, but not in the nucleus accumbens. Whereas low doses inhibit the firing rate of neurons in both brain regions, high doses of amphetamine inhibit the nucleus accumbens but excite neostriatal activity. This shift in the firing rate of neostriatal neurons appears to parallel the amphetamine-induced dose-dependent transition from forward locomotion to focused stereotypy (Rebec and Zimmerman, Brain Res., 1980, 201: 485). Since the time-course and intensity of the amphetamine behavioral response are significantly altered with long-term treatment (eg., Rebec and Segal, Pharmac. Biochem. Behav., 1980, 13: 793), we extended our electrophysiological analysis to include the effects of repeated amphetamine injections on neuronal activity in these sites.

Adult, male rats received intraperitoneal (ip) injections of saline, 1.0 or 5.0 mg/kg d-amphetamine twice daily for six consecutive days. The animals were immobilized and locally anesthetized on the following day and single unit activity was recorded simultaneously from the neostriatum and nucleus accumbens. Challenge doses of amphetamine were injected via an in-dwelling ip catheter.

In saline pretreated rats, amphetamine produced a dose-dependent biphasic effect on neurons in the neostriatum. Thus, whereas the predominant response to 1.0 mg/kg d-amphetamine was an inhibition of firing rate, neurons responded to 5.0 mg/kg with either an inhibition or an excitation. Following chronic amphetamine treatment, both doses produced a significant increase in neostriatal activity compared to saline pretreated controls. Neurons in the nucleus accumbens, however, were mainly inhibited by 1.0 or 5.0 mg/kg d-amphetamine in both saline and amphetamine pretreated animals. In fact, no differences were apparent in either the duration or intensity of this response following chronic amphetamine administration. These results suggest that the neostriatum, rather than the nucleus accumbens, is responsible, at least in part, for the behavioral alterations that accompany long-term amphetamine treatment.

This research was supported by USPHS Grant DA-02451-03 from the National Institute on Drug Abuse.

92.3 EFFECTS OF SYSTEMIC TRYPTOPHAN LOADING ON CENTRAL SEROTONIN METABOLISM IN RATS FOLLOWING CHRONIC METHAMPHETAMINE ADMINISTRATION. Violet M. Trulson* and Michael E. Trulson (SPON: R. Stillman). Dept. Psychol., Univ. of Texas at Dallas, P.O. Box 688, Richardson, TX, 75080.

The various behavioral and physiological effects of amphetamine are thought to be mediated primarily through an action on brain catecholamines. Several recent studies, however, have focused on the serotonergic effects of amphetamine. Acute administration of amphetamine increases the release and turnover of serotonin (5HT), while chronic drug administration produces a decrease in central 5HT metabolism (Trulson and Jacobs, J. Pharm. Exp. Ther. 211: 375-384, 1979). This latter effect appears to be mediated, at least in part, by a decrease in the activity of tryptophan hydroxylase (TPH), the rate limiting enzyme in 5HT biosynthesis (Trulson and Jacobs, Life Sci. 26: 329-335, 1980). To more fully explore the serotonergic effects of amphetamine treatment, in the present study we investigated the effects of chronic administration of methamphetamine (METH) on the kinetics of TPH and examined the effects of tryptophan (TP) loading on 5HT metabolism following chronic METH treatment. Adult male rats were injected with either METH HCl (20 mg/kg, i.p.) or saline (SAL, 1.0 ml) every 12 hr for 6 consecutive days, and were killed for TPH assays (using the method of Gal and Patterson, Anal. Biochem. 52: 625-629, 1973) 12 hr after the final injection. Additional groups of rats received a single injection of either METH (20 mg/kg) or SAL and were assayed 12 hr later. Chronic METH treatment produced a significant decrease (-32%) in the V_{max} of brain TPH, while a single injection of the drug had no significant effect. Neither acute nor chronic METH administration produced any significant changes in the K_m of TPH for either TP or tetrahydrobiopterin. Chronic METH treatment also produced significant decreases in brain 5HT (-33%) and 5HIAA (-27%), while brain TP levels were not significantly changed. TP loading (50 mg/kg, i.p.) resulted in similar % increases in 5HT (31 & 34%) and 5HIAA (42 & 47%) levels in SAL and METH pretreated groups. Thus, 5HT and 5HIAA returned to normal levels following TP loading in the METH pretreated group, and were elevated above normal levels in the SAL group. Chronic METH administration decreased brain dopamine (-45%) and norepinephrine (-42%), and the catecholamines were not significantly changed by TP loading. The fact that only the V_{max} of TPH is changed by chronic METH administration, with no change in the affinity for either substrate or cofactor, suggests that there are either fewer enzyme molecules present or fewer active sites per enzyme molecule. The remaining 5HT biosynthetic machinery, however, appears to respond normally to TP administration. Therefore, it may be possible to restore central 5HT function to normal following chronic METH administration by systemic TP loading.

92.4 **WITHDRAWAL FROM CONTINUOUS APOMORPHINE: A NOVEL HALLUCINOGEN.** **R. E. Davis, W. W. Sant and G. Ellison. Department of Psychology, University of California, Los Angeles, CA. 90024**

Chronic, continuous amphetamine (D-AMP) administration induces a behavioral syndrome consisting of limb flicks, wet dog shakes and other abnormal behaviors. These behaviors are seen after acute administration of hallucinogens and are not seen following the acute administration of catecholamine agonists in rats. The appearance of these behaviors is correlated primarily with alterations in the metabolism and receptor sensitivity in the dopaminergic systems of the neostriatum (NS) and nucleus accumbens (NA). To further clarify the role of dopaminergic mechanisms in the appearance of these "hallucinogen-like" behaviors, we developed techniques for chronic, continuous administration of the selective dopamine (DA) agonist, apomorphine (APO).

Twenty-four male, albino rats were implanted subcutaneously either with a silicone pellet containing 25 mg of APO base (N=16) in PEG or one containing PEG (N=8). This pellet produces a mean dosage of 1.0 mg/kg/hr in 400 g animals. Since APO oxidizes rapidly, a new pellet was implanted 24 hrs after the first implantation. Forty-two hrs after the first implant, all animals were removed from their home cages and were placed in observation cages. One-half of the animals receiving APO had their pellets removed 4hrs prior to placement in the observation cages. The frequency and duration of 18 behavior classes were scored continuously for the next 30 mins. These behavior classes can be divided into 3 subcategories: 1) normal behaviors (e.g. grooming); 2) "hallucinogen-like" behaviors (e.g. limb flicks); and 3) motor dyskinesias (e.g rapid jaw movements). Parallel groups of animals given identical treatment as above were prepared for simultaneous analysis of DA, DOPAC, HVA, 5-HT and 5-HIAA in 3 DA rich regions (NS, NA and ventral tegmentum, VT) using HPLC with electrochemical detection.

Animals so treated did not exhibit stereotyped behavior at any time nor did they demonstrate "hallucinogen-like" behavior in their home cages. When these animals were removed from their home cages and were placed in novel environments, they initially exhibited a burst of "hallucinogen-like" behaviors. These behaviors gradually subsided during the observation session being replaced in later parts of the session by intense motor dyskinesias (rapid jaw movements and respitory tics). These behavioral changes were accompanied by increases in the levels of DA and HVA in the NS but not the NA or VT. In contrast, 5-HT levels were elevated in the NA and VT but not in the NS while 5-HIAA levels were elevated in all 3 areas.

92.5 EFFECTS OF CONTINUOUS LOW-LEVEL d-AMPHETAMINE ADMINISTRATION ON VOLUNTARY ALCOHOL CONSUMPTION. Allen D. Potthoff* and Gaylord D. Ellison (SPON: J. T. Cannon). Department of Psychology, U.C.L.A., Los Angeles, CA 90024.

Rats given subcutaneous implants of pellets which released 33 mg of d-amphetamine (d-AMP) continuously over a 30 day period increased voluntary intake of 10% v/v ethanol (EtOH), from a baseline mean of 15 cc/rat/day to a mean of 24 cc/rat/day during d-AMP treatment. Choice of 10% v/v EtOH solution increased from a pre-treatment group mean of 42 % of total fluid intake to 69% of total fluid intake during d-AMP treatment. Neither rats drinking anise-water or a solution isocaloric to 10% v/v EtOH given continuous d-AMP pellets, nor rats drinking 10% v/v EtOH and given daily injections of the same amount of d-AMP showed increases in flavored fluid intake comparable to the original group. Rats withdrawn from drinking 10% v/v EtOH for six days also exhibited less of an increase in EtOH intake than the original group. Behaviorally, rats treated with d-AMP pellets showed enhanced motor activity. Daily i.p. injections of 0.25 g/kg EtOH were increasingly effective in reducing the enhanced motor activity. It is hypothesized that the continuous low-level administration of d-AMP models the type of metabolic imbalance present in cases of chronic stress and that ethanol antagonizes the effects of the continuous d-AMP. Therefore, the d-AMP pellet treated rats increased ethanol intake as a means of self-medication. (Supported by AA02969)

92.6 HALLUCINOGEN-LIKE EFFECT OF CONTINUOUS AMPHETAMINES ON STARTLE. W. W. Sant*, R. Davis, and G. Ellison (SPON: D. Glanzman). Dept. Psychol., UCLA, Los Angeles 90024

Nielsen et al. (Psychopharm., 68) reported that an acute injection of d-amphetamine (d-Amp) decreased the frequency of behaviors typically elicited by hallucinogens, but these behaviors were increased when d-Amp injections are given 12 hrs following 4 1/2 days of continuous low levels d-Amp.

It has been shown that while both hallucinogens and d-Amp increase acoustic startle response amplitude (ASRA), they do so in a different fashions. D-amp increases ASRA by decreasing habituation and increasing sensitization to repeated tones, while the hallucinogen LSD increases the sensitization effects of background white noise on ASRA. Exposing rats to white noise preceding and during the presentation of startle-eliciting tones increases the magnitude of the rat's response to each tone (increasing ASRA). This effect is augmented by acute hallucinogen administration but not by acute d-Amp administration.

Male Sprague-Dawley rats were pretreated with the d-Amp or vehicle pellet for 4 1/2 days. The pellets were then removed and 12 hrs later animals were administered either d-Amp or vehicle and placed immediately into the startle apparatus and exposed to white noise for 15 minutes prior to the onset of a series of startle-eliciting tones. The result observed was that with as with LSD administration, ASRA was greater for the first tones in the series in the animals pretreated with d-Amp pellets and then administered d-Amp than in similarly pretreated animals administered vehicle. This effect stood in stark contrast to vehicle pellet pretreated animals, where the effect of d-Amp was to decrease ASRA on the first tones of the series. (supported by DA 02312)

92.7 CHRONIC STIMULANT ADMINISTRATION RESULTS IN INCREASED ALCOHOL AND BENZODIAZEPINE DRINKING IN RATS. Linda R. Nelson, Allen D. Potthoff*, and Gaylord D. Ellison. Dept.of Psychology, UCLA, Los Angeles, CA 90024.

Previous investigations in our lab have demonstrated that after implantation of continuous slow-release d-amphetamine (d-AMP) pellets, rats with free access to water and 10% v/v ethanol (EtOH), selectively increase their consumption of the EtOH solution over baseline levels. We have hypothesized that this represents a form of self-medication, such that the rats increase their alcohol intake to counteract the stimulatory effects of amphetamine. In order to further explore this hypothesis, we have varied both the drug solution available for drinking, and the drug chronically administered via implant.

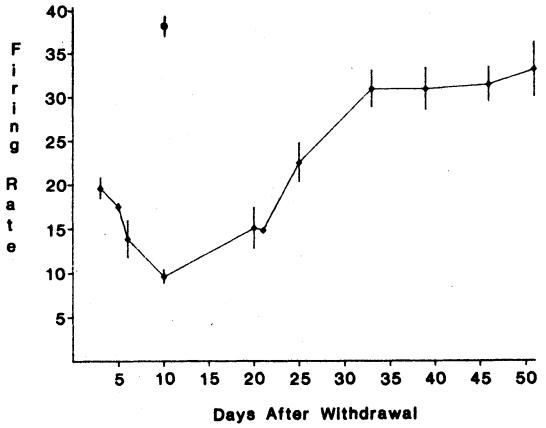
When a benzodiazepine solution (diazepam - .060 mg/ml), was substituted for the 10% EtOH, similar results were obtained. After d-AMP pellet implantation, diazepam intake rose from 25% to 40% of total fluid intake.

The following substances were administered in a chronic slow-release manner in place of d-AMP and resulted in no increase in EtOH consumption: caffeine, phencyclidine (PCP), LSD, mescaline, haloperidol, and secobarbital. However, rats treated with continuous slow-release nicotine increased their ethanol drinking from 39% to 76% of total fluid intake.

These results extend the self-medication phenomena to another stimulant: nicotine, and another depressant/anxiolytic: diazepam. Future investigations will search for neurochemical alterations produced by the chronic stimulant administration and their possible attenuation by the concomitant ingestion of alcohol or anxiolytics. (Supported by AA02969)

92.8 EFFECTS OF WITHDRAWAL FROM CHRONIC AMPHETAMINE ON SPONTANEOUS PURKINJE NEURON DISCHARGE IN RAT CEREBELLUM. S.M. Sorensen, S. Johnson* and R. Freedman. Univ. of Colorado Health Sciences Center, Denver, CO 80262.

The spontaneous discharge of rat cerebellar Purkinje (P) cells, known to be controlled by the β-adrenergic (NE) input from the locus coeruleus was studied in animals after withdrawal from chronic treatment with amphetamine (2 mg/kg per day x 21 days). Discharge rates in withdrawn animals remained significantly lower than those of controls for up to 50 days.



Disruption of central adrenergic transmission by the β antagonist propranolol or the NE-depleting agent reserpine partially restored discharge rate. P cells from amphetamine-withdrawn rats were not further depressed by acute administration of amphetamine, whereas P cells from control animals were markedly slowed. Moreover, P neurons from amphetamine withdrawn rats manifested a small subsensitivity to locally applied NE. Taken together, these data suggest that amphetamine withdrawal is associated with a long-term increase in the activity of the noradrenergic input to cerebellar cortex. (Supported by USPHS Grant # DA-02429 and NS-09199).

92.9 EFFECTS OF CHRONIC AMPHETAMINE ADMINISTRATION ON LOCAL CEREBRAL GLUCOSE UTILIZATION IN THE RAT. D. Dow-Edwards, F. Orzi*, J. Jehle*, C. Kennedy, and L. Sokoloff. Lab. of Cerebral Metabolism, NIMH, Bethesda, MD 20205.

Amphetamine, a dopaminergic stimulating agent, has many biochemical and behavioral effects when administered to rats. The effects of acute amphetamine administration on local cerebral glucose utilization (LCGU) have been described (Wechsler, et al., J. Neurochem.32:15-22, 1979). Components of the extrapyramidal motor system as well as those of other dopaminergic systems showed increased metabolic rates following i.v. administration of 5 mg/kg of d-amphetamine. In the present studies we have reproduced these earlier findings and have also examined the effects of chronic d-amphetamine administration on LCGU in conscious rats. There were two methods of administering the amphetamine chronically. Two groups of rats received daily i.p. injections of either 5 mg/kg for 2 weeks or increasing doses from 5 mg/kg to 15 mg/kg for 3 weeks. Two other groups of rats were implanted with osmotic pumps (Alzet, Alza Co.) designed to release amphetamine continuously for either 1 week or 2 weeks. The dosage approximated 15 mg/kg/day for the one week treatment and 5 mg/kg/day for the two week treatment. A fifth group of rats received an acute dose of 5 mg/kg i.v. after two weeks of i.p. saline injection. A sixth group of rats received the same acute dose after 2 weeks of receiving daily i.p. injections of 5 mg/kg drug.

At the end of the drug administration or 20 minutes following the acute injection, local cerebral metabolism was studied by the [^{14}C]deoxyglucose method. Forty-nine brain regions in 30 rats were examined autoradiographically. The acute treatment resulted in significant increases in glucose utilization in several regions including the substantia nigra, caudate nucleus, several thalamic nuclei, the subthalamic nucleus, and the red nucleus. The metabolic rate in the lateral habenula was significantly decreased. Pretreatment with daily injections followed by a single acute dose of amphetamine produced similar but enhanced effects. Daily injections of amphetamine with no acute injection (a period of 6 hours was allowed between the last injection and the initiation of the deoxyglucose method) resulted in no statistically significant increases in LCGU. Continuous administration for both 1 and 2 weeks via the osmotic pump, however, did significantly increase local metabolic rate in the n. accumbens. This increase was not seen following acute or daily i.p. injections of amphetamine. This is, to our knowledge, the first demonstration of an effect of d-amphetamine on metabolism in any component of the mesolimbic system.

93.1 NEURAL CIRCUITRY FOR MATE CALLING IN MALE SOUTH AFRICAN CLAWED FROGS, XENOPUS LAEVIS. D. M. Wetzel, and D. B. Kelley. Dept. of Psychology, Princeton Univ., Princeton, NJ 08544.

Male Xenopus laevis produce a distinctive mating call during their breeding season. The aim of these experiments was to establish the neural circuitry involved in mate calling. The larynx is the structure responsible for the production of calls in frogs. Previous work in our lab has shown that the motor neurons controlling the laryngeal musculature are located in cranial motor nucleus IX-X (n.IX-X).

Retrograde transport of horseradish peroxidase conjugated to wheat germ agglutinin (HRP-WGA) was used to investigate the connections of medullary vocal nuclei. HRP-WGA was used to produce the intense and small injection site necessary for work in frog brain. Iontophoretic injections of HRP-WGA were made into n.IX-X. After 48 hrs. survival, frogs were sacrificed and perfused. Frozen sections were reacted with tetramethylbenzidine or cobalt intensified diaminobenzidine and hydrogen peroxide. Labelled cells were located in the ipsilateral and contralateral dorsal tegmental area of the medulla (DTAM, terminology of Kelley et. al, 1975, J. Comp. Neurol.) and a small ipsilateral nucleus adjacent and medial to n.IX-X. The ipsilateral DTAM was labeled more heavily then the contralateral. Injections of HRP-WGA into DTAM resulted in labeled cells in the ipsilateral preoptic area; no labelled infundibular cells were seen. The ipsilateral and contralateral n.IX-X contained HRP- filled cells. These latter data suggest reciprocal connections between the laryngeal motor nucleus and its medullary afferent nucleus, DTAM.

On the basis of electrophysiological studies in Rana pipiens, Schmidt (J. Comp. Physiol., 1976) has proposed a neural model for the generation of anuran mate calls. The connections described above support several features of the proposed model, including input from anterior diencephalic nuclei such as the preoptic area, and suggest new loci for interactions in the calling circuit. In Xenopus laevis, mate calling is under the control of steroid hormones. Many of the nuclei described above, which contribute to the generation of calling, contain hormone-concentrating cells and are thus potential candidates for mediating the powerful behavioral effects of sex steroids.

Supported by HD 12126.

93.2 MALE AND FEMALE LARYNGEAL MOTORNEURONS IN XENOPUS LAEVIS. P. C. Hannigan* and D. B. Kelley. (SPON: S. Reingold). Dept. of Psychology, Princeton Univ., Princeton, NJ 08544.

The sex typical vocal behaviors of male and female Xenopus laevis differ. These differences are paralleled in the vocal organ, the larynx. Adult, sexually mature, males typically have larger and more muscular larynxes than adult, sexually mature females. Mean weight for males is 0.42g (range 0.30 to 0.52) and mean weight for females is 0.13g (range: 0.11 to 0.15). The objective of our studies was to determine if there is also sexual dimorphism in the motorneurons which innervate the larynx. These cells comprise cranial nerve IX-X (n.IX-X) and are located caudal to cranial nerve V (n.V) and VII and rostral to the spinal cord neurons. These neurons are found lateral to the medullary grey in a column approximately 1mm long. Adult male and female Xenopus laevis received injections of horseradish peroxidase (HRP) into the laryngeal muscles. Motorneurons labelled with HRP of females were found in approximately the same location in the medulla as those of males. Female motorneurons appeared smaller and were located more medially than their male counterparts. These differences were examined further in normal Nissl stained material. Males were found to have larger motorneurons than females. The mean cell size for males is 420.44μ^2, while the mean cell size for females is 341.63μ^2. Males were further found to have more motorneurons than females. Mean number of motorneurons for males is 620 while mean number of motorneurons for females is 373. We also counted the motorneurons of n.V. The mean number of motorneurons for males is 389 and the mean number of motorneurons for females is 299. We calculated a difference score ($\frac{X - Xf}{Xm + Xf}$). Using this measure, the number of IX-X cells were 25% greater in males than in females. The number of V cells were 13% greater in males than females. Thus laryngeal motorneurons in Xenopus laevis were found to be sexually dimorphic with response to cell number, cell size and cell position. This dimorphism may contribute to differences in vocal behavior. Differences in motorneuron number could reflect an overall difference between male and female brains as n.V neurons were also fewer in females than in males. The mechanism for these sex differences remains to be determined.

Supported by HD12126.

93.3 A 2-DEOXYGLUCOSE AUTORADIOGRAPHIC INVESTIGATION OF SENSORY INPUTS TO THE OPTIC TECTUM OF RANA PIPIENS. D.L. Gorlick, M. Constantine-Paton and D.B. Kelley. Depts. of Biology and Psychology, Princeton Univ., Princeton, N.J. 08544.

Radioactively labelled 2-deoxyglucose can be used to identify areas of elevated metabolic activity in the CNS (Sokoloff,L., J. Neurochem.,29:13,1977). We wanted to use this technique to investigate sensory input to the brain during prey-catching behavior in frogs. Prey-catching is comprised of a small number of discrete behavior patterns whose performance appears to be mediated in areas of the optic tectum. A visual stimulus apparatus was constructed to mimic the motion of "prey". Frogs placed in the apparatus showed orientation and strikes towards its moving spots that resembled normal prey-catching behaviors. Rana pipiens (1-5 months postmetamorphic) were injected with 2.5μCi of 14C-2-deoxyglucose and placed in the visual stimulator for 90 minutes. Autoradiograms of 12μm brain sections were prepared and all heavily labelled areas identified from counterstained source sections. Densitometry profiles of optic tecta were constructed by measuring optical density (O.D.) radially across tectal layers. Autoradiograms of intact animals (S=sighted,n=4) showed heavy labelling of all primary visual projection areas of the diencephalon and optic tectum. Areas associated with acoustic input also showed high activity, probably due to the sound of the motors of the visual stimulator. One-eyed (OE,n=5) and Blind (B,n=3) animals were prepared by cutting optic nerves 2-7 days prior to visual stimulation. OE animals showed behavior similar to that of S animals, while no behavioral response was observed in B animals. Analysis of O.D. data indicated that cutting one optic nerve significantly reduced label in the superficial neuropil and cell layer 6. However, there were no differences in cell layers 4 and 1+2 (Potter,H.D.,J. comp. Neurol.,136:203,1969) between tecta ipsilateral and contralateral to the cut optic nerve. In an attempt to remove confounding inputs, both acoustic-vestibular nerves were sectioned in two OE animals (AV+OE,n=2). These animals did not strike, and showed few orienting movements. O.D. comparisons between tectal pairs produced results similar to those of OE animals for layer 6, but suggested that differences in the visual input to cell layers 4 and 1+2 had been partially masked by acoustic-vestibular or motor activity. Tectal cell layers 4 and 1+2 may thus be involved in sensorimotor integration of prey catching behavior in frogs.
(D.L.G. supported by NIMH fellowship MH 15799).

93.4 AUDITORY RESPONSE PROPERTIES OF DORSAL THALAMIC NUCLEI IN THE BULLFROG, Rana catesbeiana. Andrea L. Megela and Robert R. Capranica, Sect. of Neurobiology & Behavior,Cornell Univ., Ithaca, NY 14853.

The dorsal thalamus has been physiologically identified as an auditory center in anurans (Mudry et al.,J.Comp.Physiol. 114: 1-13,1977). Our HRP injections into the dorsal thalamus of the bullfrog, Rana catesbeiana, reveal extensive interconnections with forebrain and midbrain areas known to be responsive to acoustic stimuli. This suggests that, as in mammals, many different thalamic nuclei may receive auditory input. We therefore recorded neuronal evoked potential and multi-unit activity in the various thalamic nuclei to determine if all areas exhibit identical auditory response properties.

Two characteristics of auditory activity were studied in detail: habituation and nonlinear facilitation. In the mammalian auditory system, habituation effects are most pronounced in thalamic sensory relay nuclei but not in intralaminar nuclei (Wester, EEG,30:52-61,1971). We found similar results in the anuran dorsal thalamus. Neural responses from the central nucleus (possible homolog of the medial geniculate) habituated in response to acoustic stimuli repeated at rates of one every 10 seconds or faster. These habituation effects were much less obvious and reliable in the anterior, lateral, and posterior thalamic nuclei, and in the ventral thalamus, all of which receive multimodal sensory input. Moreover, the time course of habituation in the central nucleus was similar to the time course of behavioral habituation of evoked calling in adult male bullfrogs. These results suggest that neuronal processing in the central nucleus plays a significant role in mediating the evoked calling behavior of the bullfrog.

Mudry et al. found that evoked potentials from the dorsal thalamus show nonlinear facilitatory responses to tone combinations that maximally excite both of the auditory (amphibian and basilar) papillae in the inner ear simultaneously: these responses were significantly larger than a simple summation of the responses to each tone separately. Our analyses of nonlinear facilitation indicate that these response patterns are most pronounced in the central nucleus, with only minor or no facilitation present in the other thalamic nuclei. Furthermore, we confirmed that facilitation does not occur in any of the auditory midbrain nuclei of the torus semicircularis.

Supported by NIH fellowship NS06036 to ALM and NIH grant NS09244 to RRC.

93.5 SPECIES TYPICAL DISPLAY BEHAVIOR ELICITED BY ELECTRICAL BRAIN STIMULATION IN THE WESTERN FENCE LIZARD, SCELOPORUS OCCIDENTALIS. R. S. Tarr, Department of Physiology, Chicago College of Osteopathic Medicine, Chicago, Illinois 60615.

Previous investigations have shown that complete social displays or fragments of displays can be elicited from stimulation of the Iguanid central nervous system. However, these investigations have failed to demonstrate localization of function relative to these displays. This lack of clear localization is unusual in light of the specific localization revealed by lesion studies in Iguanid lizards. In our experiments we stimulated 166 points in the fence lizard brain (monopolar electrodes, less than 50 micron exposed tip, 96 animals). Stimuli were biphasic square waves, 1 msec duration, delivered via a Grass constant current dual stimulator. All observations were made on unrestrained animals, both while alone in the test cage and in the presence of a conspecific and food. Assertion displays were elicited (criteria was over 50% of applied stimulations resulted in a display) from 14 sites in the basal telencephalon but were absent in all but one site outside of the telencephalon. In several lizards over 80% of the applied stimulations elicited assertion display at low (less than 40 microamp) thresholds. In contrast, elementary locomotor effects (such as stimulus bound turning and circling) were confined to sites in the diencephalon and brain stem. Threshold currents eliciting these effects ranged from 3 microamps to over 100 microamps. Elements of the challenge display, such as a raised posture or lateral compression, were elicited from eight animals. In six of these eight animals the electrode was in a small area just anterior and dorsal to nucleus sphericus.

The results of these preliminary studies indicate that localization of function is a characteristic of the reptilian brain as it is in other vertebrates. It is further concluded that the lizard telencephalon functions primarily as an integrator of complex behavior and not as a primary motor system. Surprisingly, stimulation of the striatum elicited assertion displays rather than the challenge displays one would predict from lesion studies. The present results indicate that challenge behavior is integrated primarily in the posterior amygdala.

93.6 SPECTRAL SENSITIVITY IN HAPLOCHROMIS BURTONI (CICHLIDAE) E. E. Allen* and R. D. Fernald (SPON: M. Menaker). Inst. of Neurosciences, Univ. of Oregon, Eugene, Oregon 97403.

The African cichlid H. burtoni, many of whose social interactions are visually mediated, possesses three distinguishable vitamin A_1 based cone pigments, each confined to a single cone type, interspersed with rods in a regular retinal mosaic. Spectral absorbance peaks occur at 500nm for the rods, 455 nm for the single cones, and 523 nm and 562 nm for the paired cones (Fernald, R. D. & Liebman, P. A., Vis. Res. 20: 857-864, 1980). Experiments are underway to determine if this retinal pigment organization corresponds to trichromatism. Measurement of the Luminous Efficiency Function (LEF) under both dark and light adapted conditions, combined with knowledge of pigment absorption characteristics, should allow specific predictions. The LEF is determined through a two-choice, food reward operant conditioning paradigm. The dark adapted LEF conforms closely to the nomogram for a $P500_1$ pigment. The precise form of the light adapted LEF should reveal if the interactions of the various cone types are subtractive in nature, as is the case for other color opponent visual processing systems.

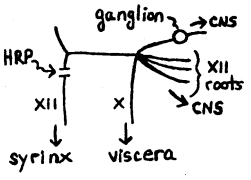
This research is being supported by National Institute of General Medical Sciences National Research Service Award 5T32 GM 07527 in Systems and Integrative Biology.

93.7 **IDENTIFICATION OF AFFERENT NEURONS IN THE HYPOGLOSSAL NERVE OF THE ZEBRA FINCH.** **S.W. Bottjer and A.P. Arnold, Department of Psychology, University of California, Los Angeles, CA 90024.**

The possible role of proprioceptive feedback from the vocal organ (syrinx) in song learning and maintenance of song by passerine birds has not been evaluated sufficiently, and anatomical pathways for such feedback are heretofore unknown. In order to determine the existence of afferent fibers from the syrinx in the zebra finch (Poephila guttata), horseradish peroxidase (HRP) was applied to the cut peripheral branch of the hypoglossal nerve that innervates the left side of the syrinx. Following tetramethylbenzidine reaction of the HRP, labeled cell bodies were found in the ganglion of the left vagus (X) nerve, but no transganglionic (anterograde) transport into the CNS was observed at transport intervals of one to five days. (As expected, motor neurons of the posterior portion of the ipsilateral hypoglossal nucleus were labeled; cf. Nottebohm et al., J. Comp. Neurol., 165: 457, 1976.) Applying HRP to the cut descending branch of the vagus also produced labeled cell bodies in the vagal ganglion as well as heavy anterograde label extending into nucleus solitarius. The reason for this discrepancy in terms of anterograde transport is unclear.

Sectioning the lower two roots of the hypoglossal nerve and applying HRP to the hypoglossus distally eliminated labeling of hypoglossal motor neurons, but not of vagal ganglion cells. Sectioning the vagus rostral to the hypoglossal roots eliminated labeling of ganglion cells, but not of motor neurons. Finally, sectioning the descending branch of the vagus caudal to the X-XII anastomosis had no effect on labeling of motor neurons or ganglion cells.

Auditory feedback is essential for song learning in young zebra finches but not for maintenance of song in adult birds (Price, J. Comp. Physiol. Psychol., 93: 260, 1979), suggesting that proprioceptive feedback from the syrinx may contribute to singing behavior. Unilateral section of either the left or right vagus rostral to the X-XII anastomosis in non-deaf adult zebra finches had no significant effect on singing behavior.



This preliminary result may imply that adult song doesn't require peripheral feedback via the afferents of the XIIth nerve.
Supported by NSF grants BNS 77-05973 and BNS 80-06798 to A.P.A., USPHS grant RR07009 to UCLA, and NIMH-NRSA 1T32 MH15345 to S.W.B.

93.8 NEURAL DETERMINANTS OF VOCAL BEHAVIOR IN BIRDS THAT DO NOT EXHIBIT VOCAL LEARNING. J. Cohen and F. Nottebohm. The Rockefeller University, New York, NY 10021.

Bird species differ with respect to how adult vocalizations are acquired. Oscine songbirds (e.g. canary) learn to sing by reference to auditory information. In contrast, the doves produce normal adult vocalizations even when deprived of auditory information. Previous studies (Nottebohm et al., JCN 165: 457, 1976) have shown that canaries possess a series of interconnected nuclei which functionally determine the bird's song. This telencephalic to motor end organ pathway takes the following form: HVc → RA → n.XIIts → syrinx. Although there are many potential sources of variability that may account for the presence of vocal learning in one species, and the lack of it in another, one likely possibility is the underlying neural substrate. We therefore report preliminary observations on the neural pathways mediating vocal behavior in ring doves, birds that do not exhibit vocal learning.

In ring doves, the proximal tracheosyringealis branches (ts) of each hypoglossal nerve extend down the trachea; the two ts branches anastomose above the syrinx and give rise to two nerves, each of which innervates one syringeal half. When horseradish peroxidase (HRP) is applied to the cut muscles of one syringeal half (distal to the anastomosis), retrogradely labeled neurons are seen, bilaterally, in the caudal portion of each hypoglossal nucleus (n.XIIts) of the medulla. When HRP is administered to one ts branch (proximal to the anastomosis), labeled neurons are seen unilaterally, in the same caudal portion of n.XIIts.

With the identification of n.XIIts, we sought to determine its afferents. N.XIIts is localized with the aid of stereotaxic coordinates and antidromic electrical stimulation. When the nucleus is found, an iontophoretic injection of HRP is made unilaterally into n.XIIts. The brain is then processed for HRP histochemistry. HRP filled axons are visualized in the occipitomesencephalic tract. Several HRP filled cells are seen, bilaterally, in lateral portions of the archistriatum.

These experiments, while still in progress, allow comparisons between canaries and doves. Although the n.XIIts to syrinx projection is present in both canaries and doves, the projection is unilateral in canaries and bilateral in doves; this probably contributes to differences in syringeal function. In canaries the projection from RA to n.XIIts is unilateral, while in doves, first indications suggest that the archistriatum → n.XIIts projection is bilateral. Such differences in laterality, as well as the structural variations in the nuclei themselves, may contribute to the neural determinants responsible for the disparate patterns in the acquisition of adult vocal behavior.

93.9 SPATIAL LEARNING ALTERED BY EXPERIENCE DURING SUCKLING. C. P. Cramer*, E. R. P. Breitinger*, D. S. Olton, and E. M. Blass (SPON: S. Mitchell). Dept. of Psychology, The Johns Hopkins University, Baltimore, MD 21218.

Numerous studies have focused on behavioral differences following differential post-weaning rearing. Suckling, the predominant waking behavior of neonatal rats, has recently been demonstrated as a suitable forum for various types of learning. In this experiment, the suckling situation was altered to allow either easier or more difficult access to the mother's nipples. At weaning, rats were tested on an 8-arm radial maze, a task with conceptual similarity to the suckling situation.

Male rats were reared from Day 5 postpartum to weaning at Day 25 in one of three maternal conditions. In the first, pups were raised in litters of 5 by normal mothers with all 12 nipples available to present a high density of nipples. In the second, siblings of pups in the first condition were raised in litters of 5 by mothers with only 4 nipples (the additional 8 nipples having been surgically removed prior to mating) to present a low density of nipples. In the third, pups were reared in normal litters of 10 by a mother with 12 nipples to provide baseline performance.

At weaning on Day 25, rats began maze acquisition testing. The maze consisted of a set of 8 arms radiating outward, like the spokes of a wheel, from a central platform, with a single food pellet at the end of each arm. The rat was placed in the center of the maze and allowed to choose freely among the arms, with choices separated by a brief period of confinement in the central platform, until all 8 pellets had been retrieved or 10 minutes had elapsed. Each rat was given one daily test session until reaching a criterion of 7 correct responses in the first 8 choices for 5 consecutive sessions.

Rats from normal litters and rats reared in litters of 5 by mothers with all 12 nipples reached criteria far more quickly than their siblings reared by mothers with only 4 nipples. Mean days to criterion were 6.8, 4.7, and 16.8, respectively.

These results demonstrate the importance of experience during suckling for later spatial learning. Rats presented early in the suckling period with contingencies characteristic of the radial maze later learned the maze task faster than their siblings whose suckling situation did not present those contingencies. The relatively poor performance of the rats reared in litters of 5 with only 4 nipples available does not reflect inadequate nutrition, as their body weights were not different from those of pups raised in normal litters. Moreover, the phenomenon appears to be task-specific, as performance on a variety of lever-pressing tasks which did not include a spatial component was the same between the groups.

93.10 EFFECTS OF CHOLINERGIC ANTAGONISTS ON INTERSPECIFIC AGGRESSION IN THE RAT. B.C. Yoburn* and M. Glusman. Dept. of Psychiatry, Columbia Univ., and N.Y. State Psychiatric Institute, New York, NY 10032

Microgram quantities of cholinergic agonists injected into lateral hypothalamic structures increase interspecific aggression in the rat. These findings suggest that a cholinergic substrate exists for the modulation of interspecific aggression. However, in the absence of data indicating suppression of this form of aggression by cholinergic antagonists, it may be premature to assume control by a cholinergic system. The present experiment investigated the effects of intracranially applied nicotinic and muscarinic antagonists on interspecific aggression. The specificity of these effects on aggression were evaluated by concurrent observations on eating, drinking and irritability.

Five male rats which spontaneously killed mice when maintained at 80% of their free-feeding weight were implanted with a 22ga guide cannula in the right lateral hypothalamus. Following recovery, 0.5 µl injections of atropine sulfate (2.5-20.0 µg) or d-tubocurarine (0.2-1.0 µg) dissolved in buffer (.1M phosphate, pH=7.0) were given through a 28ga internal cannula. Buffer was used for control injections. Animals received a series of tests at 10,20,30,40,50,60 min following an injection. Each series consisted of: (1) an aggression test, in which a white mouse was placed in the test cage and the latency to attack and kill was recorded. (2) An irritability test, in which response to handling was rated; (3) an eating test, in which the latency to eat 2, 45-mg pellets of food was recorded, and (4) a drinking test, in which the amount of water consumed every ten minutes was noted.

Atropine failed to systematically affect the behavior of any of the animals on any test. There was no evidence of suppression of aggression, even at the highest dose used. Curare suppressed aggression in a dose-dependent manner for three rats. However, suppression of aggression was accompanied by concurrent dose-dependent suppression of feeding, and no change in drinking and irritability. For the remaining two rats, Curare had no effect on aggression, but produced a dose-dependent increase in drinking, a slight facilitation of eating, and no effect on irritability.

The finding that curare suppressed aggression concurrently with feeding in 3 rats suggests a nonspecific drug effect, although a direct modulation of feeding cannot be ruled out. Together with the lack of effect of atropine, these results indicate that neither muscarinic nor nicotinic antagonists specifically inhibit interspecific aggression in the rat. (Supported by NIH Grant MH 15174.)

93.11 THE ISOLATION SYNDROME IN MICE: CHARACTERIZATION OF ISOLATION INDUCED CANNIBALISM. W. R. Pfister and D. Polito*. Pharmacology Control Section, Hoffmann-La Roche Inc., Nutley, NJ 07110.

Prolonged isolation induces compulsive, aggressive and fighting behavior in many animal species (Yen et al., Arch. Int. Pharmacodyn., 123:179-185, 1959; Welsh and Welsh, The Physiology of Aggression and Defeat, 91-142, Plenum Press, 1971), and precipitates a spectrum of behavioral alterations termed the Isolation Syndrome which is characterized by decreased exploratory and sexual activity (Valzelli, Psychopharm. (Berl.), 31: 305-320, 1973), and learning deficits.

We report for the first time the phenomenon of isolation induced cannibalism (IIC) in mice. Previously group housed (10/cage) male (17-30g) CF_1 mice were isolated, given food and water ad libitum, and either exposed daily for 24 hours to a CO_2 euthanized mouse or paired and evaluated for incidence (%) and intensity (scale: 0-4) of cannibalism or fighting behavior. Measurements of incidence - intensity of cannibalism were 15%-0.4, 67%-2.5, and 93%-3.0 following 1, 3, and 19 days of isolation, respectively. The time to development of a 50% incidence (RT_{50} and 95% CI) was 4.0 (3.3-4.7) days. In a parallel study the RT_{50} values for development of IIC and fighting behavior were similar, 2.4 (0.7-4.1) and 3.6 (2.1-6.1) days, respectively. IIC can be dissociated from other stress induced behaviors (i.e. irritability, defensive and aggressive behavior). Cage size does not alter the development of IIC, suggesting that physical isolation is sufficient to induce the behavior. IIC is facilitated by prior experience since the incidence in mice isolated for 16 days in the presence and absence of a 24 hour euthanized mouse was 75% vs 25% (χ^2=10.0, df=38, P=.002), respectively. Food deprivation (1 day) was as effective as 4 days of isolation in producing a 60% incidence of cannibalism, but cold swim stress was not. The incidence of IIC after 7 days of testing was 80%. Following an intertest interval of 3 weeks in mice that were regrouped (10/cage) or 8 weeks in mice remaining isolated, the incidence of IIC was 90% and 86%, respectively. This suggests that, in contrast to other stress induced behaviors, cannibalism is irreversible once established.

This study further demonstrates differences between individually and group housed animals, and suggests that isolation induced cannibalism, like fighting behavior, may be correlated with the development of aberrant behavior in man and may be useful in the screening of potential psychotherapeutic agents.

94.1 SENSORY SPECIALIZATION OF FREE NERVE ENDINGS IN THE RABBIT CORNEA. D.L. Tanelian and R.W. Beuerman, Division of Ophthalmology, Stanford University School of Medicine, Stanford, CA 94305.

The ability of free or morphologically unspecialized nerve endings to respond to different modalities of sensory stimulation was examined in the corneal epithelium. Albino rabbits (2-3 kg) were anesthetized with urethane (1.5g/kg) and placed in a head holder. The conjunctiva was excised just posterior to the cornea and secured over a ring to provide stability during recording and stimulation. A long ciliary nerve was isolated and sectioned centrally so an average conduction distance of 25 mm was obtained. Conventional recording techniques were utilized. Mechanical stimulation was produced by calibrated nylon filaments (170-2000 dynes). Thermal stimulation over the range of 20°C-50°C was by a jet of normal saline. The small mechanical component of the jet was subthreshold. Fifty-six identified units have been tested with both types of stimuli.

The mechanical units (n=46) were not spontaneously active, and their frequency of firing was dependent on the stimulus magnitude. The mechanical threshold for these rapidly adapting units ranged from 170-350 dynes. Thermal stimulation between 20°C and 45°C was not excitatory. The receptive fields of 20 units ranged from 5-40 percent of the total corneal surface area (213 mm^2) with an average of 12.5±9.3 percent. Conduction velocities were determined by bipolar electrical stimulation of the cornea, resulting in an average value of 2.71±1.4 m/sec, n=41. Near the limbus, where nerve trunks supplying the cornea emerge, larger conduction velocities (8.1±1.5m/sec, n=5) were obtained.

Thermal units (n=10) were spontaneously active, 10-25 spikes/sec, at the 33°C adapting temperature. Temperature changes in the cooling direction were excitatory for all units, whereas warming caused a decrease in the spontaneous firing frequency. Small temperature changes (ΔT=0.5°C) in the cooling direction produced a vigorous discharge. The time frequency histogram profiles for all temperature changes in the cooling direction were similar for these units, and the maximal frequency was proportional to the size of the temperature change. This relationship was also true for temperature changes in the warming direction except that the decrease in spontaneous activity was inversely proportional to the temperature change. These results indicate that a basis exists for functionally different receptor types in the cornea.

Supported by NEI Grant EY 02108.

94.2 ADAPTATION OF TYPE I AND TYPE II CUTANEOUS MECHANORECEPTORS IN THE CAT. K. W. Horch and P. R. Burgess. Dept. Physiol., Univ. Utah Col. Med., Salt Lake City, UT 84108.

When a type I receptor (Haarscheibe) is abruptly indented and the indentation maintained, there is an increase in impulse frequency that declines over a period of minutes to levels of about 3-5 impulses/sec. Subsequent indentations cause increases in frequency that decline gradually to the level produced by the first indentation. Type II receptors (Ruffini endings) with little or no resting discharge behave in a similar fashion, except that the rate of adaptation may be slower. Type II receptors with resting discharge rates above 15 imp/sec may give a long-term signal of skin indentation or stretch, but the signal is not greater than 2-3 imp/sec.

We conclude that tonic cutaneous mechanoreceptors in hairy skin do not have a persistent discharge that varies appreciably with the position of the skin, an unexpected finding in the case of type II receptors because their resting discharge can be both increased and decreased by stretching the skin in different directions. The return of the discharge to a common resting level in the presence of different maintained stimuli appears to require different coupling mechanisms between the receptor and surrounding tissue for resting and evoked activity. Functionally, this may serve to allow these receptors to signal directional changes in skin position for a time without compromising the level of resting activity to be modulated.

94.3 STIMULUS-RESPONSE RELATIONSHIPS FOR A MECHANORECEPTOR: THE PACINIAN CORPUSCLE. S. J. Bolanowski, Jr. and J. J. Zwislocki. Center for Brain Research, University of Rochester and Institute for Sensory Research, Syracuse University.

Stimulus-response relationships in both the intensity and frequency domains were obtained for single, isolated Pacinian corpuscles of cat mesentery. The resulting characteristics interrelate the vibratory stimuli, the generator potentials and the firing rates of action potentials. The stimuli consisted of 300 msec bursts of sinusoidal displacements (20Hz-1.25KHz; 50 msec rise-fall time), and the generator potentials and firing rates were measured in the steady state. The magnitude characteristics of averaged generator potentials are linear at low displacement amplitudes and saturate at higher levels. At these higher intensity levels, full-wave rectification is often found, but is asymmetric. The degree of asymmetry varies among receptors. The generator potentials also show a time-dependent hysteresis in response to each stimulus cycle at moderate and high stimulus displacement amplitudes. The intensity characteristics for action potential firing rates are steep at low intensity levels and plateau at multiples of the stimulus frequency, as stimulus intensity is increased. Post-stimulus-time and interval histograms reveal that the plateaus occur as a result of phase locking to the stimulus. The multiples of stimulus frequency at which the phase locking occurs and the length of the plateaus depend on stimulus frequency. The plateaus that are found at firing rates of 2 spikes per stimulus cycle are produced by the asymmetric full-wave rectification. The amplitude-frequency characteristics for both a constant firing rate and a constant amplitude of the generator potential below action-potential threshold are U-shaped functions. The phase-frequency characteristics of the generator potentials below the threshold show two populations of responses. Both populations undergo phase changes of about 300° as the stimulus frequency is increased but are separated by 180° . The underlying suprathreshold generator potentials were measured in the presence of tetrodotoxin (TTX). TTX is shown not only to eliminate the action potentials but also to decrease the amplitude of the generator potential. The percentage decrease is frequency dependent but is constant at any given frequency, indicating that the measured characteristics can be corrected by a multiplicative factor. The action potential results are consistent with in situ measurements from monkey nerve fibers believed to innervate Pacinian corpuscles (Talbot et al., J. Neurophysiol. 31, 1968). This indicates that the generator potential characteristics found are not an artifact of excision. (Supported by Grants NS-03950 and NS-09940, NIH).

94.4 SPATIAL ORGANIZATION OF RECEPTIVE FIELDS OF SINGLE GUARD HAIR AFFERENT NERVE FIBERS AS REVEALED WITH MOVING AIR JET AND PUNCTATE MECHANICAL STIMULATION. R. H. Ray, L. E. Mallach and L. Kruger. Depts. of Anatomy and Anesthesiology and the Ahmanson Laboratory of the Brain Research Institute, UCLA, Los Angeles, CA 90024.

The response properties of single guard (G) hair afferent nerve fibers innervating the hairy skin of the hindlimb were studied in acute, barbiturate anesthetized cats. The purpose of the study was to identify and analyze the relative contribution of those stimulus features determining the discharge patterns evoked in single afferents by a fine air jet stimulus moving across the skin and varying in force, velocity, position, direction and orientation. Additionally, the discharge properties as elucidated with the air jet were compared with those revealed with stationary ramp indentations varying in displacement, force, area, velocity and position within the receptive field.

Punctate displacement of a locus within the receptive field (RF) of a guard hair fiber reveals a characteristic low (G_1), medium or high (G_2) velocity sensitivity, but inhomogeneities are evident within the RF. Iso-sensitivity contours constructed for constant velocity punctate stimuli similarly reflect spatial inhomogeneity within the RF.

The response of single guard hair afferents to moving air jet stimuli reveals that the responsiveness of each fiber to stimuli with arbitrary orientation, direction and position within the RF displays an optimum velocity sensitivity which is not predictable from punctate velocity data. Although the response pattern is remarkably consistent for each controlled moving stimulus condition, there are apparent significant differences in response as a function of stimulus orientation, direction and velocity. RF 'maps' constructed from the responses evoked as the air jet traverses the skin reveal multiple zones of high and low sensitivity. The distribution of sensitive and non-sensitive zones is remarkably consistent for maps constructed with stimuli varying in orientation, direction and velocity. It is apparent that the principal determinant of the response for a given stimulus traverse is the spatial distribution of sensitive spots throughout the RF. Small but significant differences in directional selectivity are probably related to hair shaft orientation and length in linking hair displacement to receptor discharge.

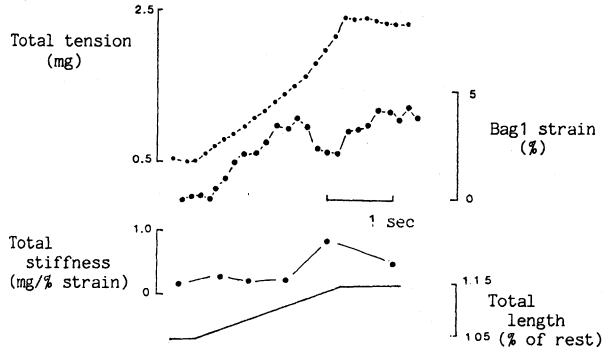
These preliminary findings indicate that guard hair afferent nerve fibers respond more vigorously to moving stimuli than to stationary displacement and display complex receptive field inhomogeneities which must be taken into account for the study of central neuronal information processing and feature extraction. (Supported by USPHS grant NS-5685)

94.5 STRETCH INDUCED CONTRACTION OF BAG1 FIBERS IN THE CAT MUSCLE SPINDLE. R. E. Poppele and D. C. Quick*, Laboratory of Neurophysiology and Department of Anatomy, University of Minnesota Medical School, Minneaplois, MN, 55455

The sensitivity of the primary endings of muscle spindle receptors to stretch is several fold greater than that of the secondary endings. This difference may be related to the mechanical properties of intrafusal muscle. In particular it may relate to a difference between the bag1 fiber which has abundant primary endings and few secondary endings and the other bag and chain fibers which have both. To test this hypothesis, we measured spindle tension and stiffness as well as intrafusal muscle strain (or the change in length per unit length) and sarcomere lengths in individual intrafusal fibers during a ramp stretch of isolated muscle spindles from cat tenuissimus muscle.

The results support the conclusion that there is a stretch induced contraction of the bag1 intrafusal muscle leading to an increased strain in the primary sensory area during a stretch. The observations leading to this conclusion are diagrammed below. There is an increasing slope of spindle tension in response to a ramp stretch that coincides with an increase in total stiffness and a decreasing slope of bag1 fiber strain. In addition there is a shortening of bag1 sarcomeres that parallels the decreased strain. Bag2 and chain fibers do not show this behavior.

Supported by grants from NSF (BNS 7825165) and NIH (NS 10969).



94.6 RECEPTOR ORGANIZATION IN HUMAN MUSCLES: A DETAILED STUDY OF RECEPTORS IN RECTUS CAPITIS POSTERIOR MAJOR. D.A. Bakker* and F.J.R. Richmond, Dept. of Physiology, Queen's University, Kingston, Ontario, Canada, K7L 3N6.

In the cat, small muscles surrounding upper cervical vertebrae are extraordinarily rich in sensory receptors. The receptors including muscle spindles, (up to 500/g in some muscles) Golgi tendon organs and paciniform corpuscles are organized in elaborate complex groupings which may include up to 15 receptors linked together (Bakker and Richmond, 1980). Early descriptions of the sensory apparatus of human neck muscles suggest that large populations of muscle spindles are also present in muscles of the suboccipital region. However the number and distribution patterns of receptors in such muscles have never been systematically examined in the adult.

In the present study the receptor content of rectus capitis posterior major was examined in tissue obtained postmortem. Tissue was fixed in formol-saline and double embedded in celloidin and paraffin. Serial 10 um sections were cut and stained with Holmes' silver method and picrofuchsin. The distribution patterns of receptors were mapped and reconstructed diagrammatically in three dimensions.

Human muscles usually contain about 10 spindles/g. Muscle spindles were most frequently observed in rectus capitis posterior major in densities of about 35 spindles/g. More than half (58%) of the spindles were organized in complex forms with at least one other spindle. Spindle complexes often comprised 2-3 spindles linked in tandem configurations (33%) which extended well over half of the muscle length. Frequently spindles at one end of the tandem linkage contained only one nuclear bag fibre in their intrafusal fibre complements while the other spindles contained at least 2 nuclear bag fibres. Other complex forms consisted of 2-3 spindles linked by paired (40%) and parallel (1%) formations. Most single spindles and spindle complexes were attached at one end to tendinous tissue. Golgi tendon organs and pacinian corpuscles were occasionally seen in human muscle. Golgi tendon organs were located in musculotendinous junctions at sites of attachment to the skull or vertebral processes. Pacinian corpuscles were associated with the fascia surrounding the muscle.

These results suggest that the complex spindle organization may be a significant feature of human neck muscles and may be important in the sensory function of the neck muscles.

Bakker, D.A. and Richmond, F.J.R. (1980) Distribution of receptors around neck vertebrae in the cat. J. Physiol. 298: 40-41 P.

Supported by Canadian MRC.

94.7 SENSATIONS FROM SELECTIVE ACTIVATION OF SINGLE HUMAN SENSORY UNITS WITH MYELINATED FIBERS. J.L. Ochoa and H.E. Torebjörk*. Dept. of Neurology, Dartmouth Medical School, Hanover, N.H. 03755, and Dept. of Clinical Neurophysiology, University Hospital, Uppsala S-75014, Sweden.

Introduction: The microneurographic technique of Vallbo and Hagbarth (1968) has substantially increased knowledge of the stimulus-response characteristics of single cutaneous receptors in humans. However, virtually nothing is known about the sensations evoked by activity in single sensory units. The aim of this work was to answer the fundamental question: what is felt when mechanoreceptive sensory units of various types are activated in isolation at a range of frequencies?

Method: Tungsten electrodes were used for microneurographic recordings of impulse activity in single afferent A fiber units in the median nerve in alert subjects. Such electrodes could also be used for electrical intraneural microstimulation (INMS) of single, identified sensory units.

Results: On INMS at liminal intensity for sensation, a pure and specific sensation (pure tapping, pure vibration, pure pressure) with a defined temporal dimension (intermittent or continuous) was typically referred to a focal area in the glabrous skin of the hand. Analysis of such sensation allowed to predict the type of stimulated unit, and the location of its receptive field. Such predictions were regularly verified when the stimulating electrodes were used for recording. Proof that the stimulated and the recorded unit was one and the same was obtained by demonstration of post-tetanic changes in excitability confined to the stimulated and recorded fiber. It was found that a single impulse in a single Rapidly Adapting (RA) unit innervating fingertip is regularly perceived. Temporal summation of several impulses is required to arouse sensation from RA units from other regions, as well as from all Slowly Adapting type I (SA I) and Pacinian Corpuscle (PC) units. RA units mediate tapping, which becomes flutter or vibratory "buzzing" at higher frequencies. PC units signal vibration. SA I units evoke sensation of pressure, its intensity being frequency dependent. No sensation is reported when individual SA II units are stimulated, regardless of frequency.

Conclusions: The sensory representation in the brain of the afferent innervation of the glabrous skin in the hand is discriminative enough to detect modality, frequency or intensity, and location of sensation from the input of individual mechanoreceptive units of certain types. This provides proof for a high degree of specificity in the sensory nervous system.

94.8 A SOMESTHETIC CODING MECHANISM FOR TEXTURE LIKE COLOR CODING IN VISION Kenneth O. Johnson and Ian Davidson Dept. Physiology, The Johns Hopkins Sch. Med., Baltimore, Md. 21205 and Dept. Physiology, Melbourne Univ., Melbourne, Australia.

The available evidence (Johnson & Lamb, J.Physiol. 310:117-144 1981; Darian-Smith, Davidson & Johnson, J. Physiol. 309:135-146, 1980) indicates that the spatial features of a repetitive surface with spacings finer than approximately 1.5 mm are not coded in the spatial structure of the afferent discharge of any of the mechanoreceptive fiber populations when a finger is scanned across the surface; however, a wide range of textured surfaces with spatial features finer than 1.5 mm are discriminated easily. Some other coding mechanism must account for this discriminative capacity. The discharge patterns of SA, QA, and PC afferents innervating the fingers of monkeys (M. nemestrina) were assessed using 9 embossed dot arrays (center-to-center spacings ranging from 0.525 to 3.25 mm; dot dia. = 0.3xspacing) applied at each of 6 combinations of contact force (20 & 60g) and scanning velocity (40, 80, & 150 mm s^{-1}). There was no detectable spatial structure in the responses of any of the afferents for spacings less than 1.5 mm; however, the mean impulse rates within and between fiber groups provided considerable information that might be used to discriminate these surfaces. Although there were a large number of variables in the experimental design (9 surfaces x2 forces x3 vel. x3 fiber types), the results are described fairly completely by the following summary:

1. The mean impulse rate, summed over all three fiber types, was approximately a linear function of center-to-center dot spacing at all forces and velocities; however, the slope generally increased with increasing force and velocity.

2. At dot spacings greater than 2.0 mm the ratios of the mean impulse rates in the three fiber groups were relatively constant at about 0.4:1.0:0.8 (SA:QA:PC relative mean rates). These ratios were affected little by force, scanning velocity or dot spacing.

3. At dot spacings less than 2.0 mm, the relative rates changed in a systematic way with dot spacing: on a relative basis PC rates increased while QA rates decreased for finer surfaces. For the finest surface, which had a smooth "feel", the relative QA and PC rates fell while the relative SA rate rose. These differences presumably represent the different frequency sensitivity profiles of the different mechanoreceptive classes.

These results indicate that the mean response rates of the three main afferent classes (perhaps four in man) provide a basis for coding both qualtitative (texture) and quantitative aspects of surface features. The relative engagement of the three classes was, like the perception of texture, relatively unaffected by changes in contact force or scanning velocity.

94.9 PSYCHOPHYSICAL FRACTIONATION MEASURES FOR CUTANEOUS THERMAL STIMULI. Sherry L. Berg* (SPON: R. S. A. Tindall). Florida State University, Tallahassee, FL 32306 and DeVry Institute of Technology, Irving, TX 75062.

Five male subjects performed a fractionation task for contact cooling stimuli presented to the right dorsal forearm. Additional warm-cool comparisons with observations from stimuli presented to both the forearm and the forehead were obtained from one subject. The stimulus site was preadapted to 32.6°C and fourteen stimulus intensities (0.3° to 6°C) were produced from thermal transients of 3 sec duration for fourteen corresponding rates of temperature change (0.1° to 2°C/sec). All five stimulating surfaces (1,2,4,7.5 and 18cm^2) were used in the primary cooling fractionation task.

Each of the fourteen stimuli were initially presented in an ascending then a descending series, afterwhich, a descending order of fractional comparisons began by introducing two consecutive presentations of the Standard Stimulus followed by a Trial Stimulus. Subjects indicated if the Trial Stimulus was greater or less than half the Standard, so that the Experimenter could manipulate the Trial Stimulus in the appropriate manner until a suitable Comparison Stimulus for one half the Standard could be obtained.

All observers demonstrated subjective fractional comparisons of one half or greater than the numerical equivalent of the Standard Stimulus when thermal cooling was measured. Reports from the one subject making fractional comparisons for warming on both the forearm and the forehead were even greater than those demonstrated in the cooling tests. Another subject showed an orderly array of psychophysical cooling functions correlating with decreases in stimulus surface area when fractionation measures were "intuitively" derived.

In a post-experimental debriefing and questionnaire, all five subjects rated the fractionation task as having a high degree of difficulty.
(Supported by USPHS Grant NB-02992 and Training Grant MH 11218)

94.10 TWO THERMAL INFLUENCES ON TOUCH SENSATION. J. C. Stevens. John B. Pierce Fndn. Lab. & Yale University, New Haven, CT 06519.

In 1834 the German physiologist Ernst Weber noted that a cold coin placed on the forehead feels heavier than a warm one. Although the observation has been casually confirmed from time to time, not until recently has a systematic study of this phenomenon been undertaken. Weights of various temperatures and masses were applied to seven different regions of the body -- forehead, palm, forearm, upper arm, back, midriff, and thigh, and subjects judged their subjective heaviness by the method of magnitude estimation. In all regions tested cool weights were judged to feel considerably heavier than weights near skin temperature -- sometimes as much as several times heavier. Warm weights were also judged heavier -- but only in the arm and hand regions. This difference between warm and cold seems to rule out a "cognitive summation" explanation, i.e., that subjects irresistably add the thermal sensation to the weight sensation -- contrary to instruction. It also turned out that the sensitivity of the various body regions to weight correlates very strongly with measures of spatial acuity such as the two-point limen and point-localization. That is, those regions to which the weights feel heaviest also show the best spatial resolution. It seemed likely, therefore, that weight sensation and acuity are mediated by the same receptor system. If this is so then temperature of the test objects might influence the skin's acuity. Indeed, two-point limens and two-edge limens improved by an average of 41% when the stimulators were warmer or cooler than the skin of the forearm. Since the thermal senses on their own show virtually no spatial resolution the improvement probably results from warming and cooling the mechanoreceptors that mediate pressure and spatial acuity.

This research was supported by NIH Grant #NS15419.

95.1 THE ELECTRICAL PROPERTIES OF ENZYMATICALLY DISSOCIATED MÜLLER CELLS. J. D. Conner*, P. V. Sarthy, P. B. Detwiler*. Physiology & Biophysics and Ophthalmology Depts., Univ. of Washington Sch. of Med., Seattle, WA 98195.

In order to understand the electrical properties of Müller cells and to examine their role in visual function, we have studied the membrane properties of isolated Müller cells from the fresh water turtle, Pseudemys scripta elegans.

Retinae were dissected and incubated in Ca++, Mg++-free Ringer containing papain pre-activated with 10 mM cysteine. After 45 min. of incubation at 37°C, the retinae were dissociated in normal Ringer supplemented with BSA and deoxyribonuclease. Dissociated cells were mechanically stablized with chilled agarose (0.4%) and transferred to a microscope stage where they were perfused with oxygenated, normal Ringer at about 15°C. Müller cells were identified by visual inspection and impaled with intracellular electrodes

The resting potentials of freshly isolated Müller cells ranged from -60mV to -100mV. In some cases the initial resting potential was smaller, but it usually grew larger and stabilized during the first 5-10 minutes of the recording. Isolated cells continued to have large resting potentials for a period of time ranging from four to six hours post-dissociation. The observed resting potentials are comparable to those recorded from Müller cells in the intact retina.

The input resistance of Müller cells was measured using two electrodes: one for passing current and one for recording voltage. The current-voltage relation is non-linear showing inward-going or anomalous rectification. The steady-state slope resistance is 60 MΩ for depolarizing currents and 15 MΩ for hyperpolarizing currents.

The time course of the voltage change produced by current steps depends on the polarity of the applied current. The charging curve for hyperpolarizing currents is fit by a single exponential with a time constant of 9 msec. Depolarizing potential changes follow a time course described by the sum of two exponentials having time constants of 9 and 42 msec.

These observations suggest that enzymatically-isolated Müller cells are viable and may be useful for further studies of Müller cell function.

Supported by grants from the National Eye Institute.

95.2 REGIONAL DIFFERENCES IN RETINAL MÜLLER CELL MEMBRANE PROPERTIES. Eric A. Newman* (SPON: A.R.Adolph). Eye Research Institute of Retina Foundation, Boston, MA 02114.

Müller cells, like other glial elements, are believed to have high conductances to K^+ relative to other ion species. I have recorded intracellularly from these cells in the frog retina (Rana pipiens) in order to study their K^+ conductance properties. Intracellular recordings were made at the level of the inner nuclear layer in perfused, light-adapted retinal slices (~150 µm thick). Cells were identified by intracellular injection of the fluorescent dye Lucifer Yellow CH. At a control $[K^+]_o$ of 2.5 mM, Müller cell resting potentials ranged from -80 mV to -85 mV. The membrane potentials of individual Müller cells were monitored as extracellular K^+ was altered using a series of 9 isotonic perfusion solutions. Over a range of 5 mM to 30 mM the membrane potential varied linearly with log $[K^+]_o$, and had a Nernstian slope of 58 mV/decade. At lower $[K^+]_o$ (2.5 mM to 0.625 mM) Müller cell potentials deviated slightly from this idealized relation. These results demonstrate that, like other glial cells, the Müller cell membrane potential is controlled by K^+ concentrations.

Previous current source density studies of current flow patterns within the retina (Newman, Vision Res. 19:227; J. Neurophysiol. 43:1355) suggested that the endfoot region of Müller cells possesses specialized conductance properties. I have explored this possibility by monitoring intracellular Müller cell responses to localized increases in $[K^+]_o$. An isotonic 100 mM KCl solution was pressure-ejected from a second (extracellular) pipette positioned at the surface of the retinal slice. The amplitude of the resulting depolarizing K^+ responses varied with the location of the K^+ source. The largest (15 mV to 35 mV) responses were elicited when K^+ was ejected in the optic fiber layer. Responses were smaller for more distal K^+-ejection locations, although large responses were sometimes seen for K^+ ejections in the inner plexiform layer. Responses to K^+ stimuli in the fiber layer were 4 to 30 times greater in amplitude than were responses to stimuli in the inner nuclear layer. These regional differences in Müller cell K^+ responses could be due to localized differences in the cell's K^+ conductance properties, and suggest that K^+ conductance is greatest in the endfoot region of the cell.

95.3 A BEHAVIORAL DETERMINATION OF COLOR MECHANISMS IN THE TURTLE, PSEUDEMYS, USING THE TWO-COLOR THRESHOLD TECHNIQUE OF STILES. D. F. Sisson*, A. M. Granda and J. H. Maxwell. Inst. of Neuroscience, Newark, DE 19711.

We have applied the two-color threshold technique of Stiles to specify receptor mechanisms for color in the visual system of the fresh-water turtle, Pseudemys scripta elegans. Wire loops were implanted into the jaws of five turtles. A wire threaded through this loop enabled automatic positioning of the head and monitoring of responses. The turtles were trained to retract their heads in response to flashed targets of light. The experimental paradigm determined absolute and increment thresholds for 650 nm light projected onto background fields ranging from 450 to 650 nm. Threshold data for each background-test stimulus pair were used to construct threshold vs. field intensity (tvi) curves. The spectral sensitivity of the mechanism detecting the test stimulus was calculated by determining the inverse of the adapting field intensity necessary to produce an increment threshold 0.5 log units above absolute threshold using the tvi curves.

The absorption spectra of single visual pigments found in the Pseudemys retina were calculated from a nomogram for vitamin A_2-based pigments and corrected for the transmission characteristics of associated oil droplets. These curves were too narrow to account for the broad spectral shape of the behaviorally derived curve. A better correlation was found with curves derived from coupled 518 and 620 nm pigments. Such coupling is known to occur in two instances in the Pseudemys retina: Double cones contain an accessory 518 nm pigment as well as a principle 620 nm pigment, and the signals from red cones and rods are physiologically linked in later stages of the afferent pathway.

95.4 REGIONAL VARIATION IN THE PHOTORECEPTOR LAYER OF THE TURTLE, *PSEUDEMYS SCRIPTA*. E.H. Peterson School of Anatomy Univ. New South Wales Sydney, Australia 2033

Recent evidence suggests that the ganglion cell layer of vertebrate retinas exhibits regional variation in its composition and that this regional variation is maintained in the projection to central visual targets (Peterson, '81, JCN 196). In the red-eared turtle, *Pseudemys scripta*, e.g., there is a local accumulation of small ganglion cells into a horizontally aligned visual streak with a peak density area embedded in the streak at the center of the retina (Peterson and Ulinski, '79, JCN 186).

To clarify the intraretinal origin of this regional variation, I examined the composition of the photoreceptor layer in *P. scripta*. In this species, approximately 95% of the photoreceptors are cones. There are four functional classes of cones, and each is uniquely associated with a different color of oil droplet. These oil droplets completely fill the inner segment and filter light before it reaches the outer segments. Red oil droplets are found exclusively in red sensitive single cones, yellow droplets in green sensitive single cones, clear droplets in blue sensitive single cones, and orange droplets in the red sensitive major member of double cones. Thus it is possible to characterize the composition of the photoreceptor layer by describing the sizes and relative frequencies of each color of oil droplet.

This analysis suggests that the retina of *P. scripta* can be divided into three functionally distinct regions based on the composition of the photoreceptor layer. Dorsal retina is characterized by a high relative frequency of orange droplets, and droplets of all colors are larger than elsewhere in the retina. For example, red, yellow and clear droplets are larger than those at comparable distances below the streak and are 150-250% larger than in the streak. Ventral retina is characterized by a high relative frequency of yellow droplets; their density remains high in the streak and drops sharply in dorsal periphery. In contrast, the frequency of clear droplets is relatively low in ventral retina; their frequency and especially their size increase above the streak. The visual streak differs from both dorsal and ventral hemiretina. Here there is a sharp increase in the relative frequency of red, and a decrease in orange, droplets. The sizes of droplets associated with single cones decrease abruptly in the streak. In contrast, the sizes of orange droplets vary relatively little with retinal locus.

These data suggest that dorsal retina, ventral retina, and visual streak of *Pseudemys* retina are differentially specialized, and that topographic specializations in the ganglion cell layer of vertebrate retinas may be organized more distally than the ganglion cell layer, perhaps in the photoreceptor layer itself.

95.5 ADAPTATION AND THE ULTRASTRUCTURE OF RAT PHOTORECEPTOR TERMINALS. C. Brandon and D.M.K. Lam, Department of Anatomy, University of Oregon Health Sciences Center, Portland, Oregon and Department of Ophthalmology, Baylor College of Medicine, Houston, Texas.

Brief exposure of a dark-adapted retina to light causes a dramatic decrease in its sensitivity to subsequent light stimulus (Ernst & Kemp, Vision Res. 11, 1197 (1971)). The process of adaptation, whereby this sensitivity is regained, depends partly on slow photopigment regeneration and partly on a rapid neural process; at least a part of the latter occurs in the photoreceptor (PR) cell itself (Grabowski et al, Science 176, 1243 (1972)). We have studied this process in rat rod synaptic terminals by EM examination under conditions of light- and dark-adaptation. Rat retinas were fixed by immersion in phosphate-buffered glutaraldehyde, or were immersed in phosphate-buffered sucrose solution for several minutes before glutaraldehyde fixation in the same medium. The latter method preserves the extracellular space of neural tissue (Cragg, Tissue & Cell 12, 63 (1980)), and results in improved preservation of rod horizontal cell (HC) synaptic vesicles. In light-adapted rod terminals, HC processes were invaginated by finger-like and ridge-like protrusions of PR cytoplasm, which always occurred on the side of the HC process away from its point of contact with the PR ribbon. Underlying the HC plasma membrane at such invaginations was a flocculent layer, about 60A thick and 80A away from the cell membrane; this specialization was inevitably present under areas of concavity of the HC membrane. Synaptic vesicles were present throughout the HC process, but appeared to cluster around the invaginations. In dark-adapted retinas, HC membrane areas showing the specialization were straight or convex, and PR invaginations into HC's were markedly reduced in number. We found similar specializations in goldfish cone PR terminals, in agreement with Wagner (J. Neurocytol. 9, 573 (1980)), who suggested that they were neurofilamentous in nature. We speculate that the floccular specialization is a contractile apparatus which deforms localized areas of the HC membrane in response to light, altering the three-dimensional relationship between HC and PR, and that this plasticity may mediate rapid photoreceptor adaptation by altering the efficacy of the HC→PR synapse.

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95.6 PHAGOCYTOSIS BY THE RETINAL PIGMENT EPITHELIUM IS STIMULATED BY THE PEPTIDE TUFTSIN. L.J. Fisher, G. Stevens, Jr.* and P.M. McCann*. Dept. of Ophthalmology, Henry Ford Hospital, Detroit, Michigan 48202

Phagocytosis by the retinal pigment epithelium of photoreceptor outer segments is an essential process in normal retinal maintenance. Tuftsin, a naturally occurring tetrapeptide, is a potent stimulator of phagocytosis by neutrophils and macrophages. Does this peptide also stimulate phagocytosis of rod outer segments (ROS) by the retinal pigment epithelium? To answer this question we tested the prediction that an intravitreal injection of tuftsin would increase the number of phagosomes (phagocytized ROS fragments) in the retinal pigment epithelium of treated over control eyes before and after the normal peak shedding period. Using 21 day-old RCS rats from a strain without retinal degeneration, we injected 2 µl of tuftsin (70 µg/ml through a 30 gauge needle on a Hamilton syringe) into the vitreous of one eye, and 2 µl of balanced salt solution into the vitreous of the other eye one hour prior to the usual onset of light. Eyes were injected in dim red light and were harvested one, two, three, and eight hours post injection.

The number of large phagosomes (diameter >1µm) within the retinal pigment epithelium were analyzed using the light microscope. The number of phagosomes in control eyes were increased at 1 and 2 hours after light onset. The number of phagosomes was considerably less than that seen in similarly treated Charles River rats. Eyes injected with tuftsin and harvested at light onset had a large number of phagosomes, while the control eyes had almost none. At 1 and 2 hours after light onset, the tuftsin treated eyes had more phagosomes than their corresponding controls. At 7 hours after light onset eyes had few phagosomes.

The results of these experiments are twofold: (1) tuftsin results in the appearance of phagosomes at light onset, (2) tuftsin stimulated the pigment epithelium to increase its phagocytosis of ROS. These results demonstrate that the tetrapeptide tuftsin stimulates phagocytosis of ROS by the retinal pigment epithelium.

95.7 A POSSIBLE CHOLINERGIC TRANSMISSION FROM CONES TO HORIZONTAL CELLS IN THE TIGER SALAMANDER RETINA. J. Skrzypek* (SPON: H. Ripps). Dept. of Ophthalmol., New York Univ. Med. Ctr., New York, N.Y. 10016.

The aim of this project was to clarify the role of acetylcholine (ACh) in the synaptic transmission to horizontal cells (HC's). These cells receive three synaptic inputs: from rods (R), cones (C), and other horizontal cells. Synaptic transmission between pairs of visually identified HC's was examined by passing current into one cell and measuring the voltage response from the other cell in the presence of ACh or cobolt. Synaptic inputs from photoreceptors to HC's were analysed by measuring current-voltage relationships of the HC's membrane in the dark and light and in the presence of acetylcholine.

It was found that neither cobolt nor ACh disrupts the coupling between neighbouring HC's and that the fluorescent dye injected into one cell often migrates, probably via coupling pathways, and stains the surrounding HC's.

Based on response spectra recorded from HC's, photoreceptor inputs to HC's can be spectrally separated into two components; R's contribute maximally when illuminated with blue/green (434-514 nm) light, and the C's dominate the HC's response when the stimulating light is red (600-650 nm). In the presence of ACh the peak of the response spectrum for the HC's shifts from long toward short wavelenghts, indicating that ACh blocks the cones' but not the rods' input to HC's.

Neither R's nor C's are affected by exposure to cholinergic medium. But it was found that HC's are depolarized by ACh and hyperpolarized by atropine. This depolarizing response of HC's to ACh can be reversed at about -70 mV and is associated with a conductance decrease. Cone-dominated response of the HC's has a reversal potential also near -70 mV and is associated with a conductance increase. On the other hand, HC's response to blue/green light, which excites mainly rods, reverses at membrane potentials near +30 mV and is associated with a conductance decrease.

These results suggest that HC's response to light consists of two spectrally separable, rod and cone components which have different reversal potentials and are associated with opposite conductance changes. The coupling between HC's is electrical and is not affected by exposure to ACh. Furthermore it seems that the rod to HC's pathway is noncholinergic while the cone to HC's transmission might be muscarinic.

95.8 THE DISTRIBUTION OF ACETYLCHOLINESTERASE ACTIVITY IN RAT RETINA. D.D. Dunning*, C.D. Ross and D.A. Godfrey. Dept. of Physiology, Oral Roberts University, Tulsa, OK 74171 (SPON: J.D. Dunn).

Acetylcholinesterase activity was determined in samples microdissected from the center of 7µm freeze-dried serial sections cut tangentially through rat retina. Dissected samples measured about 100-200 µm linear dimension. Data were plotted vs. section number, affording a possible 7 µm resolution across the retinal layers, with an error of about 1 µm because of the curvature of the retina.

Data below are averages of AChE activity (mmoles/kg dry wt/min @ 38°) ± S.E.M. in samples dissected from 4 eyes from 4 rats (n = number of samples)

			n
pigment epithelium,	outer	49 ± 4	3
	middle	90 ± 6	5
	inner	49 -	1
photoreceptor	outer	25 ± 4	13
segments	inner	23 ± 2	12
photoreceptor	outer	10 ± 1	5
nuclear layer	middle	9 ± 2	9
	inner	8 ± 2	6
outer plexiform layer		15 ± 1	3
inner nuclear layer,	outer	15 ± 3	10
(INL)	middle	84 ± 29	8
	inner	208 ± 46	5
inner plexiform layer (IPL)		323 ± 19	27
ganglion cell layer (GCL)		123 ± 42	3
fiber layer		46 ± 8	5

This distribution of AChE activity, particularly in the inner layers, is very similar to the pattern of distribution of choline acetyltransferase activity in rat retina (Ross & McDougal, 1976). A higher resolution presentation of the IPL data reveals a peak of AChE activity approximately 12 µm from the beginning of the GCL. AChE activity in serial 7 µm-thick samples of IPL beginning at the border with the INL:

		n
1st 7 µm lamina (outermost)	272 ± 11	3
2nd 7 µm lamina	285 ± 17	4
3rd 7 µm lamina	250 ± 17	5
4th 7 µm lamina	307 ± 17	5
5th 7 µm lamina	464 ± 36*	5
6th 7 µm lamina (innermost)	331 ± 57	5

(*differences significant between this value and all those from more superficial laminae, $p < .01$)

The position of this peak of AChE activity is consistent with the localization of enzyme activity by histochemical staining methods (Nichols and Koelle, 1969).

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95.9 CLASSES OF CHOLINERGIC RETINAL NEURONS IN CELL CULTURE. Donald G. Puro. Laboratory of Vision Research, National Eye Institute, NIH, Bethesda, MD 20205.

Defining neurons according to their information molecules (e.g. neurotransmitters and receptors) helps in understanding how neuronal circuits assemble and function. To assess the effects of putative neurotransmitters on cholinergic neurons of the chick retina, a cell culture system was used. In culture, some embryonic retinal neurons transiently form functional synapses with striated muscle cells (Puro et al, PNAS 74:4977,1977). Myotubes are useful as postsynaptic targets since their response to acetylcholine has been well studied and because their large size permits prolonged intracellular recording. By plating a low density ($2x10^4$ µm/cell) of dissociated retinal cells onto cultures of short myotubes(~300µm), it is possible to have each innervated muscle cell receiving synaptic input from only one cholinergic neuron.

Rat myoblasts were cultured in 35mm dishes for 5-12 days under conditions which limited fusion and thus myotube length. Retinal cells from day 8 chicks were dissociated with 0.05% trypsin; $5x10^4$ cells were added to a muscle culture. After 30-50 hours, 4.2% (5/120) of randomly sampled myotubes had spontaneous synaptic input as demonstrated by intracellular recordings. Once an innervated muscle cell was found, the synapsing neuron could usually be localized by microscopic examination. Identification of the presynaptic neuron was further established by evoking synaptic input with the iontophoresis of glutamate from a high resistance micropipette positioned near the nerve cell. Glycine, γ-aminobutyric acid (GABA) or dopamine were then sequentially delivered from a multibarrel micropipette.

To determine the effect of these neurotransmitters on the output of the cholinergic neuron, the frequency and amplitude of the depolarizing responses of the postsynaptic muscle were measured. Glutamate excited 88% (44/51) of the neurons tested. Some of the glutamate responsive neurons were inhibited by GABA (43%, 9/21), glycine (29%, 6/21), or dopamine (33%, 3/9). No cholinergic neuron examined thus far has been inhibited by GABA as well as by glycine. Based on these responses, at least 5 classes of cholinergic neurons have been isolated from the chick retina.

		Responses to neurotransmitters			
		GLUTAMATE	GABA	GLYCINE	DOPAMINE
Classes	1	+	-	0	0
	2	+	0	-	0
	3	+	-	0	-
	4	+	0	0	
	5	0			

95.10 GABA INHIBITION OF ACH RELEASE FROM RABBIT RETINA: EFFECTS OF 2-AMINO-4-PHOSPHONOBUTYRIC ACID (APB). *S.C. Massey*, M.L.J. Crawford and D.A. Redburn (SPON: H.G. Sperling).* Department of Neurobiology and Anatomy, University of Texas Medical School, Houston, TX 77025.

There is strong evidence for the role of ACh as a neurotransmitter in the rabbit retina. Cholinergic enzymes are present and localized in the inner portion of the rabbit retina, and ACh is released by light stimulation. In addition, both light-evoked release and resting efflux of [^{3}H]-ACh from the *in vivo* rabbit eye-cup are inhibited by GABA (1mM) or muscimol (1µM) but strikingly increased by µM concentrations of GABA antagonists. This suggests that cholinergic neurones in the retina are influenced by both tonic and phasic release of endogenous GABA.

Slaughter and Miller (Science 211: 182) have recently described the actions of 2-amino-4-phosphonobutryic acid (APB) on the mudpuppy retina. At µM concentrations this drug selectively and reversibly inhibited depolarizing bipolar cells. Complete inhibition of these cells with APB eliminated the ON pathways thus leaving only OFF responses in the inner retina. Our ERG and ganglion cell recordings have confirmed these results in the rabbit retina.

We have used APB in our examination of GABA-ACh interactions. APB (100µM) reduced the light-evoked release of ACh by 70% but did not antagonize the large increase in resting efflux elicited by GABA antagonists. This result implies that the tonic GABA input to cholinergic amacrines is unchanged by APB. Thus it appears that GABA-releasing neurones receive OFF input from hyperpolarizing bipolar cells.

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95.11 Neuropeptide containing amacrine cells in the retina of a turtle, Pseudemys scripta. William D. Eldred and Harvey J. Karten, Dept. of Neurobiology and Behavior, SUNY, Stony Brook, N.Y. 11790.

The inner plexiform layers of many retinas have anatomically specialized horizontal subdivisions or bands. Recent electrophysiological studies indicate the outer bands relate to center-off responses and the inner bands relate to center-on responses. We studied the arborization patterns of identified amacrine cells to help determine the role amacrine cells play in visual processing and provide a morphological basis for future physiological and biochemical studies.

Immunocytochemical methods were used to selectively label amacrine cells which have neuropeptide-like (leu-enkephalin, glucagon, and neurotensin) immunoreactivity. Percentages can be used to represent various levels of bands of the inner plexiform layer. We chose 0% to represent the region near the amacrine cells and 100% to represent the region near the ganglion cells.

Antisera directed against leu-enkephalin label pear-shaped amacrine soma (8µm dia.) that send delicate dendrites into the 0-20% levels and the 65-100% levels. An antisera directed against glucagon labels amacrine cells with a rounded soma (10µm in dia.) and dendritic processes that ramify in levels 0-20% with thinner bands at the 40% and 80% levels. Antisera directed against neurotensin revealed two different amacrine cell types. One type has a large pear-shaped soma (10x14µm dia.) which sends a single 2µm process that divides within levels 45-70%.

The present results indicate that various neuropeptides are present in amacrine cells of the turtle retina, and that there are specific arborization patterns for each type of neuropeptide containing amacrine cell studied. In addition an individual neuropeptide can be found in more than one morphological type of amacrine cell. Our results provide the basis for ultrastructural studies to determine the pre- and postsynaptic neurons contacting identified amacrine cells. Such functional analysis will help define the roles of the horizontal bands of dendritic arborization in the inner plexiform layer. This research supported by EY02164 to HJK and EY07039 to WDE.

95.12 FOUR TYPES OF AMACRINES ACCUMULATE GAMMA-AMINOBUTYRIC ACID (GABA) IN THE CAT RETINA. M. A. Freed*, Y. Nakamura*, and P. Sterling. Dept. of Anat., Sch. of Med., U. of Penn., Phila., Pa., 19104.

Retinal neurons which accumulate GABA comprise more than one quarter of the neurons in the amacrine cell layer. Some of the GABA-accumulating neurons (8%) were previously shown to be interplexiform cells; the remainder are true amacrines but have not yet been characterized. We have partially reconstructed from serial electron microscope autoradiograms 24 of these cells and have followed an additional 18 cells through the series. Four types of GABA-accumulating amacrines were distinguished on the basis of size, cytoplasmic appearance, shape of soma, and by the appearance of primary and secondary processes.

Type 1 (21 cells) was dark with a large soma (550-700 μm^3), which was flattened against the inner plexiform layer (IPL). Multiple, moderately thick (0.6 µm) processes splayed from the margins like the appendages on a crab. Stemming from these processes, as well as from the soma margins, were thinner processes (0.4 µm) which were directed more vertically into the IPL. GABA accumulation was high (5-14X nonspecific).

Type 2 (9 cells) was pale with a middle-sized (320-430 μm^3) mitral soma. One moderate diameter (0.8 µm) process descended vertically from the center of the inner soma surface and sent off horizontal processes at either the inner or outer half of sublamina a. One or two thin (0.4 µm) processes stemmed from the soma margin. GABA accumulation, with two exceptions, was moderate (3-4X nonspecific).

Type 3 (10 cells) was dark with a small to middle-sized (200-410 μm^3) generally pyriform soma. In six cells the inner soma surface tapered into one stout (3.0 µm) process which bifurcated in the middle of sublamina a. One or two fine processes descended from the soma margin and in one case ran for 9 um in the outermost IPL. Two cells were exceptions, having loaf-shaped somas, each with one moderately thick (0.8 µm) process which descended 3 µm into the IPL without branching. Two other cells were spherical and emitted only fine (0.2 µm) processes. A diversity of morphologies may require subclassification of Type 3 after further study. GABA accumulation ranged broadly (3-8X nonspecific).

Type 4 (2 cells) was pale with the largest soma (850-870 μm^3). This was pyriform, tapering into a stout (2.0 µm) process which descended into the middle of sublamina a where it emitted smaller horizontal processes. One moderate diameter (0.6 µm) process stemmed from the soma margin. GABA accumulation was moderate (3-5X nonspecific).

It is to be expected that these four different types of amacrines, which accumulate GABA at differing levels, will have distinct functions in retinal circuitry. To understand these functions it will be necessary to characterize in detail their arborizations and synaptic connections. EY00828.

95.13 THE IDENTIFICATION AND DEVELOPMENT OF AMACRINE CELLS CONTAINING SOMATOSTATIN-LIKE IMMUNOREACTIVITY IN CHICKEN RETINA.
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Transverse sections of chicken retina stained with antisera to somatostatin show well-defined amacrine cell bodies situated one to two cell layers away from the boundary between the inner plexiform and inner nuclear layers. Their processes ramify throughout the inner plexiform layer, with concentrations in Ramon y Cajal's layers 1, 4 and 5. The amacrine cells were not visible after intraocular injection of kainic acid, but they were not affected by destruction of the retinal ganglion cells by axotomy or intraocular injections of colchicine. These results were confirmed by radioimmunoassay. There was no reduction of the levels of somatostatin-like immunoreactivity after destruction of the ganglion cells, while the levels of somatostatin-like immunoreactivity were markedly reduced by intraocular kainic acid. These results may indicate that the amacrine cells containing somatostatin-like immunoreactivity receive bipolar cell input, but do not synapse onto retinal ganglion cells, presumably being involved in amacrine-amacrine or amacrine-bipolar cell interactions.

The development of these cells was followed by radioimmunoassay and immunohistochemistry. Somatostatin-like immunoreactivity was first detectable around 7 days *in ovo*, and developed slowly up until day 19 *in ovo*. The amount of somatostatin-like immunoreactivity then more than doubled to reach adult levels around the time of hatching. Histochemically, immunoreactive cell bodies were first visible around day 13 *in ovo* but clearly-defined processes in the inner plexiform layer were not visible until around days 17-19. These results indicate that the immunoreactive amacrine cells begin to synthesize somatostatin-like immunoreactive material before the final definition of their dendritic arborisations and synaptic interactions and that their development is not dependent on visual stimulation, since they reach a mature form prior to hatching.

95.14 SOMATOSTATIN-LIKE IMMUNOREACTIVE MATERIAL IN THE RABBIT RETINA.
S.M. Sagar, O.P. Rorstad,* D.M. Landis and J.B. Martin. Dept. of Neurology, Massachusetts General Hospital, Boston, MA.

As an initial step in the investigation of the function of somatostatin (SRIF) in the mammalian retina, the properties of somatostatin-like immunoreactive material (SLI) in the rabbit retina were studied. Using a specific radioimmunoassay, SLI was detected in the rabbit retina at a mean concentration of 1.1 $\pm$ 0.1 ng SLI/retina, or 0.17 $\pm$ 0.3 pg SLI/µg protein. Dilution curves of the retinal extract paralleled those of synthetic tetradecapeptide SRIF. On gel permeation chromatography, three major peaks of immunoreactivity were found. One peak co-eluted with synthetic SRIF, one with synthetic somatostatin-28, and the third eluted with higher molecular weight material. The SLI was found to be concentrated in a synaptosomal fraction prepared by differential centrifugation. The concentration of SLI in the retina was not diminished by the intravitreal injection of either 6-hydroxydopamine or 5,7-dihydroxytryptamine, neurotoxins which destroy catecholamine and indoleamine accumulating neuronal terminals, respectively. Retinal SLI, but not dopamine or substance P concentration, was decreased by cysteamine, an agent known to deplete SLI in other neural tissues. As in the investigation of other retinal neurotransmitter candidates, the rabbit retina offers a convenient system for further investigation of SRIF function.

Supported by PHS grant AM 26252. SMS is an NIH post-doctoral fellow supported by the National Eye Institute.

95.15 DOPAMINE RECEPTOR REGULATION IN AVIAN RETINA BY LIGHT/DARK ENVIRONMENT.
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Avian retinas respond to dopamine with an increase in cAMP concentration (DeMello, F.G., J. Neurochem., 31:1049, 1978). It recently has been shown that long-term (6 days) dark adaptation of post-hatch chicks increases the maximal elevations in cAMP in retina elicited by dopamine (DeMello, F.G., and DeMello, M.C. F., Pont. Acad. Sci. Sc. Var., 45:343, 1980). We have investigated the possibility that this increased response is due, at least in part, to a physiologically-induced increase in dopamine receptors.

Chick neural retinas, dissected free of pigment, homogenized and incubated at 37° in 50 mM TrisCl pH 7.4 with 120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, and 1 mM $MgCl_2$, specifically bound both 3H-ADTN and 3H-spiroperidol. High affinity 3H-ADTN binding had a K_d of 6.7 x 10^{-9} M and a B_{max} of 220 fmol/mg protein when determined using 10^{-4} M dopamine to block specific binding. 3H-Spiroperidol binding was highly sensitive to buffer composition and the use of Tris and HEPES buffers without additional salts abolished specific binding. When 10^{-4} M dopamine was used to block specific binding, the K_d for 3H-spiroperidol was 7.8 x 10^{-10} M with B_{max} equal to 40 fmol/mg protein. When 10^{-5} M fluphenazine was used to block specific binding, two components of 3H-spiroperidol binding were observed (#1: K_d = 3 x 10^{-10} M, B_{max} = 50 fmol/mg protein; #2: K_d = 4.3 x 10^{-9} M, B_{max} = 240 fmol/mg protein). When 10^{-4} M dopamine was used to block the specific binding of 5 x 10^{-9} M 3H-spiroperidol, we observed nearly a two-fold increase in binding to retina homogenates from chicks maintained in a dark-environment for 10 days (69 fmol/mg protein) compared to levels found in retinas from chicks maintained in a light-environment for the same period (36 fmol/mg protein.

Our results show that environmental stimuli can physiologically induce changes in dopamine-sensitive 3H-spiroperidol binding sites, and they provide further support for the hypothesis that dopamine receptor regulation is a modifier of synaptic communication in the central nervous system. (Supported in part by a grant to WLK from the Brazilian-American Institute of Rio de Janeiro and NIH grant NS 15299.)

95.16 EM AUTORADIOGRAPHIC LOCALIZATION OF OCTOPAMINE TO EFFERENT FIBERS OF LIMULUS VENTRAL EYE.
J.A. Evans* and B-A. Battelle. Laboratory of Vision Research, National Eye Institute, NIH, Bethesda, MD.

Previous studies suggested that the biogenic amine octopamine is synthesized and stored within efferent fibers of Limulus ventral eye (Battelle and Chamberlain. Neurosci. Abstr. 6: 720, 1980). The EM autoradiographic localization of sites of octopamine synthesis described here provides direct evidence in support of this suggestion. Ventral eyes were incubated in vitro with ^{3}H-tyramine using conditions which optimize octopamine synthesis and accumulation. The preparation was fixed, and thin sections were processed for EM autoradiography. After an 8-week exposure, sections were developed using gold latensification in combination with a physical developer (Kopriwa. Histochem.44:201 1975) to ensure formation of small, 480 A silver grains which minimally obscure underlying ultrastructure. Efferents were identified by (1) Size: efferent fiber diameter ranges from 0.16-0.77 µm. (2) The presence of pleomorphic, 0.2 µm dense granules having a periodic 110 A substructure. (3) The presence of a surrounding glial sheath.

Clusters of silver grains were localized over efferent profiles inside and near the surface of photoreceptor cell bodies and in the ventral nerve. Only low levels of diffuse label were seen elsewhere. Close examination of efferent profiles within 8 different photoreceptor cell bodies revealed that 90% were labeled; 80% of these contained diagnostic dense granules. Labeled efferents were concentrated near rhabdomes and were frequently in direct apposition to microvillar membrane at sites where the glial envelope was interrupted. Efferent axons in the ventral nerve were more difficult to recognize because profiles containing granules were infrequent. Identification was made primarily on the basis of size (0.5 µm compared to 6 µm photoreceptor axons). Virtually all labeled structures in the ventral nerve could be identified as efferents, however a systematic search for unlabeled efferent profiles in this region was not made. An unexpected observation was that labeled efferents run in bundles of 2-4 axons surrounded by glia. Results of this and previous studies implicate octopamine as a possible neurotransmitter in efferent fibers of Limulus ventral eye.

Preliminary EM autoradiographs of lateral eyes incubated with ^{3}H-tyramine and processed as described above also show clusters of silver grains over efferent fibers. This suggests that octopamine is synthesized within lateral eye efferents as well. We hypothesize that octopamine is a neurotransmitter common to efferent fibers in the Limulus visual system and that it plays a role in modulating visual function.

95.17 EFFECTS OF ASPARTATE ON RETINAL RESISTANCE AND LIGHT TRANSMITTANCE. H. Shimazaki, C. J. Karwoski and L. M. Proenza. Univ. of Georgia, Vision Res. Lab., Athens, GA 30602.

Prior to the usually reported isolation of the ERG a-wave, mudpuppy (Necturus maculosus) eyecup preparations perfused with 3.0 - 30.0 mM Na-aspartate (Asp) commonly show ERG amplitudes which more than double over control values (Shimazaki et al, ARVO abst. p39, 1980). Since both the a- and b-waves are similarly enhanced, it is possible that one of the initial effects of Asp is to increase retinal resistance. In order to determine changes in retinal resistance during Asp perfusion, a constant current of 3 μA was passed between electrodes in the vitreous humor and behind the eyecup, and the voltage developed by this current between a recording electrode in the retina and a reference electrode in the vitreous was measured. Light-evoked ERGs between these latter two electrodes were also measured, thus allowing simultaneous recordings of ERG and retinal resistance. When ERG amplitude increased during Asp perfusion, there was also a rapid increase in resistance (by a factor of from 2 to 5) between the vitreous and the depth where the maximal negative b-wave was recorded. Thus, ERG enhancement during Asp perfusion is likely due, at least in part, to an increase in retinal resistance.

Asp application to frog (Rana pipiens) retina caused changes similar to those observed in mudpuppy--specifically, ERG amplitude usually increased prior to a-wave isolation, and retinal resistance increased when the ERG was increased. Since the frog sclera is almost transparent, and the pigment epithelium layer can transmit some light through it, measurements of the effect of Asp on light transmittance through the retina were obtained by positioning a microphotometer behind the eyecup. When ERG amplitude increased, light transmittance through the retina gradually decreased and reached a minimum of about 35% when the a-wave was isolated. This decreased light transmittance was accompanied by the retina taking on a milky appearance.

These observations suggest that Asp has complex effects on the retina and ERG amplitude which must be considered when interpreting results obtained after application of this drug: On the one hand, Asp increases retinal resistance which probably contributes to the increase in ERG amplitude; on the other hand, light transmittance to the photoreceptors is diminished, which must result in some decrease of ERG amplitude. Asp effects such as the well-known lack of post-receptoral responsivity associated with a-wave isolation, the resistance and light transmittance changes, and the previously described changes in extracellular DC potentials and K^+ (Shimazaki et al, 1980), indicate that Asp induces a prolonged spreading depression type of reaction in the retina.

95.18 ABNORMAL VISUAL FUNCTION INDUCED BY DEPLETION OF RETINAL TAURINE IN THE RAT. Norma Lake, Departments of Physiology & Anaesthesia Research, McGill University, Montréal, Québec, Canada.

Taurine-free diets lead to abnormal vision in cats (Schmidt et al. Invest. Ophthal. 15: 47 (1976) but it is not known if this is a phenomenon peculiar to cats, since taurine-free diets in other species are ineffective in reducing tissue taurine levels because of increased biosynthesis and decreased excretion. This difficulty of experimentally manipulating tissue taurine levels has hindered studies of its functions. However Lake has demonstrated that in vivo administration to rats of a taurine analogue, guanidinoethyl sulfonate (which competes with taurine for transport sites) results in specific depletion of retinal taurine to 30% of control (Neurosci. Abst. 6: 345 (1980). In the present studies assessment of the functional consequences of this biochemical change was carried out by electroretinographic (ERG) studies after treatment of rats with 1% guanidinoethyl sulfonate in their drinking water for 60 days. Control animals consumed water without additives. The ERG response to 10 μsec white full field flashes at 1 Hz was recorded with wick electrodes on the cornea of ketamine-anaesthetized animals. The amplitude of the b wave of the ERG was 600-800 μV in 5 control animals while it was significantly reduced to 200-250 μV in the 6 guanidinoethyl sulfonate -treated animals. This study demonstrates the importance of the transport of taurine across the blood-retinal barrier for the maintenance of retinal function in the rat, and with the data from the cat studies suggests that this may be a mechanism important to vertebrate vision in general.

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95.19 TONIC EXCITATORY AND INHIBITORY SYNAPTIC INPUTS TO MUDPUPPY RETINAL GANGLION CELLS. J. S. McReynolds, D. R. Dvorak* and J. H. Belgum*. Department of Physiology, University of Michigan, Ann Arbor, Michigan 48109.

The synaptic basis of the sustained light responses of ON- and OFF-center ganglion cells to illumination of the receptive field center was studied using intracellular recording in the superfused mudpuppy eyecup. Current-voltage relations were measured under three conditions: in steady darkness, during steady illumination of the receptive field center, and when synaptic transmission was blocked with 4 mM cobalt chloride.

In darkness, blocking synaptic input causes depolarization of ON-center cells and hyperpolarization of OFF-center cells. These changes are accompanied by large increases in input resistance and show that both types of ganglion cells receive tonic synaptic input in darkness. The net effect of this input is inhibitory in ON-center cells and excitatory in OFF-center cells.

In ON-center cells, light causes a sustained depolarization which is due to an increase in excitatory input as well as a decrease in the inhibitory input which is dominant in darkness. In OFF-center cells, light causes a sustained hyperpolarization which is mainly due to an increase in inhibitory input. It is likely that the excitatory input which is dominant in darkness is decreased during illumination, but any such change (disfacilitation) is small relative to the increase in inhibitory input.

The tonic excitatory and inhibitory inputs each act by increasing membrane conductance. The reversal potential of the excitatory input is more positive, and that of the inhibitory input more negative, than the membrane potential in darkness.

Thus it appears that in both ON- and OFF-center ganglion cells the membrane potential in darkness and during the sustained response to light are determined by the balance of tonic excitatory and inhibitory inputs acting in a push-pull manner. This is contrary to the widely held belief that the sustained responses of ganglion cells are due entirely to modulation of excitatory input from bipolar cells. The existence of tonic inhibitory inputs may result in more integration of information at the ganglion cell level than previously supposed. It is likely that bipolar cells are the source of the tonic excitatory input and that sustained amacrine cells are the source of the sustained inhibitory input.

This work was supported by NIH grants EY 01653 and EY 07022.

95.20 THE EFFECT OF STRYCHNINE ON CURRENT-VOLTAGE PROPERTIES OF MUDPUPPY RETINAL GANGLION CELLS: EVIDENCE THAT GLYCINE MEDIATES TRANSIENT INHIBITION. D. R. Dvorak,* J. H. Belgum* and J. S. McReynolds (SPON: T. J. Morrow). Department of Physiology, University of Michigan, Ann Arbor, MI 48109.

In darkness and during steady illumination, the membrane potential of mudpuppy retinal ganglion cells is determined by the combined effects of tonic excitatory and inhibitory synaptic input (McReynolds, et al., Neurosci. Abstr., 1981). Changes in luminance activate additional excitatory and inhibitory synapses which contribute transiently to the response. We reported previously that the transient inhibitory input can be selectively blocked by strychnine, a pharmacological antagonist of glycine (Dvorak, et al., Neurosci. Abstr., 1980). In the present study, the properties of transient inhibition were investigated in greater detail by measuring current-voltage (I-V) relations of ganglion cells in normal Ringer and in the presence of 10 μM strychnine.

Intracellular recordings were made from ON-center, OFF-center and ON-OFF ganglion cells in superfused mudpuppy eyecups. I-V relations were measured in steady darkness and during the response to a spot of white light (200 μm dia) positioned in the receptive field center. Long duration (3-5 sec) light stimuli were used in order to easily separate the transient and maintained components of the response.

I-V relations measured at short times after the onset and termination of the light stimulus had steeper slopes and were displaced to more positive potentials in the presence of strychnine. Measurements made in darkness or during steady light showed no significant difference between the presence or absence of strychnine. The average conductance increase associated with the transient inhibitory input was 2-10 nS. Control observations on bipolar and amacrine cells indicate that the observed effects of strychnine are occuring at the ganglion cell level.

The results provide strong evidence that the transient inhibitory input to the three classes of ganglion cells is mediated by a glycinergic synapse. This synapse is inactive in steady darkness and during maintained illumination, and it is active only briefly (for less than one sec) following a step change in illumination. The data support the premise that this input derives from transient amacrine cells.

This work was supported by NIH grants EY 01653 and EY 07022.

95.21 NEW CELL TYPES AT THE RETINAL GANGLION CELL LEVEL IN THE TURTLE, PSEUDEMYS. J.E. Fulbrook, J.H. Maxwell, and A.M. Granda. Inst. for Neuroscience, University of Delaware, Newark, DE 19711.

We have examined some of the spatial, temporal, chromatic, and movement-sensitive properties of retinal ganglion cells (RGCs) in the turtle. Over several years of single unit data collection, a classification of cell types has been made which includes most of the RGC types known in other vertebrates. In turtle, most receptive fields (RFs) had center and surround regions but RGC surrounds were generally silent and did not respond to flashed or moving stimuli. The RF organization of most RGCs was complex, usually asymmetric, and frequently mutable to changes in adaptation level, stimulus wavelength, or stimulus velocity. All of the cells were movement-sensitive. Most units were at least 50% more sensitive and responsive to moving rather than stationary stimuli. About 40% of the units were directionally-sensitive (DS). In addition to the above cell properties and types, several new RGC types have been characterized in the turtle. Here, we report on a few of these cell types.

LARGE-FIELD CELLS: Several cells (n=6) had RF sizes in excess of 25^{o} visual angle on any side. The largest RF was shown by a DS, ON-OFF cell with a crescent-shaped field about 75^{o} in length. Three large-field cells with only blue cone input were observed. These cells were oval-shaped, 20^{o}-40^{o} in width and length limits, gave weakly sustained ON responses to stationary, flashed stimuli (450 nm), were non-DS, and preferred slow-moving stimuli.

ANNULAR CELLS: These cells (n=3) did not respond to any type of center stimulation but gave robust responses to surround stimulation, especially to stimuli moving less than 2.0^{o}/sec. Annular cell centers were circular, 5^{o}-6^{o} in diameter; surrounds were 2^{o}-3^{o} thick. Two cells were red-sensitive (640 nm), the other was green-sensitive (560 nm). Surrounds gave ON responses to stationary, flashed stimuli, required light adaptation for any response to occur, and were non-DS.

BAR-SHAPED CELLS: These cells (n=8) resemble the simple cortical cells seen in cat and monkey. Length-to-width size ratios ranged from 2:1 to 10:1. Most units were DS with the preferred-null directions arranged orthogonal to the long axis of the RF. The RF organization consisted of 1, 2, or 3 parallel, bar-shaped response regions which produced robust ON, ON-OFF, or OFF responses to moving or flashed stimuli. The RF organization of most bar-shaped cells was independent of adaptation level. Color opponency was not observed in any of the above cell types.

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95.22 MORPHOLOGY OF RABBIT RETINAL GANGLION CELLS, D.H. Rapaport, J.M. Provis*, and B. Dreher*. School of Anatomy, Univ. of New South Wales, Dept. of Ophthalmology, Sydney Eye Hosp., and Dept. of Anatomy, Univ. of Sydney, Sydney, Australia.

Clear correlations have been established in the cat retina between physiological and morphological classes of ganglion cells. A number of functional classes of rabbit retinal ganglion cells have been described physiologically, and while some of these seem to be analogous to those distinguished in the cat, others are quite different.

The morphology of rabbit retinal ganglion cells was examined in both Golgi stained and HRP filled whole-mounted retinae. The data obtained with either method were essentially the same, so we can be confident that we are not including displaced amacrine cells in our ganglion cell classification. Class 1 cells had large cell bodies (20-40μm dia.), thick axons (0.8-2.0μm dia.) widely extending dendritic fields (200-660μm dia.), usually arising from 3-5 thick primary dendrites branching radially. These cells remind us of the alpha cells of the cat retina, and therefore are likely to be the morphological counterparts of rabbit Y cells. Class 2 cells had small to medium somas (10-24 μm dia.) and large dendritic fields (250-680μm dia.), in many ways they seemed continuous with Class 1 cells, but they had distinctly thinner primary dendrites which spread radially from the soma and branched somewhat less often. Class 3 cells were clearly distinct from 1 and 2, although soma and dendritic field diameters overlapped considerably with those of the other two classes (13-30μm soma dia.; 100-500μm arbor dia.). The dendritic tree of Class 3 cells formed a meshwork of highly branched, thin, wavy dendrites around the soma. Both Class 2 and 3 cells had finer (0.4-0.8μm dia.) axons than those of Class 1 cells.

Within each morphological class a wide range of soma and dendritic field diameters are present, and there is only a slight correlation between these two parameters. Class 1 cells in temporal retina were larger than their counterparts in other retinal areas, but we have yet to notice any systematic variation in soma or dendritic field diameter within a class that can be related to distance from the visual streak.

A small proportion of cells were found which did not fit easily into our classes. If they represent truly separate classes their scarcity in our sample suggests that they form only a small proportion of the overall ganglion cell population.

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95.23 RETROGRADE LABELING OF GANGLION CELLS AND THEIR DISSOCIATION FROM THE MOUSE RETINA. G.W. Balkema and P.V. Sarthy. Dept. of Ophthalmology, Univ. of Wash., Seattle WA 98195.

We have labeled ganglion cells in the mouse retina by retrograde transport of fluorescent dyes injected into the brachium of the superior colliculus. In animals injected with the fluorescent dyes, DAPI (4'-6-diamidino-2-phenylindol-2HCl) and Primulin, and allowed to survive for 1-2 days, the whole mounts showed labeled somata across the entire retina. While Primulin appeared as bright yellow specks throughout the ganglion cell cytoplasm often filling the dendritic processes, DAPI fluorescence was confined mainly to the nucleus. In transverse sections almost all of the labeled cell bodies were found in the ganglion cell layer; occasionally, however, some labeled cells were found to be displaced into the inner plexiform/inner nuclear layer. As in the whole mounts, DAPI fluorescence was limited to the nucleus while the cytoplasm and dendrites showed Primulin fluorescence.

When animals were allowed to survive longer than 2 days, blue DAPI label could be occasionally found in cell somata within the inner nuclear layer. This DAPI fluorescence was less intense than the label found in the ganglion cells. In addition, no Primulin specks were found within the cytoplasm of these cells.

Other mice with similar injections (survival time=2 days) were enucleated and the labeled retinae were dissociated into single cells by papain treatment and mechanical dissociation. In the dissociated cell suspensions we found about 1,000-5,000 labeled cells per retina. These cells had a large labeled nucleus with a thin layer of surrounding cytoplasm. Cells with bipolar, amacrine, photoreceptor or Muller cell morphology never showed any label. During our search for fluorescent cells, we looked extensively for cells which had DAPI/Primulin fluorescence as well as ganglion cell-like morphology; we failed to find them. All brightly fluorescent cells showed only a rounded morphology.

These experiments establish that ganglion cells labeled by retrograde transport of DAPI/ Primulin retain their fluorescence even after dispersion by papain treatment and mechanical dissociation.

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95.24 CONTRALATERAL AND IPSILATERAL RETINAL PROJECTIONS IN NORMAL GOLDFISH. A. D. SPRINGER. Dept. of Anatomy, New York Med. Coll. Valhalla, N.Y. 10595.

Cobaltous-lysine applied to the distal stump of a severed optic nerve was used to study the retinal projections of normal adult goldfish. Both the termination areas of optic axons, as well as the pathways they traveled were established. Contrary to previous descriptions of the goldfish visual system, the optic nerves do not decussate completely at the optic chiasm. Fascicles that entered the ipsilateral optic tract innervated targets in the ipsilateral thalamus and optic tectum. Other optic fibers crossed the posterior commissure from the contralateral side of the brain and also innervated the ipsilateral tectum and thalamus. In addition, optic fibers bilaterally innervated a hypothalamic target in close proximity to the infundibulum that may correspond to the nucleus lateralis tuber. The contralateral preoptic region contained two discrete areas of innervation, each served by separate fascicles. The ipsilateral preoptic region was similarly innervated, but more sparsely. Fibers that entered the contralateral ventral thalamus originated from three fascicles and terminated in three distinct targets. In contrast, three targets in the contralateral dorsal thalamus were served by one fascicle, and fibers passed from one nucleus to the other two. Innervation of the ipsilateral thalamus was similar to that seen contralaterally. Each main optic tract divided into three tracts, two of which entered the optic tectum, while the other innervated several pretectal areas. Other fibers innervated an accessory optic nucleus located near nucleus glomerulosus. The contralateral tectum contained numerous radially oriented optic fascicles. These fascicles represented optic fibers that left thalamic and pretectal targets to enter the optic tectum from beneath the stratum periventriculare. Optic fibers were also observed in the transverse commissure, tractus rotundus, horizontal commissure, tectobulbar tract and fasciculus retroflexus. Therefore, it appears that many of the anomalous projections seen after tectal ablation or after optic nerve crush are not in fact aberrant. Such projections probably reflect the presence of unusually large numbers of optic fibers in tracts that normally contain optic axons, as well as increased innervation of areas that normally receive sparse retinal projections Filled tectal cells that could represent cells projecting to the retina were not observed in either tectal lobe. The ipsilateral retinal projections could not be attributed to cobaltous-lysine being transneuronally tranported in readily detectible amounts.

95.25 THE OPTIC NERVE OF THE NORTH AMERICAN OPOSSUM: AN ELECTRON MICROSCOPIC STUDY. M.A. Kirby*, L. Clift-Forsburg*, S.C. Rapisardi, and P.D. Wilson. Dept. of Psychology, University of California, Riverside, CA 92521; Dept. of Anatomy, Howard University, Washington, D. C., 20059.

The retinal ganglion cell population (from retinal whole mounts) of the North American opossum (Didelphis virginiana) has been estimated at approximately 101,000 (Rapaport, Wilson, and Rowe, J. Comp. Neur., 199, 1981, In Press). This value differs from an earlier light microscope study of the optic nerve of this species in which the total fiber population was estimated at 82,100 (Brusch and Arey, J. Comp. Neur., 77:631,1942). Analysis of the fiber population of the optic nerve of this species was undertaken (three cross sections from two animals) in order to examine this apparent discrepancy.

Electron microscopic (EM) examination of the optic nerve indicated a mean total count of 103,000 fibers (range: 91,850 to 122,742), of which less than 2% were unmyelinated. Diameters of myelinated fibers ranged between .25 um to 6.2 um (mode at .85 um) while unmyelinated fibers ranged between .25 um to 2.13 um (mode at .53 um). Frequency distributions of fiber diameters were found to be unimodal and positively skewed.

Our estimate of 103,000 fibers is within 2% of the estimated retinal ganglion cell population reported for whole mounted retinas (Rapaport, et al., 1981). This estimated fiber population represents a value 31% larger than the previous light microscopic study of Didelphis virginiana, a difference attributed to the inability of resolving small diameter myelinated and unmyelinated fibers at the light microscopic level. In addition, the present finding of a less than 2% unmyelinated fiber population contrasts sharply with the previous light microscopic estimation of 33%. We are currently exploring the possibility that the large estimated unmyelinated population of the earlier light microscopic study may have resulted from examination of the optic nerve sectioned in the region of the lamina cribrosa, as has been previously proposed (cf. Hokoc and Oswaldo-Cruz, J. Comp. Neur., 178:773, 1978).

In a similar EM study of a closely related subspecies, the South American opossum (Didelphis marsupialis aurita), the fiber population of the optic nerve was estimated at 74,700 of which approximately 20% were unmyelinated (Hokoc and Oswaldo-Cruz, 1978). Therefore, based on EM examination of the optic nerve fiber populations and light microscopic study of retinal ganglion cell populations, it would appear that major differences are evident in both the myelinated and unmyelinated fiber populations of these two closely related subspecies. (Supported by University of California Research Grant, and NIH Grant #RO1-EY02953-03.)

96.1 A PROCEDURE TO ENHANCE AUDITORY BRAINSTEM RESPONSE RELIABILITY IN UNRESTRAINED, UNANESTHETIZED RATS. Julia A. Lee*, Richard A. Abbott*, Donald W. Nielsen and Robert F. Berman. Psychology Dept., Wayne State University and Otological Research Laboratory, Henry Ford Hospital, Detroit, Michigan, 48202.

Reliability of the auditory brainstem response (ABR) is achieved through control over stimulus variables including rate, polarity, intensity, and frequency spectrum. Traditionally, control over the stimulus is achieved by anesthetizing the animal and delivering the stimulus through hollow ear bars. However, it is not always possible to anesthetize or restrain the animal. For instance, if the ABR is used as an electrophysiological measure of drug effects, there could be drug-anesthetic interactions, without apparent effects of the anesthetic alone. Restraint is a stressful procedure that may interfere with the pharmacological agents being studied. One solution to these difficulties has been to present the stimulus free-field, a procedure with less control over the stimulus reaching the ear. Even though sound pressure levels throughout an empty chamber may vary minimally, sound pressure levels at the ear do change as a function of body position. It is possible to wait for an animal to achieve a certain orientation before recording. However, the drug being studied could affect posture by altering muscle tone, so that drug and no-drug postures would differ. With our procedure these concerns are minimized. Distance between sound source and ear is standardized, and the stimulus is monitored at the entrance to the ear. The speaker-ear distance is held constant by attaching a miniature electroacoustic transducer to the rat's head. The base for the speaker unit is mounted permanently to the acrylic supporting the electrode connector. The speaker itself is fastened in the same position for each rat prior to ABR recording. The same transducer is used for all subjects. Stimulus features, including sound pressure level, time waveform, and frequency spectrum, are measured at the ear entrance via a small plastic tube. The tube is inserted, under general anesthesia, subcutaneously so that one end terminates in the entrance to the ear canal, and the other end is secured to the acrylic platform at the top of the head. At the latter end, a small, calibrated, probe microphone is inserted to measure stimulus characteristics. Since the ABR appears to be influenced by temperature, brain temperature is also measured. With our procedure it is not necessary to anesthetize the animal or wait for the animal to assume a certain orientation. It is necessary to wait for the rat to stop moving; therefore, the rat is observed on a video screen, and the EEG is monitored on an oscilloscope.

96.2 HYPOTHERMIA EFFECTS ON CAT BRAINSTEM AUDITORY EVOKED RESPONSES. G.T. Rossi and R.H. Britt. Neurosci. Dept. and Neurosurg. Div. R-155, Stanford Sch. of Med., Stanford, CA 94305 and Palo Alto Veterans Hospital, Palo Alto, CA 94304.

Five pentobarbital anesthetized cats were surgically prepared and placed on total cardiopulmonary bypass for periods of up to 8 hours. Systemic hypothermia was achieved by slowly cooling the bypass blood from 37° C to 19° C through a water heat exchanger. Brainstem auditory evoked responses (BAERs) to monaural clicks (90 dB, 10 per sec) were filtered (150-3 KHz) and averaged from differential recordings between vertex and mastoid electrodes. Arterial pressure controlled by the rpm speed of the bypass pump head (Bio-Medicus user specified) was maintained above 50 mm Hg. Temperatures were recorded from 3 thermocouple probes (Bailey) located in the left parietal lobe and in the arterial and venous return cannulas and general purpose temperature probes (Hewlett-Packard) in the esophagus and rectum. Esophageal rather than rectal temperature was found to be the more accurate indicator of temperature of the brain. Serial BAERs were recorded at 1 minute intervals as brain temperature was lowered from 37° C to 22° C and then rewarmed to 37° over a 1 hour period.

Two significant effects were produced by controlled systemic deep hypothermia. First, the latencies of each BAER wave (I-V) were linearly increased as brain temperature was lowered to 26° C. Latencies of early waves (I-III) increased less than those of late waves (IV-V) in the range from 0.7 msec for wave I to 4.5 msec for wave V. Plots of latency versus temperature for each wave are linear to first approximation. Below 26° C this function becomes non-linear. Differences do occur between the slopes (latency in msec/° C), calculated to be 0.08, 0.17, 0.19, 0.26, and 0.30 for waves I-V, respectively. Second, the wave peak amplitudes were initially increased, then decreased as brain temperature was lowered. Amplitudes increased to their maximum values between 32°-33° C; then decreased at a similar rate to 26° C for all waves and were lost completely below 26°-23° C (brain temperature). Amplitude-temperature plots for waves I-V appear to: 1) have a somewhat parabolic shape; 2) have nearly linear decreases in amplitude occurring after the maximum values and 3) have similar slopes (μV/° C) between waves. Gradual rewarming restored the BAER to its original latencies and amplitudes. The same results were repeated for sequential cooling and rewarming.

This study suggests that the BAER does not significantly change in configuration over a wide temperature range. Analysis indicates that the increase in latencies and the decrease in amplitudes of the BAER wave components can be expressed as mathematical functions over the temperature range of 37° to 26° C. Loss of the BAER occurs abruptly with temperatures below 26°-23° C.

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96.3 GENERATORS OF THE FREQUENCY FOLLOWING RESPONSE. LESION STUDIES. R.L. Davis* and R.H. Britt (SPON: W.D. Neff). Div. Neurosurg., R155, Stanford Med. Sch., Stanford, CA 94305 and Palo Alto Veterans Hospital, Palo Alto, CA 94304.

An ongoing series of lesion experiments (15 to date) are designed to identify the generators which contribute to the amplitude and complexity of the frequency following response (FFR) in the cat. Histologically confirmed lesions were made in areas of the afferent auditory brainstem pathway by aspiration or radiofrequency thermocoagulation. The brainstem auditory evoked response (BAER) elicited with click stimuli was monitored throughout the experiments as an estimate of the extent of each discrete lesion. The FFR was elicited with 11 ms tone bursts (2 ms rise-fall time) presented through shielded earphones at 10 per sec. Farfield BAER and FFR responses (bandpass filtered from 150 to 8K Hz) were averaged from differential electrodes placed at the vertex and mastoid. Round window electrodes were used to record the cochlear microphonic, N1 and N2 responses.

Sequential lesions of inferior colliculi and superior olivary complexes on both sides resulted in a 500 Hz waveform envelope which maintained 50 to 75% of the baseline amplitude. Preliminary findings from similar experiments demonstrated that the 500 Hz waveform maintained 100% of the baseline amplitude when only the cochlear nucleus was lesioned; care was taken to not disrupt blood flow to the cochlea. Assuming that the cochlear nucleus was completely transected (as evidenced by the the waveform change in the BAER) these results suggest that the amplitude decreases observed after the higher level auditory brainstem structures were lesioned can be attributed to factors other than the loss of FFR neural generators. Although lesions of the cochlear nucleus did not substantially affect the waveform amplitude at any of the frequencies, a considerable decrease in waveform complexity was observed at 500 Hz. This finding suggests that the complexity of the waveform is due to neural activity in the cochlear nucleus superimposed on a cochlear microphonic sinusoidal waveform. Lesions peripheral to the cochlear nucleus which also disrupted the cochlear blood supply resulted in dramatic decreases in FFR amplitude at all test frequencies. The residual FFR and CM were completely abolished after the ipsilateral cochlea was destroyed indicating that the recordings were not influenced by stimulus artifacts.

These lesion studies suggest that the major amplitude component of the FFR lies peripheral to the cochlear nucleus, most likely from the cochlear microphonic. The complexities of the 500 Hz waveforms observed at high stimulus intensities are thought to originate from cochlear nucleus activity.

[Supported by VA Merit Review and NIH (NS15860) Grants]

96.4 EFFECTS OF ANESTHETICS ON THE BRAINSTEM AUDITORY EVOKED RESPONSE. M. Cohen* and R. Britt (SPON: R. Dooling). Div. of Neurosurg. R155, Stanford Medical Center, Stanford, Ca. 94305 and Palo Alto Veterans Hospital, Palo Alto, Ca. 94304.

A parametric study was performed comparing the effects on the brainstem auditory evoked response (BAER) of four anesthetic agents commonly used in the operating room and in the laboratory. Sodium Pentobarbitol, Ketamine, Halothane and Chloralose were administered individually to each of four cats in a different sequence. Click-evoked BAER's were compared for differences in waveform configuration, latencies and amplitudes of the component peaks, five of which (I-V) could be identified in each animal and with each anesthetic agent. There were no statistically significant differences between anesthetic agents in mean latencies of the component peaks. Amplitude differences between animals using the same anesthetic agent were too large to be compared meaningfully. This study is consistent with the use of the BAER as a useful means of monitoring the integrity of the ascending auditory pathway with little ambiguity added by the use of a variety of parenteral and inhalation anesthetic agents. (Studies supported by VA RAG/Merit review and NIH(NS 15860) Grants and the Neurosurgery Research Fund)

96.5 CHANGES IN THE AUDITORY EVOKED POTENTIALS OF THE NUCLEUS ACCUMBENS AND AMYGDALA INDUCED BY REPEATED DOSES OF d-AMPHETAMINE. C. C. Turbes, G.T. Schneider*, R. J. Morgan and J. M. Simard*. Dept. of Anatomy, Creighton Univ. Sch. of Med., Omaha, NE 68178 and Dept. of Biophysics and Physiol., Colorado State Univ., Fort Collins, CO 80521.

Slow wave brain potentials flowing in the extracellular space are important since they can be shown to alter the excitability in neuronal populations. There may be a substantial class of cooperative neural interactions which depend on synaptic and non-synaptic influences that act on synchronizing and desynchronizing mechanisms in the brain.

In these studies we investigate the action of repeated doses of d-amphetamine on auditory evoked potentials (AER) of the nucleus accumbens septi and the basal nucleus of the amygdala. The AER of the two brain regions are compared using cross correlation analysis. Data from eleven cats are used in these experiments. Recordings are made with a polygraph and an FM tape recorder using hardwire and radio telemetry methods. Auditory evoked potentials are selected and analog to digitally converted. The Fast Fourier Transform (FFT) algorithm processing is done in the frequency domain. With three repeated daily doses, there is a progressive decrease of percent normalized cross correlation. The action of d-amphetamine is associated with increasing time delays (tau) between the wave potential trains of the nucleus accumbens and the basal nucleus of the amygdala. There are also shifts in average taus, the wave trains of the nucleus accumbens leading in pre-drug period. The basal amygdala leads during the action of d-amphetamine. It is apparent that d-amphetamine at dosages used alters electrochemical processes that have a rôle in synchronization and desynchronization mechanisms of neuronal assemblies that process sensory information and function in behavior.

96.6 THE T COMPLEX OF THE HUMAN AUDITORY EVOKED POTENTIAL (AEP): RATE DEPENDENCE. S. Satya-Murti*, J.R. Wolpaw, C.A. Schaffer* and A.T. Cacace*. (SPON: R.S. Bourke). Dept. of Neurology, Albany Med. Coll., Albany, NY 12208 and Div. Labs. Res., N.Y.S. Dept. Hlth., Albany, NY 12201.

The AEP recorded from the temporal scalp contains components probably produced in auditory cortex, an 80 ms positive peak (TP 80) and a 120 ms negative peak (TN 120). These waves, designated the T complex (TC), are simultaneous with the conventional vertex potential (VP) which dominates the AEP over most scalp regions. It is unresolved if the VP originates in auditory cortex, other areas of cortex and/or subcortical regions, and if it is separate in origin from the TC. To date, the TC has been studied only at slow stimulation rates. We studied the rate dependence of the TC and compared it to that of the VP to determine 1) if the TC may be reliably recorded at faster rates and 2)if the TC and the VP display different rate dependence which could aid in their separation.

Subjects were 6 adults, ages 22-36. The stimulus was a binaural click (0.5 ms pulse, 66 dB above HTL) delivered at rates of 2, 1, and 0.3/s in successive runs followed by a second 2/s run (to monitor stability). AEPs were recorded from Cz, Fp/z (halfway between Fp and Fz), T3/5 (halfway between T3 and T5), and T4/6, all referred to a balanced noncephalic reference. A Nicolet-Apple system sampled 1 pt/ms and averaged 150 (all rates) and 300 responses (2/s rate). VP latency and amplitude were measured from the Cz AEPs. The bipolar AEPs, T3/5-Fp/z and T4/6-Fp/z, were constructed off-line. The TC latency and amplitude were measured from the bipolar AEPs. Topographic studies indicate that these bipolar AEPs show the TC in relative isolation.

The TC amplitude was 6.8 (±2.3) uV, and the VP amplitude 11.5 (±3.3) uV at .3/s. At higher rates both TC and VP were markedly smaller, by 35 (±16)% and 34 (±9)% at 1/s and 73 (±17)% and 66 (±16)% at 2/s respectively. Neither TC nor VP latencies showed consistent dependence on stimulus rate, though TP 80 occurred slightly later in 3 of 6 subjects at higher rates. VP latencies displayed more intra-individual variability than did TC latencies.

Results indicate that 1) relatively slow rates of stimulation, such as 0.3/s are preferable for recording the TC since, like the VP, it is much smaller at faster rates and 2) there is no marked difference between the rate dependences of TC and VP.

96.7 SIMULTANEOUS RECORDING OF PHOTOPIC ELECTRORETINOGRAMS AND VISUAL EVOKED RESPONSES BEFORE AND AFTER RETINAL DAMAGE BY MONOCHROMATIC LIGHTS.
R.B. Rosenberg*, R.S. Crockett and T. Lawwill* (Spon. K. Reid) Depts. of Physiol. & Biophys. and Ophthalmol. University of Louisville, Health Sci. Cen., Louisville, Ky. 40202.
Photopic electroretinogram (ERG) b-waves and visual evoked responses (VER) were simultaneously recorded by computer assisted signal averaging from Rhesus and Cynomolgus monkeys to determine the functional effect of intense monochromatic light exposure. The stimulus was a bar-pattern alternating at 1 Hertz. The dark and light bars had a 1.6 log contrast and a retinal spatial frequency of 0.367 cycle per degree. The bar-pattern was illuminated with 550 nm light providing a corneal irradiance of 0.1 $uWcm^{-2}nm^{-1}Sr^{-1}$ (5 x 10^4 Trolands). A round 38.1° test field was centered upon the macula. After pre-exposure testing the eyes were exposed for 4 hrs to monochromatic laser light of 457.9 nm (5 $mWcm^{-2}$, 4 eyes), 514.5 nm (10, 15, and 20 $mWcm^{-2}$, 2, 1, and 2 eyes, respectively) and 590 nm (20 and 40 $mWcm^{-2}$, 2 eyes each). Laser irradiances chosen were near those required to produce non-thermal or photochemical threshold retinal injuries as defined by scotopic ERG, histology and indirect ophthalmoscopy. The pre-exposure ERG b-wave amplitude was 7.0 ±0.14 uV (SEM). Pre-exposure VER was 55.4± 1.54 uV (SEM). Significant reductions in b-wave amplitude ($p< 0.05$) were found after the 457.9 nm and after the 40 $mWcm^{-2}$ 590 nm exposures. Significant reductions in VER were found after the 457.9 nm and after the 20 $mWcm^{-2}$ 514.5 nm exposures. These results are consistent with the ERG and VER changes expected after photochemical damage to the retina. Under some conditions the VER may provide a more sensitive measure of retinal insult than the ERG.

96.8 ENHANCED SOMATOSENSORY EVOKED POTENTIALS ADJACENT TO FOCAL INJURY OF RAT CORTEX. R. T. Linn, D. M. Feeney, M. G. Boyeson, W. G. Dail and H. M. Murray. Departments of Psychology and Anatomy, University of New Mexico, Albuquerque, NM 87131.

Somatosensory evoked potentials (SEPs) were recorded following stimulation of the forepaw (FP) or hindpaw (HP) in acute barbiturate-anesthetized rats with either a craniotomy (control) or a unilateral undercut laceration of the cortical HP somatosensory area. Animals were studied in independent groups immediately post or at 1, 4, 15 and 30 days post injury. Recordings were made simultaneously from both the HP and FP primary projection areas.

In control animals HP stimulation produced SEPs in contralateral HP cortex with two positive peaks (P1 at 7-8 msec; P2 at 25-34 msec) and two negative peaks (N1 at 15-17 msec; N2 at 55-75 msec). These were absent immediately post laceration and never returned to the undercut cortex.

In control animals the HP SEPs recorded from the FP area were smaller in amplitude and similar in waveform to those recorded in HP area. In injured animals, beginning at one day post (L+1) and continuing through 30 days post (L+30) injury, the HP SEPs recorded in FP area (adjacent to undercut cortex) consisted of two, sharp, high amplitude positive peaks (P1 at 13-17 msec; P2 at 22-23 msec). These responses were never seen in controls.

Normally, the FP SEP recorded in contralateral cortex was bimodal with two positive peaks (P1 at 13-16 msec; P2 at 19-23 msec) and two negative peaks (N1 at 15-18 msec; N2 at 45-70 msec). Immediately post laceration of the HP cortex the N2 component of FP SEP was greatly increased in amplitude. An enhanced FP SEP was present in L+1 through L+30 preparations. The amplitude of this FP SEP increased linearly with time post injury, reaching a maximum of 1 mV at L+30.

This abnormal, high amplitude SEP produced by stimulating HP or FP recorded in contralateral (injured) cortex was consistently found in a circumscribed location which corresponded to the center of the FP projection region at the edge of undercut cortex. The waveform of this response after HP stimulation was identical to that seen with FP stimulation, suggesting that the same generator sources were involved. Although a denervation supersensitivity hypothesis could explain the increasing amplitude of the SEP over time, such an explanation is not congruent with the immediate appearance of the enhanced response. The rapidly appearing effect may be related to a release of the FP area from inhibition and also may represent an "unmasking" of HP afferents to FP cortex. Supported by NIH Grant NS 13684-03 and NIMH National Research Service Award MH15142-04.

96.9 PERONEAL EVOKED RESPONSE IN THE DOG - EFFECTS OF SPINAL CORD LESIONS. D.H. York, R. Gaines* and C. Watts. Dept. of Physiology & Surgery, University of Missouri, Columbia, MO 65212.

The question of which specific sensory tracts carry signals ultimately constituting the scalp recorded peroneal-evoked cortical response (PECR) is of considerable interest in prognostic applications of evoked potentials in spinal cord injury. The present study was undertaken in 12 adult mongrel dogs of either sex anesthetized with either pentobarbital or halothane-oxygen. A thoracic laminectomy was performed with the removal of T dorsal process exposing the spinal cord. The PECR was recorded from two scalp pin electrodes, one inserted at the occipital promience on the midline (positive electrode) and one 3 cm. anterior to this site (negative electrode). A ground electrode was placed on the upper forelimb. The signal was amplified by 104 through a bandpass of 1-250 Hz and averaged by a Nickolet CA-1000 for 36-64 stimulus presentations. Stimulus consisted of square wave pulses (100 μsec duration, 1 pulse/2 sec) of constant current (8-12mA), delivered to the peripheral nerve through pin electrodes placed 4 cm apart inserted through the skin of the lateral aspect of the distal femur. The stimulus strength was adjusted to just produce a toe twitch consistently with each stimulus. The PECR response consisted of three waves which were reproducible across animals. The initial response was a positive going wave at 18.2±1.5(SD) msec followed by a larger negative wave at 26.6±2.1(SD) msec followed by a smaller positive wave at 42.8±2.0(SD) msec. In some animals consistent waves at longer latencies were also evident. Transection of the dorsal colums in 3 animals by scalpel cut 3-4 mm deep resulted in a transient loss of the PECR, but quickly returned to previous values within 5-10 minutes. Histological sections of the spinal cord confirmed total transection of the dorsal columns. Upper quadrant spinal cord section ipsilateral to the leg stimulated was also ineffective in abolishing the PECR response. An ipsilateral cord hemisection, or combined with a contralateral upper quadrant section was also without effect. However, total transection of the spinal cord abolished the PECR response. Thus the presence of an intact anterior quadrant contralateral to the leg stimulated appears sufficient to maintain the PECR response. Further experiments are underway to more precisely define which specific areas of the anterior spinal cord are necessary for conduction of the PECR response.
(We are most grateful to Humberto Caiaffa, MD of Barranquilla, Columbia for his surgical expertise in these experiments.)

96.10 FAR FIELD SOMATOSENSORY EVOKED POTENTIALS IN THE CAT. M. A. Cordova-Salinas*, J. G. Blackburn*, T. H. Boyer*, and S. Katz. Department of Physiology, Medical University of South Carolina, Charleston, South Carolina 29425.

Far field somatosensory evoked potentials have been previously described in the cat in response to percutaneous stimulation of the forelimb. The purpose of this study was to describe the far field potentials obtained by stimulating the hind limb of the cat.

Adult cats were anesthetized with nitrous oxide and paralyzed with pancuronium bromide. The left superficial peroneal nerve was stimulated percutaneously at a frequency of 3 Hz, 0.1 msec pulse duration and 8 mA intensity. Far field potentials were recorded from a steel screw electrode located on the mid-line of the skull at AP=0. The reference electrode was placed on the ipsilateral ear and the ground on the contralateral ear. The potentials were amplified, filtered (300 Hz to 3 KHz), summated with a microprocessor (2048 sweeps) and displayed on a digital plotter.

Five stable potentials were identified preceding the cortical component of the somatosensory evoked potential. The mean latencies (± SEM) for each of these components were:

I	5.8 ± 0.01 msec
II	7.5 ± 0.04 msec
III	8.2 ± 0.20 msec
IV	10.4 ± 0.10 msec
V	12.6 ± 0.30 msec

With the exception of component V, these values are in close agreement with latencies reported by other investigators for forelimb evoked responses, taking into account differences in conduction distances. The apparent stability of these responses indicates that far field potentials may be more useful than near field somatosensory evoked potentials in studying the effects of trauma in the central nervous system. (Supported by NINCDS 3P50 NS11066)

96.11 EVOKED POTENTIALS WITH SPINAL STIMULATION. J. Myklebust,* A. Sances, Jr., J. Cusick and S. Larson. Neurosci. Res., USVA Med. Ctr., Wood, WI 53193 and Dept. Neurosurg., Med. Coll. Wis., Milwaukee, WI 53226.

Studies in the rhesus monkey were conducted to compare the characteristics of averaged evoked potentials recorded rostrally due to lower thoracic dorsal column spinal cord stimulation (1-800Hz) with those produced by peripheral stimulation.

Potentials recorded at the upper thoracic level with spinal stimulation demonstrated less complex waveforms than those with peripheral nerve stimulation. However, in either case, the responses were unaltered at stimulation rates up to 200-300Hz and followed with diminished amplitude up to 600-700Hz.

The potentials evoked at thalamic nucleus VPL and sensorimotor cortex with spinal stimulation are larger in amplitude and are recordable through a larger volume than those with peripheral stimulation. The waveforms are similar, and the responses behave similarly with increased stimulation rates. Late portions of the waveforms are reduced at 6-10Hz, while the primary components are intact at 20-30Hz and follow at reduced amplitude up to 50-60Hz.

A short latency spike is recorded in the anterior sensorimotor cortex. The latency is consistent with a conduction velocity of approximately 50-60m/s and is similar to that of the corresponding cortex-to-cord (corticospinal) response. The short latency cord-to-cortex potentials and the corticospinal potentials respond similarly to mechanical and vascular stress. Both the short latency cord-to-cortex and cortex-to-cord potentials are unaffected by increases in stimulus frequency up to 300-400Hz. They follow at reduced amplitude up to 700-900Hz.

The latency, waveform and frequency response imply that the short-latency potential is nonsynaptic. The behavior in ischemia suggests that it is dependent upon the integrity of a cell body. Although the spinal stimulation is applied over the dorsal columns, it is reasonable to hypothesize that spinal stimulation activates the pyramidal tract. While direct spinocortical afferent connections have been reported, this response is probably due to antidromic conduction in the pyramidal tract.

The evoked potentials recorded rostral to spinal stimulation are similar to those with peripheral stimulation. Although several neural systems may be activated, the response characteristics and the stimulus level used suggest that the responses primarily reflect activity in the dorsal columns and pyramidal tract, and that spinal stimulation is a useful experimental method to monitor the integrity of primary afferent and efferent systems.
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96.12 THE EFFECTS OF GRADED SPINAL TRAUMA ON SOMATOSENSORY EVOKED POTENTIALS IN CATS. J.G. Blackburn*, M. Cordova-Salinas*, R.K. Simpson, H.F. Martin and S. Katz. Department of Physiology, Medical University of South Carolina, Charleston, S.C. 29425.

The effects of graded spinal trauma on the somatosensory evoked potential were studied in adult cats anesthetized with nitrous oxide and paralyzed with pancuronium bromide. The superficial peroneal nerve was stimulated percutaneously at 0.2-0.3 Hz, 0.3 msec pulse duration and 7-10 mA intensity. Evoked potentials were taken from epidural electrodes over the contralateral leg area of the somatosensory cortex, summated with a microprocessor and displayed on a digital plotter. The reference electrode was placed on the ear contralateral to the monopolar recording electrode. Following control recordings (both before and after laminectomy) the spinal cord was subjected to impact trauma at the level of T_6. Trauma forces ranged from 150 to 220 gm-cm. Recordings were taken under acute (time of impact to 4 hours) and chronic (1 day to 2 weeks) conditions.

The SEP disappeared, then returned within 15 minutes at the 150 gm-cm trauma level. At 220 gm-cm, the SEP failed to return following impact injury. The responses between the 150 and 200 gm-cm levels were somewhat variable. However, an interesting phenomenon was observed in the SEP following trauma between 200-210 gm-cm. In some of the cats, the evoked potential disappeared, reappeared within one hour, only to disappear after several hours to 1 day following the injury. This unique response to trauma may serve as a model for future studies of spinal injury. Establishment of this model will make it possible to judge the efficacy of therapeutic intervention as determined by the duration of the period during which conduction returns following immediate loss. Extending this plateau of returned activity should provide a measure of the efficiency with which a chosen therapy retards or prevents the irreversible sequence of events reported to occur following spinal trauma. (Supported by NINCDS 3P50 NS11066)

96.13 Studies of Neural Generators of Lumbosacral Evoked Potentials in Man. P.J. Delwaide*, Milan R. Dimitrijevic, J.A. Halter*, L.D. Lehmkuhl*, Arthur M. Sherwood. Clinical Neurophysiology, The Institute for Rehabilitation and Research, Houston, Texas, 77030.

Electrical events arising from neural structures can be recorded from the skin over the lumbo-sacral portion of the spinal cord, following stimulation of peripheral nerves. A comparison with subdural recordings suggested that these potentials were generated in nerve roots and spinal cord. We have conducted a series of studies on intact, healthy adult humans to re-examine the origin of these potentials. For each trial, 128 responses to tibial nerve stimulation at the popliteal fossa were averaged from electrodes placed over the T12, L2, L4, and S1 vertebrae, referenced to an electrode at T6, while monitoring soleus EMG responses. Following control trials with single stimuli, test studies were made 1) with H-reflex recruitment techniques using double pulse separations from 2 to 150 ms, 2) conditioning of the H-reflex response with vibration, and 3), with a series of recordings in which the stimulus strength was systematically increased. The results of these studies showed that, in contrast to the previous assumption, the potentials recorded by the lower leads do not reflect simply electrical activity generated in spinal nerves nor do potentials recorded by the upper leads reflect electrical activity of a single spinal generator. We have found the second peak, or A wave, is not simply related to efferent output, but has some characteristics of a standing wave as well. In addition, the S wave, rather than being a simple homogeneous response, can be differentiated into two peaks under conditions of low stimulus intensity. Thus, it appears that the generators involved are minimally related to the peripheral neurogram, given the great distance and small fibers involved. Instead it is far more likely that the observed responses arise in several pools of neurons and their associated fibers.

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97.1 MORPHOLOGICAL DIFFERENCES BETWEEN THE CEREBRAL HEMISPHERES IN THE FEMALE RAT. M.C. Diamond, D. Young*, S. Sukhwinder Singh*, and Ruth E. Johnson* Department of Physiology-Anatomy, University of California, Berkeley, CA 94720.

We recently noted that throughout the lifetime of the male rat, the right cerebral cortex was thicker than the left. Specifically, this was true from birth to 650 days of age in areas 10, 4, 3, 2, 18, 17, 18a and 39, with areas 17, 18a and 39 reaching a statistically significant level. We measured the female's brain at one age only, 90 days of age, and found her left hemisphere was thicker than the right, in 8 out of 9 areas, but the results were nonsignificant in all areas. This intriguing finding led to the present group of experiments: to study if the left-right pattern was similar to the males in the early period of the life of the female.

Twenty micra, coronal, frozen, brain sections were taken from formol-saline perfused, female, Long-Evans rats at 7, 14, and 21 days of age (N=3-7 rats/area). The sections included the same areas as in the male studies. Cortical thickness was measured on thionin stained sections on microslide projected images.

At 7 days of age no dominant hemispheric pattern was noted in the 9 areas measured in the female, but by 14 and 21 days, the most consistent left-greater-than-right-pattern was evident in areas 18, 17, 18a and 39 with other areas showing less consistent differences.

Previously, we had ovariectomized the female at day-one and discovered that her cortical thickness pattern closely resembled that of the male by 90 days of age. More recently we ovariectomized the female at day-300 and examined her brain at 390 days of age. The right-left pattern in the female at 390 days of age is not as clearly left sided as at 90 days and ovariectomy does not significantly alter her pattern at this time. However, area 3 is consistently statistically significant in the left hemisphere in both the control and ovariectomized females. This finding was not evident in the 90 day old female.

These results support our previous work noting that sex steriod hormones alter cortical structure and indicate that the cortex is not as responsive to ovariectomy in the older animal as it is in the newborn.

97.2 SEX DIFFERENCES IN DENDRITIC STRUCTURE IN THE PREOPTIC AREA OF JUVENILE MACAQUE MONKEYS. William T. Greenough, David M. Ayoub*, and Janice M. Juraska. Depts. Psychol. & Anat. Sci. & Neural & Behav. Biol. Prog., Univ. IL, Urbana-Champaign, IL 61820 and Dept. Psychol., Indiana Univ., Bloomington, IN 47401.

While structural sex differences have been well documented in the rodent brain, particularly the preoptic area (e.g., Gorski, et al., J. Comp. Neur., 193:529, 1980; Greenough et al., Brain Res. 126:63, 1977; Raisman & Field, Sci. 173:731, 1971), fewer studies have examined such differences in the monkey brain. We have studied the preoptic area in juvenile macaque monkeys since this area has been implicated in male sexual behavior (Slimp et al., Brain Res. 142:105, 1978).

We examined Golgi-Cox stained neurons in the preoptic area (defined as a rectilinear solid extending 0.2mm anterior to anteriormost midline anterior commissure (AC), 0.2mm posterior to posteriormost midline AC, ventrally from AC to a distance equal to 40% of the distance from midline corpus callosum to midline AC, and laterally beginning 0.3mm from the third ventricle and extending to a distance equal to half the distance to medialmost internal capsule, to adjust for brain size differences) of 4 male and 4 female Macaca fascicularis which were 8-9 months old. All well-stained neurons not seriously truncated by sectioning (at 100μ) were included in sample, 980 neurons from males and 809 neurons from females. Camera lucida drawings of neurons were categorized by soma shape (round, oblong, intermediate) and analyzed in terms of spine density ($\leq$ .1/μ,>.1/μ to .5/μ,>.5/μ), dendritic thickness ($\leq$ or >.2μ, thickest dendrite at one soma diameter from soma) and cell size. Dendrites were analyzed in terms of lengths of branches and numbers of bifurcations.

Neurons of males had approximately 20% more bifuractions than those of females ($p<.01$). This difference was most pronounced in intermediate shaped cells, although a tendency in this direction was also apparent in the oblong cell type. There was no sex difference in mean dendritic length per neuron overall, nor were there differences related to soma shape. Females also had a higher proportion of sparsely spined neurons. This difference (significant overall) was specific to large and oblong cells and not evident in small and round cells. Dendritic density distribution, which differs by sex in the hamster (Greenough et al.) is currently under investigation, since a difference in numbers of bifurcations without a difference in total dendritic length suggests a difference in dendritic pattern.

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97.3 EXPERIMENTAL SEX REVERSAL OF THE SEXUALLY DIMORPHIC NUCLEUS IN THE PREOPTIC AREA OF THE FEMALE RAT BRAIN. A. Coquelin, K.D. Döhler*, R.A. Gorski and J.E. Shryne*. Dept. of Anatomy and Lab. of Neuroendocrinology, Brain Research Inst., UCLA School of Medicine, Los Angeles, Ca. 90024.

There is a morphological sex difference within the medial preoptic area of the rat brain (Gorski et al., Brain Res. 148: 333, 1978). The volume of an intensely staining component, now called the Sexually Dimorphic Nucleus of the Preoptic Area (SDN-POA), is several fold larger in adult male rats as compared to that of females. The development of this nucleus depends largely, if not totally, on the hormonal environment during the critical period of sexual differentiation. Treatment of neonatal females with a single injection of 1 mg testosterone propionate (TP) increased the volume of the SDN-POA, but did not result in complete sex reversal. Neonatal castration of males reduced SDN-POA volume, but again did not completely sex reverse it (Gorski et al., 1978). The present study was designed to determine whether development of the SDN-POA is exclusively hormone dependent, or relies, in addition, on a genomic component. From day 16, pregnant Sprague-Dawley rats were injected sc daily with 2 mg TP in sesame oil or with oil only. After birth the pups were injected sc with 0.1 mg TP/day or with oil throughout the first 10 days of life. By giving multiple injections, which covered the total period of sexual differentiation, we tried to imitate as closely as possible the natural influence of testicular testosterone. At 60 days of age all rats were perfused with 10% neutral formalin. The brains were sectioned at 60 μm and stained with thionin. SDN-POA volume was determined as described previously (Gorski et al., 1978). The results are listed in the following table:

Sex (N)	Male(5)	Male(6)	Female(6)	Female(8)
Treatment	OIL	TP	OIL	TP
g Body Wt	278±11	232± 7	204± 6	209± 6
g Brain Wt/100g BWt	0.64±0.02	0.80±0.02	0.85±0.02	0.87±0.02
mg Gonad Wt/100g BWt	913±26	448±42	32.6±1.7	12.5±0.8
SDN-POA Vol ($mm^3 x10^{-3}$)	18.5±1.3	18.1±0.5	5.8±0.9	17.5±1.1

All TP treated females were anovulatory. The TP treated males had very small testes and 33% of them lacked spermatazoa. Oil treated rats showed the sexual dimorphism observed in previous studies. TP treatment resulted in complete sex reversal of SDN-POA volume in females, but it did not increase SDN-POA volume any further in males. These results demonstrate that differentiation of the SDN-POA seems to be totally dependent on the hormonal environment during the critical period of sexual differentiation. The involvement of a genomic component seems unlikely. (Supported by NIH grant HD 01182 and by the Heisenberg Program.)

97.4 FORMATION OF THE SEXUALLY DIMORPHIC NUCLEUS OF THE PREOPTIC AREA OF THE MALE RAT. Carol D. Jacobson, Fred C. Davis*, Erna Freiberg* and Roger A. Gorski. Dept. Vet. Anat., Iowa State Univ., Ames, Iowa and Dept. Anat., UCLA Sch. Med., Los Angeles, CA.

In the Sprague-Dawley rat, the Sexually Dimorphic Nucleus of the Preoptic Area (SDN-POA) is larger in volume in the male sex. This sex difference is at least partially dependent upon gonadal hormones during early postnatal life. Moreover, the neurons which reside in the adult SDN-POA can be specifically labeled by the prenatal administration of 3H-thymidine (*thy). We have used this fact to describe the pattern of formation of the SDN-POA during the perinatal period. A sperm-positive vaginal smear on estrus was defined as day 1 postfertilization (PF). On day 13PF, each pregnant rat was implanted with a right atrial cannula through which an injection of *thy (5μCi/g. B. wt.) was given on day 18PF. Fetal and neonatal male pups were sacrificed and perfused with neutral formalin either 2 hours after injection (day 18PF) or on day 20, 22, 24, 26, 28 or 32 PF (days 18, 20, 22 of gestation and 2, 4, 6 or 10 of postnatal life (PN), respectively). Autoradiograms were prepared on 6μm coronal sections. The location of labeled neurons within the MPOA was analysed during this period of formation of the SDN-POA. Two hours after injection of *thy, labeled cells are concentrated in the ependymal lining of the third ventricle (3V); the SDN-POA is not visible at this age. Two days after injection (day 20PF), there is a dense accumulation of labeled cells in the ventral aspects of the ependymal lining of the 3V and the adjoining region of the base of the brain dorsal to the optic chiasm (OC). From day 20 the SDN-POA can be identified. In general, labeled neurons are located in the more posterior parts of this nucleus. Four days (day 22PF) after *thy injection, labeled neurons are scattered between the OC, 3V and SDN-POA, but there are very few labeled neurons dorsal to the SDN-POA. From day 24PF through day 32PF the number of labeled neurons between the 3V and the SDN-POA decrease to such an extent that by day 10PN, labeled neurons are restricted to the region of the SDN-POA. Furthermore, the labeled neurons form a dense cluster within the nucleus. These data suggest that the SDN-POA is formed both by the migration and loss of neurons during the perinatal period. During the late prenatal period presumptive SDN-POA neurons migrate into the forming SDN-POA from the ventral aspects of the 3V and the base of the brain just dorsal to the OC. Moreover, death postnatally of neurons in the MPOA in general appears to contribute to the specific labeling o the SDN-POA. Future studies of the pattern of formation of the SDN-POA in the female as well as quantitative analyses of neuron al labeling in both sexes may elucidate the mechanisms which lea to this anatomical sex difference. NIH Grants HD-01182, RR07034

97.5 TRANSPLANTATION OF NEONATAL MALE PREOPTIC TISSUE INTO THE PREOPTIC AREA OF NEONATAL FEMALES INCREASES MASCULINE SEXUAL BEHAVIOR. G. W. Arendash* and R. A. Gorski. Laboratory of Neuroendocrinology of the Brain Research Institute and Department of Anatomy, UCLA School of Medicine, Los Angeles, California 90024.

Recent studies indicate that perinatal brain tissue can survive transplantation into a host brain. Since the medial preoptic area (MPOA) has been strongly implicated in the neural regulation of male sexual behavior, the MPOA of 1-day old male rats was transplanted stereotaxically into the MPOA of 1-day old female rats using a 0.7mm metal cannula for tissue punching and implantation (transplant volume about 0.2mm^3). Additional females received transplants of amygdala (Amyg) or caudate (Caud) tissue from male donors or were sham-operated. Immediately following bilateral transplantation, the recipients were injected sc with either oil or 8µg testosterone propionate (TP) in oil. After ovariectomy in adulthood, they were tested for female sexual behavior, then implanted sc with a testosterone-filled silastic capsule for subsequent male behavioral testing. Those females that received male MPOA transplants plus oil neonatally showed dramatically higher levels of masculine sexual behavior compared with those of the sham-oil or Caud-oil groups. Similarly, MPOA-implanted females given 8µg TP neonatally also had substantially elevated levels of copulatory activity compared to those of sham-TP controls. Not surprising in view of the modulatory influence exerted by the Amyg on male sexual behavior, some female hosts implanted with male Amyg tissue (plus oil or TP) showed behavioral scores comparable to those of MPOA-implanted animals. Female sexual behavior after minimal priming with estradiol benzoate (2µg EB/3 days) was markedly enhanced by the neonatal transplantation of male MPOA or Amyg in combination with 8µg TP. Interestingly, those females that received MPOA tissue plus oil neonatally (but not Amyg plus oil) also showed a significant, though less dramatic, increase in receptivity after minimal EB priming. Histological examination of the MPOA in all host brains indicated that tissue from the great majority of transplants survived and was characterized by apparently normal neurons. These studies show that the neonatal transplantation of male brain tissue bilaterally into the MPOA of female recipients can dramatically affect sexual behavior in adulthood, thus suggesting the development of functional connectivity between the transplanted neurons and host brain tissue. Note that the implanted tissue included the Sexually Dimorphic Nucleus of the MPOA, the development of which has been shown to be modified by gonadal hormones. Functional connectivity of exogenous neurons from this specific nucleus could contribute to the present behavioral observations. (NIH Grant HD-01182.)

97.6 THE EFFECT OF GONADECTOMY ON HALOPERIDOL-INDUCED CATALEPSY IN THE RAT. C.A. Hardy* and L.P. Spear. Dept. of Psychology, SUNY-Binghamton, Binghamton, N.Y. 13901.

Previous research has suggested a differential sensitivity to haloperidol-induced catalepsy during development. Periadolescent rats (P42) have shown enhanced cataleptic sensitivity to haloperidol when compared to weanling age (P30) or to young adult animals (P54) (Spear, Shalaby, and Brick, 1980). One possible explanation for the enhanced cataleptic response to dopamine antagonists during this period of development may be the state of hormonal fluctuation in the puberal animal. In the present study, this hypothesis was tested. Male and female Sprague-Dawley rats were either bilaterally gonadectomized or sham operated at weaning (P21). Each litter was assigned to be tested at either 28, 38, or 48 days of age. On the day of testing male and female, gonadectomized and control animals were given 0,1,2,or 4 mg/kg/2 cc haloperidol, IP. The first catalepsy testing session began 30 min from the time of injection and subsequent test sessions were spaced 30 min apart, for a total of 8 testing sessions over a period of 4 hrs. Testing was always conducted between 11:00 am and 4:00 pm. Each testing session consisted of individually placing the animal's forelimbs over a raised horizontal bar, adjusted for each age, such that the animal was at a 45° angle to the testing table. An animal was considered cataleptic if it remained stationary with both forelimbs over the horizontal bar for a period longer than 30 sec; 60 sec of catalepsy was the maximum amount of time allowed for each animal in all testing sessions. The total number of cataleptic test periods and total seconds of catalepsy were summed across the 8 testing sessions for each animal.

Results indicate a systematic decreasing sensitivity to the dose-dependent effects of haloperidol on cataleptic behavior with age. Moreover, adult male and female gonadectomized rats were consistently less cataleptic than their control counterparts, and adult males were less cataleptic than adult females. A similar trend was found in periadolescent rats, but not in weanling animals. These results indicate that gonadal hormones do appear to play a role in psychopharmacological sensitivity to haloperidol, but only in post-puberty animals that are sexually mature. Further implications of these results will be discussed.

97.7 PRENATAL CANNABINOID EXPOSURE ALTERS PITUITARY-GONADAL FUNCTION IN ADULT MALE MICE. S. Dalterio,* G. Chamness,* A. Bartke,* A. Ruiz* and D. Mayfield.* (SPON: W.B. Stavinoha) Depts. of Pharmacology and Ob-Gyn, The University of Texas Health Science Center, San Antonio, TX 78284.

Marihuana and its major psychoactive component, Δ^9-tetrahydrocannabinol (THC) have been reported to suppress the function of the pituitary-gonadal axis in male laboratory animals and in men. In our earlier studies, we have observed a decrease in plasma testosterone (T) levels in adult, immature and fetal male mice after THC exposure. In addition, perinatal exposure to THC or cannabinol (CBN), a non-psychoactive component of marihuana, results in long-term alterations in body weight regulation, pituitary-gonadal function, responsivity of vas deferens *in vitro* to norepinephrine and enkephalin and decreased sexual behavior in the adult (Subs. Alc. Act./Misuse 2:1, 1981). The present studies examined effects of prenatal exposure to THC or cannabidiol (CBD), a non-psychoactive cannabinoid, on pituitary-gonadal function in adulthood. Female mice received THC or CBD (50 mg/kg) or sesame oil (20 µl) daily by oral feeding starting day 18 of pregnancy until parturition, which occurred after 2 or 3 treatment days. Litters were culled to male pups. In adulthood, there were no significant differences on any parameter in controls dependent on treatment length. However, in cannabinoid-exposed males, testis weights were increased after 3, but not 2, prenatal treatments (366±23 THC; 318±16 CBD vs. 279±9 mg OIL; $p < .05$), although body weights were not different. In contrast, in animals prenatally exposed to CBD for 3 days, testicular weights were reduced and body weight was increased, so that testis:body weight ratio was decreased (6.9±.09 vs. 8.00±.08, $p < .02$). Concentrations of estrogen receptor (ER) in pooled hypothalami appeared to be reduced in 2-day, but not 3-day, THC-treated males; however, there were no effects of cannabinoid treatment on hypothalamic androgen receptors. The concentration of androgen receptors in seminal vesicles (SV) in all THC-exposed males was significantly reduced (105±13 vs. 160±20 fm/mg protein, $p < .05$), a finding which may be related to our observation of reduced SV weights after perinatal THC treatment. Post-castration, a single s.c. dose of 20 µg T appeared to reduce plasma LH levels to a greater extent in prenatally THC-treated mice compared to controls (62 vs. 46%), whereas plasma LH levels were reduced by only 19% in CBD-exposed males. Thus, cannabinoid effects on any specific parameter during sexual differentiation may depend greatly on the developmental events or timing of "critical periods" relative to drug exposure and may reflect both immediate changes in the hormonal milieu of the developing animal and/or alteration in receptor development which may affect later neuroendocrine function or target tissue responsivity.

97.8 SEXUAL DIMORPHISM IN THE DORSOLATERAL MOTOR NUCLEUS OF THE RAT LUMBAR SPINAL CORD AND ITS RESPONSE TO NEONATAL ANDROGEN. C.L.JORDAN*, S.M.BREEDLOVE AND A.P.ARNOLD. Dept. of Psychology, UCLA, Los Angeles,CA., 90024.

The spinal nucleus of the bulbocavernosus (SNB) in the rat lumbar spinal cord contains motoneurons innervating the bulbocavernosus and levator ani, two perineal muscles. The SNB is present in males and reduced or absent in females (S.M.Breedlove and A.P.Arnold, Science, 210:564, 1980). We report a second sex difference in the dorsolateral nucleus (DLN) of the rat lumbar cord, which is the origin of motoneurons innervating the ischiocavernosus muscle, a third sexually dimorphic perineal muscle. We also made neuron counts in the retrodorsolateral nucleus (RDLN), a group of motoneurons innervating muscles apparently not sexually dimorphic. Finally, we assessed the effect of neonatal androgen on the adult DLN and RDLN.

32 male and female rat pups received s.c. injections of 1 mg of testoserone propionate (TP) or oil on day 2 of life. On day 60, animals were sacrificed and spinal cords removed. Using thionin stained 50 um transverse sections of eight animals per treatment group, a blind observer made corrected counts of the number of nuclei of densely staining cells in the regions of the DLN and RDLN. 25 randomly chosen cells from each nucleus in each animal were traced through a camera lucida and the area of their somas and nuclei were measured.

Gonadally intact oil treated males have significantly more neurons in the DLN(360.86±28.44)than oil treated females (206.98±24.56,$p < .01$). There was no sex difference in neuron size. A single TP injection on day 2 of life had virtually no effect on DLN number in adult males(347.60±23.38), whereas the effect of early TP treatment in females was to increase the adult number of neurons(262.44±22.60) although not significantly. Neither DLN nuclei nor somas were significantly altered in size by the neonatal dose of TP. For the RDLN, no significant sex differences were found.

The deficit in motoneurons in the female DLN is probably related to the absence of neurons innervating the ischiocavernosus muscle, which females lack. Although the direction of change induced by neonatal androgen in female neuronal number is consistent with the hypothesis that androgens are involved in the sexual differentiation of the DLN, the effect of hormone treatment was not significant. Our finding no sex difference in the RDLN indicates that morphological sex differences are not characteristic of all lumbar motoneuron groups.

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97.9 THE SEXUALLY DIMORPHIC SPINAL NUCLEUS OF THE BULBOCAVERNOSUS (SNB) IS MASCULINIZED BY PERINATAL ANDROGEN. S.M.Breedlove & A.P.Arnold, Dept. Psychology, UCLA, Los Angeles, CA 90024.

In the male rat lumbar spinal cord there is a discrete nucleus consisting of motoneurons innervating two striated perineal muscles: the bulbocavernosus and levator ani. This nucleus, the spinal nucleus of the bulbocavernosus (SNB), and its target muscles are reduced or absent in normal female rats (Breedlove & Arnold, Science, 210:564, 1980). We now report that prenatal treatment of females with testosterone propionate (TP) significantly increases the number of SNB neurons. Dihydrotesterone propionate (DHTP) treatment just after but not before birth also masculinizes the SNB of females. These results support the hypothesis that androgens act both before and after birth to influence the sexually dimorphic development of the SNB.

Rat pups were treated with hormone during one of three developmental periods. Prenatal hormone was delivered by s.c. injections of the pregnant dam with 2 mg of TP or DHTP daily from days 17-22 of gestation (late prenatal). Postnatally, 1 mg TP or DHTP was injected s.c. into the pups on 3 alternate days: either days 1,3 and 5 (early postnatal), or days 7,9 and 11 (late postnatal). On the day of birth all treated pups were ovariectomized. Control rats were either gonadectomized and oil treated at birth, or sham operated and oil treated, or not disturbed with neonatal treatment. Rats sham operated at birth were gonadectomized on day 40. Beginning on day 68 all rats were treated with 2 mg/kg TP daily until sacrifice on day 102. A "blind" observer made corrected counts of the number of nuclei of densely thionin-stained neurons in the region of the SNB.

The table below presents the mean number ± standard error of the mean (N = number of animals) of SNB cells in females from each of the hormone treatment groups.

	LATE PRENATAL	EARLY POSTNATAL	LATE POSTNATAL
TP -	108.8 ±7.1(8)	82.5 ±7.9(8)	76.6 ±4.4(8)
DHTP -	55.0 ±5.0(8)	91.8 ±6.2(8)	63.0 ±3.7(8)

Females neonatally ovariectomized and oil treated had 66.6 ±4.5(7) SNB cells. Sham operated, oil injected females had 63.0 ±5.4(8), and undisturbed females had 72.3 ±3.0(8) cells. Sham operated males had 159.3 ±12.0(8) while undisturbed males had 188.1 ±13.1(8) SNB cells. The ano-genital distance in newborn females was masculinized by both prenatal TP and DHTP. DHTP was effective in masculinizing females during the early postnatal period, and TP was effective prenatally. The disparate effects of TP and DHTP suggest that the two hormones act via separate mechanisms.

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97.10 ORGANIZATIONAL EFFECTS OF SEX STEROIDS ON SYMPATHETIC AXONAL SPROUTING INTO THE HIPPOCAMPUS. Teresa A. Milner and Rebekah Loy. Dept. Neurosciences, University of California at San Diego, La Jolla, CA 92093

Removal of the septal input of the rat hippocampal formation elicits an ingrowth of vascular-associated sympathetic axons into the deafferented regions of the hippocampus. The extent of sympathetic ingrowth is influenced by both the sex and developmental stage of the animal. First, female adult rats show a greater amount of ingrowth when compared to male adult rats. This is especially apparent in the hilus and supragranular layer of the dentate gyrus and the stratum oriens of CA3. Second, the sprouting, which is reduced in adult and juvenile (postnatal day 13) males relative to females, is equivalent in the two sexes after fimbrial transections at postnatal day 3. These findings suggest that sex steroids may exert activational and/or organizational influences on the initiation and proliferation of the post-lesion axonal growth.

The activational effects of circulating sex hormones on sprouting were investigated by comparing the extent of sympathetic ingrowth following fimbrial lesions in normal or adult castrated male and female rats. The organizational effects of differential hormone exposure during development on synaptic reorganization in the adult was examined by comparing sympathetic axonal sprouting after fimbrial lesions in male and female rats which were castrated (male) or supplemented with silastic implants of testosterone (females and castrated males) at postnatal day 2. Thirty to ninety days post-lesion, the brains of these animals were analyzed using the cryostat glyoxylic acid method of fluorescence histochemistry for the demonstration of catecholamines.

We find that (1) castration of either female or male adult rats does not affect the sprouting response, but (2) neonatal castration of male rats permits sprouting equivalent to normal females, while neonatal testosterone treatment of females or castrated males results in a sprouting response identical to that of normal males.

This suggests that sex steroids exert organizational rather than activational effects on the sympathetic axonal sprouting in the hippocampus, since alterations in the hormonal environment during development but not in adulthood alters the extent of ingrowth. Sex differences in the development of the septohippocampal input could account for these effects.

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97.11 ADRENAL CHROMAFFIN CELLS FROM ADULT RHESUS MONKEYS IN DISSOCIATED CELL CULTURE: RESPONSE TO NERVE GROWTH FACTOR AND GLUCOCORTICOIDS. L. E. Lillien* and P. Claude. Neurosciences Training Program and Regional Primate Research Center, University of Wisconsin, Madison, WI 53706.

It has been reported that Nerve Growth Factor (NGF) will enhance the outgrowth of neurite-like processes from young rat, and adult human and bovine chromaffin cells in vitro, and that this NGF-dependent outgrowth is inhibited by glucocorticoids (Unsicker, K., et al., Proc.Natl.Acad.Sci.USA 75:3498, 1978; Tischler, A.S., et al., Lab.Invest., 43:399, 1980.) We have observed similar effects in cultures of chromaffin cells from rhesus monkeys. Adrenal medullae from adult females were dissociated with collagenase and trypsin and cells were plated on collagen-coated glass or plastic cover slips in Liebowitz L-15 medium supplemented with 10% newborn calf serum (NCS). In some cases the serum was stripped of endogenous steroids with dextran-coated charcoal. Cells were characterized by catecholamine histofluorescence and by electron microscopy. The major cell types observed in the cultures were rounded or cuboidal chromaffin cells containing characteristic chromaffin granules, glial-like cells, and fibroblast-like cells. The number of non-chromaffin cells was reduced by differential plating and by treatment with cytosine arabinoside. Chromaffin cells accounted for at least 90% of the cells in the culture.

Cultures were initially grown in L-15 or L-15 plus glucocorticoids (10^{-5} M dexamethasone or $5x10^{-5}$ M hydrocortisone). After four days, various concentrations of NGF were added to some of the cultures, both with and without glucocorticoids. After eight days in the different experimental conditions, cultures were processed for catecholamine fluorescence; the proportion of fluorescent cells showing outgrowth and the average length of the processes was determined. Exposure to 250 ng/ml 7S NGF in the presence of unstripped serum resulted in a 60% increase in the proportion of the process-bearing cells and an increase in the average length of processes. In stripped serum, the same concentration of NGF produced a 170% increase in the proportion of process-bearing cells. The maximum proportion of chromaffin cells bearing processes did not exceed 10%, even in stripped serum. Exogenous glucocorticoids completely inhibited the NGF-dependent increases. The stimulation of process outgrowth by NGF was not further enhanced by higher doses of NGF (500 ng/ml or 1 µg/ml). Similar results were obtained using dissociated chromaffin cells from seven- to ten-day-old rats.

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97.12 BEHAVIORAL AND STRUCTURAL MANIFESTATIONS OF PERINATAL GLUCOCORTICOID ADMINISTRATION: HIPPOCAMPAL INVOLVEMENT. A. J. Nonneman, S. T. DeKosky and S. W. Scheff. Departments of Psychology, Neurology, and Anatomy, Univ. of Kentucky, Lexington, KY 40506

Relatively large injections of steroids in the perinatal period have been associated with disrupted maturation of the cerebellum and hippocampus. Animals with such injections have been shown to manifest deficits in motor coordination, presumably because of alterations in cerebellar development. The present study was designed to assess the influence of perinatal steroid injections on morphology and behaviors associated with the hippocampus.

Litters of Sprague-Dawley rats were culled to 8-10 animals and randomly assigned to control and experimental treatment groups. Experimental animals received a single injection of dexamethasone (100 mg/kg) at 4 days of age. Some litters were sacrificed at 10 or 20 days of age for histological and microchemical analysis. Other animals received behavioral testing as young adults and were then sacrificed for microchemical and histological analyses of CNS changes associated with the behavioral results. These animals were weighed weekly prior to behavioral testing.

At 60 days of age, each animal's ability to spontaneously alternate was assessed. Alternation testing was conducted in a T-maze, 3 trials per day for 3 days. At 90 days of age the animals were tested for their ability to acquire and reverse a spatial learning task in a circular water tank. The rats were required to swim to a submerged platform, 8 trials per day. The learning criterion consisted of 6 of 8 trials with latencies to reach the platform of 5 sec or less on each of two consecutive days. Following attainment of the acquisition criterion the animals were begun on the reversal task and tested to the same criterion.

Body weight of steroid injected animals was significantly lower than that of age- and sex-matched controls throughout the experiment. The spontaneous alternation experiment revealed reduced alternation in the steroid treated animals as compared to controls. There were no detectable differences between groups in swimming ability, but there was a significant effect of steroid treatment on the spatial learning task in the water tank. Injected animals were significantly impaired, requiring more trials to criterion on both acquisition and reversal. These results indicate a change in behavior that may be due to disruption of hippocampal development.

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97.13 PERINATAL ALCOHOL EXPOSURE AFFECTS NEONATAL CORTICOID LEVELS BUT NOT DEVELOPMENT OF THE CIRCADIAN PITUITARY-ADRENAL RHYTHM IN RATS. A.N. Taylor, B.J. Branch* and R.E. Poland*. Brentwood VA Med. Ctr. and Brain Res. Inst. & Depts. of Anatomy & Psychiatry, UCLA Sch. of Med. & Harbor UCLA-Med. Ctr., Los Angeles, CA 90024.

Exposure to altered hormone levels during development exerts organizational influences on the CNS and persistent effects on adult behavior. We have shown that neonatal corticoid treatment delays development of the circadian rhythm of plasma corticosterone (CS) in rats. We therefore sought to determine whether fetal or neonatal exposure to ethanol (E), an agent which activates the hypothalamo-pituitary-adrenal axis, produces similar effects.

Fetal-exposed subjects were the offspring of Sprague-Dawley dams who were fed a 5.0% w/v E-containing casein-supplemented liquid diet (BioServ) ad lib or pair-fed (P) the isocaloric liquid diet without E from gestation day-8 to birth, when pups were cross-fostered to normally-fed (N) dams (lab chow and water ad lib). Neonatal-exposed subjects were born to N dams and were cross-fostered at birth to the E and P dams. These dams continued to receive the E and P diets during lactation week-1, when they were replaced on N diet for the remainder of lactation. Rats were housed with lights on from 0300-1700 h. Plasma was collected following decapitation of 2-3 pups from each of 5-6 litters at the time of the nadir (0800 h) and peak (1700 h) of the adult adrenal rhythm and assayed by RIA for CS.

Maternal E consumption (12 g/kg/day) did not affect number and weight of offspring at birth. CS levels at 0800 h on day-1 were significantly ($p=.004$) higher in fetal E-exposed pups (100.9±6.4 (n=10) ng/ml) than in P (53.1±8.2) or N (57.1±9.0) controls. When next examined on day-9, the differences had disappeared. The first significant ($p<.001$) nadir-peak difference in CS levels, i.e. onset of the CS rhythm, occurred on day-18 in all 3 groups.

Weight gain was significantly retarded in the neonatally E-exposed and P pups on days 7 ($p<.05$) and 14 ($p<.01$); by day-21, body weights were normal. Significant nadir-peak differences in plasma CS levels ($p<.001$) in the neonatal-E exposed rats first occurred on day-21, when neonatal P-controls did not yet show a rhythm. These delays surely reflect nutritional deficits.

These results indicate that despite elevated CS levels at birth, fetal exposure to E (in a better diet than we used in a preliminary positive report) does not affect the onset of the pituitary-adrenal rhythm. On the other hand, we have reported that adult rats exposed to E in utero respond to neural stressors with significantly greater CS responses than do P or N controls. The mechanism for this persistent action of fetal alcohol exposure, whether hormone-induced or otherwise, remains to be determined. (Supported by VA Medical Research Service.)

97.14 EARLY ADRENALECTOMY STIMULATES SUBSEQUENT GROWTH AND DEVELOPMENT OF THE RAT FOREBRAIN. Jerrold S. Meyer. Dept. of Psychology, University of Massachusetts, Amherst, MA 01003.

Male and female rats (Wistar strain) were either adrenalectomized (ADX) or sham-operated (SHAM) on day 11 of life. Groups of animals were then killed at approximately day 35 or 65 and their forebrains subjected to various analyses. ADX rats consistently displayed heavier forebrains than their SHAM counterparts (1.32 ± 0.01 vs. 1.24 ± 0.01 at day 35; 1.48 ± 0.03 vs. 1.30 ± 0.02 at day 65; all values = mean ± S.E.M.). This effect could not be explained by an increase in brain water content nor by a general stimulation of growth (ADX rats had significantly lower body weights than controls). Biochemical analyses performed just on 65-day-old subjects revealed that the whole-forebrain contents of DNA, galactocerebroside, and sulfatide (galactocerebroside sulfate) were all elevated as a consequence of early adrenalectomy. The DNA results may reflect enhanced glial cell proliferation, inasmuch as most of the neuron formation in rat forebrain occurs prenatally. Higher galactolipid levels suggest that myelinogenesis may be stimulated by the removal of adrenal hormones. This was confirmed in separate studies showing an increased recovery of purified myelin from ADX vs. SHAM forebrains. Friedrich and Bohn (Soc. Neurosci. Abst. 6: 380, 1980) previously demonstrated that neonatal hydrocortisone administration suppresses myelination in rat optic nerve. It seems likely, therefore, that the present findings can be attributed to the withdrawal of glucocorticoids from the young animal, suggesting an important role for these hormones in regulating normal brain development.

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97.15 CHARACTERIZATION OF TRANSIENTLY NORADRENERGIC CELLS OF EMBRYONIC GUT IN VIVO AND IN VITRO. G.M. Jonakait, J.A. Kessler, M. Goldstein, K. Markey and I.B. Black, Div. of Developmental Neurology, Cornell Univ. Med. Coll., New York, N.Y. 10021 and Dept. of Psychiatry, N.Y.U. Med. Center, New York, N.Y. 10016.

We have been investigating a population of cells, located in embryonic rat gut, which transiently expresses the noradrenergic phenotype. Appearing at 11.5 days of gestation (E11.5), these cells lose immunoreactivity to tyrosine hydroxylase (T-OH) and dopamine-β-hydroxylase as well as catecholamine (CA) fluorescence by E13.5. Earlier studies have shown that treatment at E11.5 with either nerve growth factor (NGF) or with agents (e.g., reserpine) that increase maternal glucocorticoids causes persistence of CA fluorescence in these cells through E13.5 (Kessler et al, 1979; Jonakait et al, 1980).

Since CA levels are affected by many non-physiologic variables, we also measured the specific gene product, T-OH, after treatment of pregnant rats with reserpine, transuterine injection of embryos with NGF or a combined regimen. Each treatment on E11.5 significantly increased T-OH catalytic activity at E13.5 in cells located at the pyloric sphincter and in paraganglia at the gastroesophageal junction. Simultaneous treatment resulted in increased paraganglion T-OH activity greater than either treatment alone, suggesting independent mechanisms of action. Sequential treatment with reserpine on E11.5 and NGF on E13.5 resulted in a dramatic increase in T-OH immunoreactivity in cells throughout the gut and persistence through E16.5. Reversal of the treatment sequence (NGF followed by reserpine) did not dramatically prolong the appearance of T-OH-positive cells, suggesting that initial exposure to glucocorticoid hormones alters the cells' ability to respond to subsequent treatment.

To examine these effects at the cellular level, we have developed a method for growing embryonic intestine in culture. E12.5 guts have been explanted to Millipore filters on wire-mesh rafts suspended over wells containing Eagles MEM with 15% rat serum. Guts grown for 4 days showed a 50% increase in T-OH activity, suggesting ongoing development of catecholaminergic cells. We are now examining the effects of glucocorticoids and NGF in culture. (Supported by NS06142, 10259, 06801 and MH15137.)

97.16 DEVELOPMENT AND THYROXINE DEPENDENCE OF THE IPSILATERAL RETINOTHALAMIC PROJECTION IN XENOPUS. S. Hoskins and P. Grobstein. Depts. of Biology and Pharmacol. Physiol. Sci., The University of Chicago, Chicago, IL 60637.

In Xenopus, the crossed retinotectal and retinothalamic projections are established during days 2-4 of embryogenesis, while the ipsilateral retinothalamic projection appears weeks later, in association with metamorphosis. We have used anterograde transport of HRP in tadpole optic nerves to define the stages during which the projection to ipsilateral rostral thalamus develops, and to examine the influence of thyroxine on its development.

Optic nerves were filled in tadpoles ranging from stages 50-66 (end of metamorphosis; 6-8 weeks). Evidence of a terminal zone in ipsilateral rostral thalamus was first detected at stages 53-54. Between stages 56 and 66, increasingly well-defined terminal zones were seen in the rostral thalamus.

Since the rise in circulating thyroxine associated with metamorphosis begins at stage 54, we examined the correlation between thyroxine presence and the development of the ipsilateral projection. Tadpoles raised continuously in 0.01% propylthiouracil (PTU) fail to metamorphose due to an inability to produce thyroxine. These animals reach stage 53-54 on a normal time course and then increase in size without progressing morphologically. We assessed the ipsilateral projections of PTU reared tadpoles after times sufficient for normal metamorphosis. The projection patterns to ipsilateral rostral thalamus were similar to those seen in normal stage 54 tadpoles. Tadpoles shifted from PTU to normal rearing medium completed metamorphosis and acquired ipsilateral projections on a normal time course.

In additional PTU reared (stage 53-54) tadpoles we made 50 nl injections of .17-.35 mg/ml thyroxine in oil into one eye. The animals were returned to 0.01% PTU, and their optic nerves filled 4-10 weeks later. At this time, most had advanced to stage 57, suggesting some systemic effects of the injection. In 6 cases, labelling in rostral thalamus ipsilateral to an injected eye was heavy, comparable to that normally seen at stage 65. In two cases in which the uninjected eye's optic nerve was filled, normal stage 57 ipsilateral terminal zones were seen. Counts of retinal ganglion cells added to the thyroxine-injected and uninjected eyes indicated that the thyroxine had much greater effect on mitosis in the injected eye.

We conclude that the ipsilateral projection to rostral thalamus is thyroxine-dependent. Refinements of thyroxine dosage may allow us to eliminate systemic effects, and determine whether thyroxine action on the eye alone is sufficient to produce an ipsilateral projection. (Supported by NSF BNS 791422.)

97.17 EFFECT OF EARLY HYPOTHYROIDISM ON NORADRENERGIC REGENERATIVE GROWTH IN THE CEREBELLUM OF RATS TREATED NEONATALLY WITH 6-HYDROXYDOPAMINE. Ranbir K. Bhatnagar. Department of Pharmacology, University of Iowa, Iowa City, IA 52242

Neonatal administration of 6-hydroxydopamine (6OHDA) produces almost total loss of norepinephrine (NE) in the cerebellum followed by an increase of up to 200% of normal. This regenerative growth of NE in the cerebellum is a useful model for the developmental growth of locus coeruleus axons and might be controlled by the target cells (Schmidt and Bhatnagar, Br. Res. 191: 173, 1980). This study was undertaken to assess the growth of noradrenergic neurons following hypothyroidism-induced alterations in the differentiation of their terminal field in the cerebellum. Early hypothyroidism produces delayed maturation, reduced density of dendritic spines and reduced dendritic arborization of Purkinje cells and reduced density of molecular layer (Nicholson and Altman, Br. Res. 44: 13, 1972; Br. Res. 44: 25, 1972). Hypothyroidism was produced by feeding 50 mg of propylthiouracil (PTU) daily to the Sprague-Dawley mother rats via a gastric tube either prenatally starting on the 18th day of gestation or postnatally starting on the day of parturition. Control rats received 3 ml of water daily. One half of each litter was injected subcutaneously with 6OHDA (100 mg/kg) on postnatal days 1, 2 and 3. The other half received vehicle injections. Rat pups were decapitated at 9 and 22 days of age. Brains were removed and dissected into cerebellum, pons and medulla for NE assay.

In PTU treated groups the weight of body and cerebellum but not that of pons and medulla was significantly reduced. PTU administration to non-6OHDA group did not alter the NE content of any tissue examined. In the group receiving PTU prenatally, at 9 days of age the regenerative growth of NE neurons in the cerebellum was significantly greater than either in the groups receiving water or PTU postnatally. At 22 days of age postnatal administration of PTU also elicited a significant increase in noradrenergic regenerative growth. In all cases the increase in regenerative growth after PTU administration was reflected in both the content and concentration of NE. 6OHDA administration resulted in a significant increase in NE content of pons and medulla. PTU did not modify the NE content or concentration of pons and medulla in rats treated with vehicle or 6OHDA. Thus, the effects of hypothyroidism are restricted primarily to the terminal field and not the field of origin of noradrenergic projections. In conclusion, the perturbation of differentiation of target tissue can alter the rate and extent of noradrenergic innervation and such alterations may have a temporal basis.

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97.18 THE SPINAL TRACT OF V IN RAT FETUSES MADE HYPOTHYROID BY PROPYLTHIOURACIL, C. H. Narayanan and Y. Narayanan*, Dept. Anat., LSU Sch. Med., New Orleans, LA. 70119.

It has been previously shown by our laboratory that there is a correlation between the progressive development of the spinal tract of V and motor neurons of cervical levels on the one hand, and the nature and extent of responses to stimulation of perioral regions in rat fetuses at the other. The present study was performed to examine the development of the spinal tract of V in rat fetuses made hypothyroid by propylthiouracil (PTU). Timed pregnant rats (Holtzman) were raised on a goiterogenic diet consisting of 0.3% PTU mixed with powdered Teklad mouse diet beginning from seven days of pregnancy. Control animals were raised on powdered Teklad mouse diet. Beginning from 12 through 20 days of pregnancy both experimental and control animals were prepared for surgery and recordings of activity were made according to established procedures in our laboratory. Recordings of the frequency of movements of the head, forelimbs and oral region were made. Evoked activity was studied at each stage using an esthesiometer. Fetuses from each age group were sacrificed, serially sectioned in toto and stained according to the reduced silver method. The experimental group of fetuses without exception showed a drastic reduction in overall activity. In particular, synchronous movements of head and forelimbs and oral area activity were greatly reduced. The response to stimulation of of the perioral region was observed only at 19 days of gestation age, but is seen as early as day 16 in the control group of fetuses. The responses to stimulation in the experimental group for all ages at best were weak and low in amplitude. The spinal tract of V was identified by following the descending fibers of the Vth nerve from the region of entrance in the pons. At 15 days of gestation age, the fibers of the spinal tract are very poorly developed and extend as far as C-1. It is only at 19 days that the fibers forming the dorsal funiculus show some differentiation into a f. gracilis and f. cuneatus and is comparable in its overall development to that seen in a 15 day control fetus. The fibers of the maxillo-mandibular division is somewhat greater in area than the ophthalmic division. These descending pathways form part of a reflex pathway that is late in its appearance in the hypothyroid fetuses. It is concluded that the descending pathways which are disrupted by PTU administration are involved in the emergence of this behavior pattern.

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97.19 EFFECTS OF THYROXINE ON THE MATURATION OF PURKINJE CELLS AND CLIMBING FIBERS IN THE FROG CEREBELLUM. K. F. Hauser* and A. G. Gona. Dept. of Anatomy, CMDNJ-New Jersey Medical School, Newark, N.J. 07103.

In bullfrog tadpoles (Rana catesbeiana) two populations of Purkinje cells can be discerned during development. One population of Purkinje cells located in the dorsal (apical) region of the cerebellum show an advanced pattern of dendritic development 1 to 2 years prior to establishment of the external granular layer (EGL), while a majority of Purkinje cells present remain immature until EGL formation during metamorphosis (Uray and Gona, J. Comp. Neur., 180:265, 1978). The purpose of this study was to examine the response of Purkinje cells to premature metamorphic change. Ultrastructural observations of the corpus cerebellum were made following thyroxine-induced metamorphosis (T4, 0.25ug/g body weight, administered intraperitoneally on alternate days during the first week, followed by increased dosages, 0.38ug/g body wt, during the last two weeks). Premetamorphic tadpoles induced to metamorphose 1 to 2 years prematurely, were sacrificed after 1, 2, or 3 weeks of T4 treatment. After the second week, immature Purkinje cells in the ventral cerebellum showed rapid maturational change. Most notably, Purkinje cell perisomatic processes (stellate stage) were observed as elaborate growth cones. These processes were frequently observed synapsing with developing climbing fiber varicosities. This study demonstrates that precocious maturation of the Purkinje cell-climbing fiber system can be induced by T_4 treatment. It is postulated that thyroid hormone acts in one of the following ways: (1) directly influences maturation of the climbing fiber system, secondarily inducing Purkinje cell development, (2) directly influences Purkinje cell development, secondarily inducing climbing fiber development, or (3) indirectly influences Purkinje cell development, e.g., by the trophic influence of newly formed parallel fibers, which in turn influences climbing fiber development.

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97.20 CENTRAL CONTROL OF THE DEVELOPING PITUITARY INTERMEDIATE LOBE. M. Duff Davis*, Walter Lichtensteiger, Margret Schlumpf and Arie Bruinink*. Pharmacology Institute, University of Zurich, CH-8006 Zurich, Switzerland.

During human fetal ontogeny, a rudimentary intermediate lobe of the pituitary forms, which is not found later in the newborn. Since the pars intermedia (PI) of other mammalian species secrete melanotropins, it is interesting to speculate on some type of developmental function for these peptides. The purpose of our current study is to investigate the development of CNS control over pars intermedia MSH release.

Fetal (from ED 18) and postnatal rat blood samples were collected and bioassayed for MSH-like peptides using adult Rana pipien skins. Pituitaries were removed and, when possible, the neurointermediate lobes (NIL) separated from the anterior lobes. Some NILs were used for release studies where the glands were incubated with or without dopamine at 10^{-6}M and the media assayed for hormone. Others were homogenized for dopamine receptor binding studies (using ^{3}H-spiperone in competition with cold spiperone, flupenthixol and sulpiride). Some specimens were processed for histofluorescence of catecholamines in order to study the stage of innervation of the PI by the tuberohypophyseal system.

A small amount of serum MSH was detected throughout the prenatal period, rising slowly on postnatal days 1-2 and more rapidly to a peak on days 4-5. Levels quickly fell to prenatal values until the end of the third week when there was another rise in hormone. NILs incubated with dopamine resulted in strong inhibition of MSH release on postnatal day 2 (the first day tested) through later stages. Basal levels of secretion showed a continual increase from the first week of birth. Dopamine binding studies confirmed previous reports of prevailing D_2 receptors in the adult PI. Catecholaminergic nerve terminals were seen infiltrating the lobe during the first postnatal days. These data indicate that CNS regulation of pars intermedia MSH probably begins in the first week postnatally. The type of neural or humoral signals which initiate the inhibitory control of MSH is under investigation.

98.1 SIZE OF MOTONEURONE POOL IS RELATED TO NUMBER OF MYOTUBES IN DEVELOPING MUSCLE. I. S. McLennan* (SPON: G. Pilar). Physiology Section U-42, Univ. of Connecticut, Storrs, CT 06268.

The extent of motoneurone cell death can be altered by varying the size of the target tissue, suggesting that the neurones are competing for some aspect of their target (Hamburger, V., Neurosci. Res. Prog. Bul. 15, Suppl. April 1977). What is limiting in the target tissue is, however, unknown. Motoneurone cell death occurs early in neuromuscular development (Stg. 29-35), at a time when the muscle consists of cell clusters composed of large primary myotubes and less differentiated muscle cells. As the muscle develops, secondary and tertiary myotubes are formed, resulting in a large increase in the number of fibers (Ontell, M., Anat. Rec. 189, 669, 1977). In this paper I have compared the size of various motoneurone pools (Landmesser, L., J. Physiol. 284, 371, 1978), in the chick lateral motor column with the number of myotubes which occur in the corresponding muscle at the time of cell death.

Stg. 29 and 35 chicken embryos were fixed in 4% glutaraldehyde and 5% formaldehyde with their hindlimbs pinned in an extended position. The limbs were embedded in wax, serially sectioned and stained for glycogen by the PAS method. The midpoint of each muscle was located and camera lucida drawings of the myotubes profiles were made.

At the onset of cell death (Stg. 29) there are 2623 ± 240 myotube profiles in the muscle mass from which the sartorius, iliotibialis and femorotibialis are derived. The number of neurones which innervate these muscles in the adult is 2852 (Landmesser, L., J. Physiol. 284, 371, 1978). At this stage there are fewer myotubes in the ventral and dorsal shank of the lower limb than neurones in their mature motoneurone pool. By the end of the period of cell death (Stg. 35), however, a one to one relationship exists between the number of myotubes in the lower limb and the number of neurones innervating these muscles in the adult.

I therefore suggest that motoneurone cell death results from a competition by the neurones for occupancy of a limiting number of myotubes (target cells), thus establishing a relationship between the size of a motoneurone pool and the prospective size of its corresponding muscle.

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98.2 DEVELOPMENTAL CHANGES IN THE LATERAL MOTOR COLUMN OF THE CHICK EMBRYO FOLLOWING EITHER SPINAL TRANSECTION OR NEURAL CREST REMOVAL. N. Okado* and R.W. Oppenheim (SPON: I-W. Chu-Wang). Lab. of Neuroembryology, Dorothea Dix Hospital, Raleigh, N.C. 27611.

Although some investigators have reported that cell number in the lateral motor column (LMC) of the chick embryo is altered by spinal transection or neural crest removal, others have failed to find such effects. Part of the reason for this apparent discrepancy may be that the effect of these manipulations only becomes manifest at a specific developmental stage. If so, then the choice of when to look for such effects becomes critical. Consequently, we have been examining the effects of such manipulations on the period during as well as following natural cell death in the LMC.

Spinal transection (cervical or thoracic) and neural crest removal (lumbar) was performed at Stages 13-15. Animals were sacrificed at various ages between 8 and 18 days of incubation. Cell number in the LMC was determined from serial sections of paraffin embedded lumbar spinal cords.

At 10 days cell number was not significantly different from controls following spinal transection. At 16 days, however, there were about 25% fewer motoneurons in the LMC of spinal animals. In the case of neural crest removal, our initial results indicate that as long as there was no significant damage to the spinal cord -- a frequent inadvertent side-effect of neural crest removal -- cell number in the LMC was unaffected, at least up to 10 days of incubation; observations on older embryos are in progress.

Our results suggest that neither spinal transection nor neural crest removal alters the course of natural cell death in the LMC, a phenomenon which begins on day 5 and is virtually over by day 10 in the lumbar spinal cord. Although at present we do not know why motoneuron numbers are decreased on day 16, after spinal transection, two explanations have occurred to us. First, some neurons in the LMC may project to the brain; spinal transection would prevent them from reaching their normal synaptic targets. A second, and more plausible, explanation is that after day 10 cells in the LMC become dependent on descending input for their maintenance. Experiments are currently in progress to determine which, if either, of these explanations is correct.

98.3 PHARMACOLOGICAL MODULATION OF NEUROMUSCULAR TRANSMISSION AND CELL DEATH IN THE LATERAL MOTOR COLUMN OF THE CHICK EMBRYO. R.W. Oppenheim and J.L. Maderdrut*. Neuroembryology Lab., Dorothea Dix Hospital, Raleigh, N.C. 27611.

Either pre- or post-synaptic blockade of neuromuscular transmission between Stage 26-28 (day 5-6) and Stage 36 (day 10) sufficient to reduce the spontaneous motility of chick embryos by more than 60-70% resulted in an increased number of neurons in the lateral motor column (LMC) at Stage 36. Both competitive (e.g., curare and benzoquinonium) and depolarizing (e.g., decamethonium) post-synaptic nicotinic receptor blocking agents increased the number of neurons in the LMC. However, doses of hexacarbacholine (a depolarizing neuromuscular blocking agent) that produced almost complete cessation of spontaneous motility had no detectable effect on the number of neurons in the LMC. The increased number of neurons in the LMC was the result of a decreased naturally-occurring loss of motoneurons between Stages 26 and 36. The decreased loss of motoneurons following either pre-synaptic (hemicholinium-3) or post-synaptic (curare and decamethonium) blockade of neuromuscular transmission was accompanied by a decrease in the number of pyknotic (degenerating) cells in the LMC at Stages 34 and 36. Embryos treated daily with 2.5 mg of curare from day 5 to day 9 that survived for two days beyond the normal time of hatching on day 20 (but were unable to emerge from the egg) had an increased number of neurons in the LMC.

Administration from day 5-6 to day 9 of doses of either nicotine, carbachol, or choline spanning a range from those that had no significant effect on spontaneous motility to those that resulted in almost complete cessation of motility had no detectable effect on either the time-course or the quantity of naturally-occurring cell death in the LMC. Administration of either 10 or 100 µg of eserine from day 5 to day 9, however, produced a small, but significant, decrease in the number of motoneurons in the LMC at Stage 36 (day 10). Daily administration of 10-100 µg of 4-aminopyridine (a potassium channel blocking agent) from day 5 to day 9 had no apparent effect on either spontaneous motility or cell death in the LMC.

Substantial reductions in spontaneous motility appear to be a necessary but not a sufficient condition for the prevention of naturally-occurring cell death in the LMC by cholinergic drugs. Substantial increases in the stimulation of nicotinic acetylcholine receptors in skeletal muscle by the chronic administration of cholinergic drugs were without apparent effect on naturally-occurring cell death in the LMC of the chick.

98.4 KINETICS OF ROHON-BEARD NEURON DISAPPEARANCE IN *Xenopus laevis* Janet E. Lamborghini. Biology Dept., UCSD, La Jolla, CA 92093

Rohon-Beard neurons, large dorsal cells in the spinal cords of embryonic fish and amphibians thought to be primary sensory neurons (Coghill, '14, DuShane, '38), disappear during development. The ∿ 200 Rohon-Beard cells found in *Xenopus laevis* are gone well before resorption of the tail during metamorphic climax (Nieuwkoop & Faber, '56, Hughes, '57). Because there is no obvious hiatus in sensory function during the time of their disappearance, whatever role Rohon-Beard cells play in the mediation of cutaneous sensitivity is considered to be assumed smoothly by dorsal root ganglion cells. The cause of the disappearance of Rohon-Beard neurons remains unknown. Before possible hypotheses can be tested, a detailed description of this phenomenon is necessary. Therefore, a study of the kinetics of disappearance of Rohon-Beard cells and associated cytological changes was undertaken.

Counts of Rohon-Beard neurons in fixed and sectioned specimens of increasing age and developmental stage revealed a gradual loss of cells starting at Nieuwkoop & Faber stages 45-46 (4-5d of development). The number of Rohon-Beard cells decreased to half by stages 49-50 (12-15d) and only a few were found after stage 57 (41d, 7d before tail resorption). Counts of Rohon-Beard cells in animals whose development was retarded by crowded conditions suggest that the rate of Rohon-Beard cell loss is stage- rather than age-dependent. Acid phosphatase activity, a common histochemical correlate of cell death, was assayed by the Gomori technique in both whole mounts and frozen sections of larval spinal cords. Coincident with the time of onset of disappearance of Rohon-Beard cells, an increase in acid phosphatase staining of the cytoplasm was observed. Increased staining was present first in cells at the anterior end of the spinal cord but by stage 47 had spread to cells over the whole length of the cord. Ultrastructural studies of Rohon-Beard cells in stage 47 larvae revealed the presence of many lysosomes in the cytoplasm, swelling of the endoplasmic reticulum and mitochondria, and a decrease in nuclear density. Participation of macrophages was not evident.

The disappearance of Rohon-Beard neurons can be attributed to autophagic cell death involving lysosomal acid hydrolases. This process begins only a day or two after the appearance and maturation of voltage- and chemically-dependent membrane conductances and electrical uncoupling of these neurons (Baccaglini & Spitzer, '77, Spitzer, '80, Bixby & Spitzer, this volume).

This work was supported by the NIH (NS15918) and the ONR (N00014-79C-0798).

98.5 THE TIME COURSE OF THE LOSS OF RED NUCLEUS NEURONS AS A RESULT OF T_{5-6} HEMISECTION IN THE NEONATAL RAT. J. Prendergast and R. Bates.* Department of Anatomy, The Medical College of Pennsylvania, Philadelphia, PA 19129.

Mid-thoracic hemisection in the neonatal rat results in a massive loss of red nucleus neurons (Prendergast and Stelzner, '76). The loss may be due to: 1) axotomy of the rubrospinal tract, 2) the inability of growing rubrospinal tract axons to reach their target, or 3) both. A plug of gelfoam saturated with HRP in 2.0% DMSO was placed in the spinal cord at birth and at fifteen days of age to determine: 1) if the tract had grown at birth beyond the point where the lesion was to be made, and 2) if a similar number of red nucleus neurons would fill at the two ages. Twenty-four to forty-eight hours following the surgery the animals were perfused. The brains and spinal cords were sectioned and reacted using the TMB procedure. At birth a few of the red nucleus neurons were filled with HRP and this indicated that at least part of the rubrospinal tract had grown beyond the mid-thoracic cord. By fifteen days more of the red nucleus neurons were filled than at birth and this suggested that at birth part of the rubrospinal tract was still growing into the thoracic cord. Other neonatal rats sustained the hemisection (T_{5-6}) and were allowed survival times of varying lengths which began at 18h. The brains and spinal cords were cut and stained with cresyl violet. Compared to controls significant reductions in the number of red nucleus neurons were evident at three days after the lesion. By five and one-half days there was an even greater cell loss than that at three days following surgery.

The loss of some red nucleus neurons following hemisection at birth may be attributed to axotomy. Death of other parent red nucleus neurons may be explained by the fact that their axons were still growing and were prevented by the lesion from reaching their targets. The protracted time course of cell death may be due: 1) to the rate that cells of differing sizes die, 2) to the time it takes for the cell body to be influenced by the loss of its target, or 3) both of these.

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98.6 DEGRADATION OF PROTEINS IN $CaCl_2$-INDUCED MYELOPATHY IN RAT SPINAL CORD. N. L. Banik, E. L. Hogan, L. J. Whetstine* and J. D. Balentine*. Departments of Neurology and Pathology (Neuropathology), Medical University of South Carolina, Charleston, S.C. 29425

We have previously shown that experimental spinal cord trauma in animals results in vesicular degradation of myelin, granular degeneration of axons, loss of myelin proteins and accumulation of calcium (Balentine, J.D., Lab. Invest., 39:254, 1978; Banik, N.L. et al., J. Neuropath. Exp. Neurol., 39:232, 1980; Happel, R.D. et al., Brain Res., in press). In order to test the hypothesis that calcium may be involved (possibly by activating proteinases) in the degradation of myelinated axons, a model of calcium-induced myelopathy in rats has been developed. Thus, rats were anesthetized and a 10% solution of $CaCl_2$ at pH 7.4 was dripped onto the exposed thoracolumbar spinal cord (after removal of dura with the leptomeninges remaining intact). This consistently resulted in paraplegia and spongiosis with ultimate necrosis of the spinal cord at the drip site. There was progressive axonal degeneration, vesiculation of myelin and phagocytosis (Balentine, J.D. and Hilton, C., J. Neuropath. Exp. Neurol., 39:339, 1980). To extend the previous study of morphological alterations the changes in proteins in the lesion sites were examined and compared to normal spinal cord at 1, 3 and 5 days following Ca application. The proteins of the cord were separated by 5-25% gradient slab and 10% disc polyacrylamide gel electrophoresis according to Laemmli. The gels were stained with Fast green, destained, scanned and the proteins quantitated by weighing peak areas. There was a loss over a period of hours of all classes of neurofilament proteins, myelin proteins, actin and other proteins. Thus, 60%, 77% and 58% of the 200,000, 150,000 and 69,000 dalton neurofilament proteins, respectively, were lost at 5 days compared to control. Large losses of myelin proteins (PLP 50%; large basic protein 68%) as well as actin and other proteins were also observed. The protein alterations observed correlate well with morphological changes we reported earlier and resemble those previously found with physical trauma. We believe that Ca++ plays an important role in the degeneration of axons and myelin sheath in both Ca++-induced myelopathy and spinal cord injury.

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98.7 DIFFERENTIAL CELL DEATH DURING DEVELOPMENT PREDICTS LATER NEOCORTICAL AREA SPECIALIZATION. M. Slattery* and B. L. Finlay. Department of Psychology, Cornell University, Ithaca, NY 14853.

The incidence of degenerating cells as a proportion of normal cells as seen with light microscopy was calculated for four areas of posterior neocortex (29, 18, 17 and 18a) for hamster pups 5-10 days old. At 5 days of age, migration into and differentiation of the external cortical laminae are still proceeding; by day 10 all layers have differentiated. Differences in both the timing and the amount of cell degeneration were observed across cortical areas and laminae.

The order of cell degeneration generally reflected the order of cell generation. Layer VI peaked in cell death before layers V, III, and II across all cortical areas. Layer IV showed a variable pattern across areas.

Immature neocortex was divided into subareas with reference to the adult neocortical map. These divisions were found to correspond to striking local differences in overall cell death rate. Presumptive area 29 had a general cell death rate 2-4 times higher than areas 17, 18 and 18a on all observed days. Areas 17, 18 and 18a, quite similar in thickness in adult hamster cortex, did not show an overall difference in cell death rate, but showed variation in the pattern of cell death by lamina over days.

The pattern of cell death by cortical lamina corresponded to the eventual thickness of that lamina in adult neocortex. In the adult, laminae II and III of area 29 are quite thin compared to the same laminae in adjacent neocortex, and the rate of cell death is 2 to 10 times higher in laminae II and III of area 29 than in adjacent areas. Laminae similar in their adult thickness are similar in cell death rate: laminae chosen for similarity in thickness (less than 5% difference) show similar cell death rates (average ratio .83). Dissimilar laminae (more than 5% difference) have more divergent cell death rates (average .62). These results suggest that cortex is generated uniformly and reaches its local adult differentiation by differential degeneration during early development.

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98.8 REGULATION OF NEURONAL NUMBER IN RETINA, THALAMUS, AND MIDBRAIN FOLLOWING EARLY MONOCULAR ENUCLEATION. D. R. Sengelaub, L. F. Jacobs*, M. S. Windrem*, and B. L. Finlay. Department of Psychology, Cornell University, Ithaca, NY 14853.

The hamster visual system shows a substantial amount of normally occurring cell death during early postnatal development. Following neonatal removal of one eye, this system also exhibits a marked plasticity in its connectivity. After the removal of one eye at birth, alterations in the normal magnitude and spatial pattern of cell degeneration were studied in the retinal ganglion cell layer of the remaining eye, lateral geniculate, and superior colliculus for the first 10 postnatal days to determine the role of cell death in early visual system plasticity.

In the remaining retina, cell degeneration in the ganglion cell layer occurred within the same period as that for normal retinas. Cell degeneration rates were lower in the temporal retina during postnatal days 1-5, resulting in an increase in ganglion cells relative to normal. This lowered cell death may reflect a reduction in competition at normally bilaterally innervated terminal areas. Retinal whole mounts from adults who had received a monocular enucleation at birth also showed increased numbers of retinal ganglion cells, principally in temporal retina.

In the superior colliculus contralateral to the enucleation, cell degeneration had a normal time course but the rate was twice as large as that of the ipsilateral colliculus. In the intermediate and deep layers of the colliculus, which are not retinal targets, no differences from normal cell degeneration were found in the period of normally occurring cell death.

Normal neuronal degeneration in the dorsal lateral geniculate coincides with degeneration in the retina, peaking at postnatal day 5. As in the superficial layer of the colliculus, cell death is greatest in areas innervated by peripheral retina. A second wave of cell death on postnatal day 8 does not show this center/periphery disparity, and may relate to the establishment of cortical connectivity. In enucleates, cell death in the contralateral geniculate was greater than in the ipsilateral geniculate and coincided with the period of normally occurring cell degeneration.

Observations of cell degeneration in mammals is a sensitive measure of size adjustments in neuronal populations following damage. Normally occurring cell death can be both reduced and increased following alterations in interconnecting populations.

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98.9 MYASTHENIA GRAVIS IMMUNOGLOBULIN AUGMENTS MOTOR NEURON SURVIVAL. G. S. Sohal, R. T. Leshner* and T. R. Swift*. Departments of Anatomy and Neurology, Medical College of Georgia, Augusta GA 30912.

A significant number of motor neurons degenerate during embryogenesis in many neural centers. The factors regulating motor neuron survival during embryonic development remain uncertain. We have studied the effects of application of sera or immunoglobulin concentrates (Ig) of patients with acquired myasthenia gravis (MG) on embryonic cell death in the trochlear nucleus of white Peking duck. A significant increase in motor neuron survival occurred following application of MG sera or MGIg during the period of embryonic cell death. Normal sera or Ig precipitates in identical dosage produced no alteration in motor neuron survival. Sera from MG or normal controls without the Ig fraction had no effect on neuron survival. Embryos treated with MG sera before or after the period of normal cell death had neuron numbers similar to that of normal embryos. No diminution of limb or extraocular muscle movement was noted during development. Trochlear motor neuron survival persisted after sera or Ig treatment was discontinued. MGIg is therefore unique in preventing motor neuron death without producing muscle paralysis and in promoting a prolonged augmentation of motor neuron survival. Previous studies have shown effects of MG sera only postsynaptically in muscle. This is the first demonstration that MGIg affects structures in the CNS. (Supported by a grant from Muscular Dystrophy Association).

98.10 ULTRASONIC EVALUATION OF THE CONTUSED SPINAL CORD OF THE CAT. D.D. Rigamonti, P.M. Gammell*, J.D. Hestenes*, D.W. Scharre* and W.E. Prominski*. Department of Anatomy, Georgetown Medical School, Washington, D.C. 20007 and the Jet Propulsion Laboratory, University of California, Pasadena, CA 91103.

Contusion of the spinal cord produces both anatomical and physiological alterations, immediately in the central gray and subsequently in the long tract axons coursing in the white matter. The extent of focal damage can be measured using behavioral (neurological testing), physiological (somatosensory evoked responses) and anatomical (histological) techniques. We have developed a procedure using ultrasound to assess the extent of damage to the adult mammalian spinal cord.

Adult cats anesthetized with pentobarbital were used in acute experiments designed to establish the normative data in animals receiving an immediate contusion produced by forceps crush of the lower thoracic spinal cord following a laminectomy. The animal was held in a rigid holding device (DKI) and a pool of saline between the probe and exposed spinal cord was formed. The A-scan ultrasound system was a Panametrics pulser receiver and the resultant signal was displayed on an oscilloscope and photographed. The transducer had a 5 MHz center frequency, 0.5" diameter crystal with a nominal 1.25" spherical focus. The 6 dB width of the beam was measured to be 1.0 to 1.2 mm at 0.5 to 1.5" range. The A-scan traces were recorded at locations 1.0 mm apart, along the spinal cord through the normal region and at 0.5 mm intervals in the injured region using a micropositioner.

In vitro experiments were performed on an isolated thoracic spinal cord segment mounted in a holding device submerged in a bath of distilled water. The transducer, a Panametrics Videoscan, 10 MHz, 0.5" diameter with a spherical focus, was moved in 1/8 mm intervals with a Beckman stepper motor. The record of the 2048 points length were digitized to 8 bits resolution at 10 ns intervals by the Biomation 8100 transient recorder. Further image enhancement techniques (Gammell, Ultrasonics, 1981) were used to produce a refined printout of the spinal cord and injury site showing the connective tissue scar and cyst formation typical of a chronic (8 week) injury. The ultrasonic image correlated with a model of the spinal cord reconstructed from paraffin embedded histological sections.

Details of the various procedures and application of these findings will be discussed.

Supported by the Georgetown Spinal Cord Injury Research Fund and NASA-Office of Technology Utilization.

98.11 FURTHER OBSERVATIONS ON THE DEVELOPMENT OF THE NUCLEUS OF ORIGIN OF CENTRIFUGAL FIBERS TO THE AVIAN RETINA. D.D.M. O'Leary and W.M. Cowan. The Salk Institute, La Jolla, California 92037.

During the normal development of the nucleus of origin of the centrifugal fibers to the retina (the isthmo-optic nucleus - ION) some neurons have been found to migrate to ectopic loci and others have been shown to send their axons aberrantly to the ipsilateral eye (Clarke and Cowan, PNAS, 1975, 72:4455). We have re-examined the number, distribution, and fate of these two classes of neurons using the retrogradely transported dyes true blue and nuclear yellow.

With this more sensitive method it is evident that the number of ectopic neurons is considerably greater than had been suggested previously and that many are located much further away from the borders of the ION (up to 1500 μm). On the 13th day of incubation (i.e., shortly after all the fibers from the ION reach the retina) about 2,000 ectopic ION neurons can be labeled on the side of the brain contralateral to the eye injected with a dye. Such ectopic cells represent approximately 10% of the total number of ION neurons generated. By the end of the period of naturally occuring cell death in the ION (on day 17), this number is reduced to about 800. The disappearance of 60% of the ectopic neurons is comparable to the percentage of naturally occurring cell death seen in the ION itself. It has been suggested that the surviving ectopic neurons persist because their axons form synapses within the retina and because they receive an input from the optic tectum, as do the neurons in the ION itself. In support of this hypothesis we have found that when large lesions are placed in the optic tectum shortly after hatching, there is both a severe anterograde transneuronal degeneration in the ION and also a proportional loss of ectopic ION cells.

Intraocular injections of true blue on day 13 label about 40 neurons in the ipsilateral ION and the immediately surrounding area. Of these roughly a fifth can be doubly-labeled by injections of nuclear yellow into the opposite eye, which suggests that at least some of the aberrant ipsilateral projections are due to axon collaterals. Only 25% of the cells with ipsilaterally projecting axons survive the period of naturally occurring cell death in the ION. Since only rarely has it been possible to doubly-label these cells in post-hatched animals with dye injections into the two eyes, it seems likely that most of the neurons with aberrant ipsilateral collaterals either die during the period of naturally occurring cell death or withdraw their collaterals to the ipsilateral retina.

This work was supported by grant EY03653 and by the Clayton Foundation for Research.

98.12 CELL DEATH AND PROLIFERATION IN THE ELECTRIC LOBES OF TORPEDO MARMORATA. G.Q.Fox and G.P.Richardson. Department of Neurochemistry, Max-Planck Inst. für biophy. Chemie, Göttingen, W. Germany.

A quantitative analysis of electromotoneurons has been made during the development of the electric lobes in Torpedo marmorata. The study was started at the 26mm body length stage shortly after the majority of the neurons have apparently differentiated as judged by the disappearance of columnar epithelia. Degenerating cells with unequivocal pycnotic profiles have been quantified and are found during a period from 38mm to 74mm embryo body length. The onset of this period coincides with the onset of differentiation of myotubes into electrocytes in the electric organ as well as the onset of differentiation of the electromotoneurons from an immature monopolar form to a mature multipolar neuron. The period also overlaps slightly with the onset of synaptogenesis in the electric organs. No obvious decline in total neuron numbers occurs during this period although following it an apparent increase in numbers has been noted which continues into adulthood. This suggests that there is a continual slow proliferation of neurons during the entire growth and development of the animal although we have not been able to locate the source of these additional cells.

The results indicate that cell death in this system can not be attributed to a failure to compete successfully for synaptic sites because it begins prior to the onset of synaptogenesis and terminates at a time when developing synapses occupy only about 15% of the available postsynaptic membrane(unpublished observations).

98.13 LOCAL ELECTROLYTE ALTERATIONS FOLLOWING ACUTE INJURY TO THE CAT SPINAL CORD. R. C. deGroof†, J. L. Alderman and J. L. Osterholm. Depts. of Pharmacology and Neurosurgery, Jefferson Med. College of Thomas Jefferson Univ., Philadelphia, PA 19107.

It has been proposed that the early block of conduction in the long tracts following spinal cord injury may be due to a substantial shift in ionic equilibrium at the site of injury. Studies by other laboratories demonstrate a significant loss of K+ from the injured cord tissue which, since blood flow is seriously impeded, presumably moved into the CSF. While those studies generally support a role for K+ in axonal blockade, they give limited information on the regional spinal cord distribution and thus the potential influence of K+ or other electrolytes in discrete functional areas of the injured cord. In order to obtain more information on the regional behavior of electrolytes in response to trauma, the spatial redistribution of the electrolytes K+ and Na+ were determined separately in the anterior, posterior, and lateral white regions, as well as in the central grey region of the experimentally injured spinal cord 1 hour after trauma. Analyses by atomic absorption spectrophotometry were made on specimens taken by the punch sampling technique from subsections taken serially along the rostral caudal axis above, through and below the actual injury site (T8). Values were corrected for protein content and expressed as percent change from remote thoracic T3-4 control regions.

K+ content of all white matter tracts was reduced by 50% from control within 5 mm both rostral and caudal to the impact site. At 10 mm in both directions, the K+ content had returned to near control level. Na+ content of white tracts was increased by 100% at the impact site and in all cases returned to near control at 10 mm both rostral and caudal. The K+ content of the central grey area was depressed by 75% at the site of impact. This depression was about 50% at 10 mm both rostral and caudal. The Na+ content of central grey depression was increased 100 to 200% near the site of impact but returned to near control level 10 mm in both directions. These results show that spinal cord injury has a profound effect on the electrolyte content of spinal tissue. This loss of potassium and increase of sodium was localized to the area near the injury, and was of generally equal magnitude in all white regions. If electrolyte alterations are responsible for depressions of conduction through the long tracts, these results indicate that conduction in all spinal areas would be similarly blocked (USPHS 5-R01-NS12287-03).

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98.14 RELEVANCE OF RETINAL GANGLION CELL DEATH TO THE PATTERN OF NEURON DEATH IN THE SUPERIOR COLLICULUS. Timothy J. Cunningham and Debra L. Giordano.* Department of Anatomy, The Medical College of Pennsylvania, Philadelphia, PA 19129.

Neuron death during development has now been documented in several areas of the normal nervous system of several different species. A number of studies have concentrated on cell death within individual cell groups but there is little information on the interrelationship of neuron death in connecting central nervous system regions. The retino-collicular pathway of neonatal rats is an ideal system for such a study because natural neuron death has been documented in both the ganglion cell layer and superior colliculus (Cunningham et al. 1981; Giordano et al. 1980), and because the topography of connections between these structures is well understood. We examined 1µ plastic sections from the normal superior colliculus and ganglion cell layer of neonatal rats. Counts of degenerating cells were made from sections of the rostral and caudal thirds of the colliculus and sections of nasal and temporal parts of the ganglion cell layer. At ages when the numbers of degenerating cells appear maximal in both structures (i.e., 6-7 days postnatal), the nasal ganglion cell layer and caudal superior colliculus (which are normally connected) have an average of 2 times as many degenerating profiles as temporal retina and rostral colliculus (which are also connected). Therefore, the patterns of natural degeneration at least to some extent, parallel the topography of retino-collicular connections. These regional differences may represent gradients of cell death and may reflect reciprocal control of neuron survival. On the other hand, studies of embryonic chick retina indicate that the nasal-temporal differences in cell death are independent of the optic tectum and may be intrinsic to the retina (McLoon and Hughes, 1979). We sought to determine if the caudal-rostral differences in collicular cell death are dependent on the projection from the retina. Rats were enucleated at birth, examined each day thereafter to postnatal day 14, and then compared to normal rats. Counts in the normal rats on days 1-8, (after which the numbers of profiles decline significantly), show more degenerating cells in the caudal third of superior colliculus compared to rostral and middle thirds. In enucleated rats, cell death has a similar time course but there is no significant difference between rostral, middle and caudal thirds of the colliculus. The results suggest that the spatial pattern of cell death in the colliculus depends on optic afferents and may indicate peripheral to central adjustments of neuron numbers in this system.

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98.15 VARIABILITY IN THE CELL DEATH OF IDENTIFIED NEURONS IN GRASSHOPPER EMBRYOS. Curtis M. Loer* and Corey S. Goodman. (SPON: J. Wine). Dept. Biol. Sci., Stanford University, Stanford, CA

Every segment in the nervous system of the grasshopper embryo has a single midline precursor 3 (MP3) which divides once to give rise to two progeny (Goodman, Bate, Spitzer, 1981). In the meso- (T2) and meta- (T3) thoracic segments, one of these two progeny differentiates into the "H" cell. In some abdominal segments, one or both of these two cells dies (Bate, Goodman, Spitzer, 1981). Although there is a trend in the segment-specific differences of survival vs. cell death, the precise pattern varies from embryo to embryo.

In the present study, we examined the variable pattern of survival vs. cell death in 14 contiguous segments of 378 grasshopper embryos, after the stage at which cell death occurs. The pattern of cell death was examined in 6 different clutches each of Schistocerca americana and S. nitens, and 11 different clutches of Melanoplus differentialis (n greater than 100 for each species). The 'typical' pattern is for one cell to survive in S3, both cells to survive in T1-T3 and A1, one or no cells to survive in A2-A7, and one or two cells to survive in A8 and A9. There is, however, considerable variability from embryo to embryo. One of the striking differences between species is the survival in the A2 segment, where two cells survive in 42% of the S. americana embryos, in only 7% of the S. nitens embryos, and in only 9% of the M. differentialis embryos. There is even more overall variability, however, between different clutches of the same species. Since the clutches of eggs are raised in nearly constant and identical environmental conditions, the clutch-specific differences suggest a genetic component to the pattern. We plan to further study the genetic component of this variable pattern of cell death by examining the pattern in embryos from different isogenic clones.

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99.1 ELECTROMICTURITION: HISTOPATHOLOGICAL EVALUATION OF SACRAL NERVES FOLLOWING ELECTRICAL STIMULATION. W.F.Agnew, T.G.H. Yuen*, L.A. Bullara*, R.A.Schmidt*, and E.A.Tanagho*. Neurological Research Laboratory, Huntington Institute of Applied Medical Research, Pasadena, Ca. and Dept. of Urology, Univ. California School of Medicine, San Francisco, Ca.

The feasibility of controlling micturition in the spinal injury patient by electrical stimulation of the sacral nerves of spinal dogs has been investigated in previous studies(Schmidt and Tanagho, Urol.Int.34:199,1979. This report describes the histopathological evaluation of stimulated and non-stimulated sacral nerves in 18 dogs of either sex. Twelve dogs were subjected to chronic electrical stimulation of the sacral nerve and six were unstimulated either with or without electrode implantation and with or without dorsal rhizotomy. The two types of electrode arrays evaluated included a silastic cuff array with bipolar platinum electrodes and a silastic spiral matrix with tripolar platinum electrodes. Animals subjected to stimulation had an electrode implanted on S2, the sacral root producing the strongest bladder contraction with stimulation. Electrodes were implanted at various sites on the ventral root of S2 with rhizotomy of the dorsal root with or without ganglionectomy and with or without division of the Pudendal trunk within the ischiorectal fossa. The animals were stimulated once daily for 60 seconds using 2.5 to 3.0 volts using an implantable receiver and transmitter system (Avery Model I-110). Micturition was assessed by use of an uroflowmeter and measurement of residual volume by fluoroscopy. The two types of electrodes were evaluated for periods ranging from 1.5 to 10.5 months following surgical implantation. At the time of sacrifice the dogs were perfused via the ascending and descending aorta with either 10% formalin (for light microscope) or a mixture of 2% GTA and 4% paraformaldehyde (for electron microscope) studies. In general, stimulation with either cuff or spiral electrode effectively produced micturition. However, the cuff-type electrode proved to be unsatisfactory for chronic stimulation due to buildup of connective tissue within the cuff lumen, often to the extent of almost complete extrusion of the sacral nerve. Despite severe mechanical distortion of the S2 nerve, micturition was readily induced in many of the animals and microscopic examination revealed a normal axon population. In some instances, however, there was a marked diminution of the number of axons, with hypomyelination of many axons, and endoneurial connective tissue accumulation. The spiral electrode array proved superior in that a good electrode-tissue contact was maintained with minimal connective tissue encroachment and both morphological and functional preservation of the nerve. (Supported by NINCDS Contract # NO-1-NS-0-2275).

99.2 CHANGES IN EXTRACELLULAR POTASSIUM ACTIVITY DURING STIMULATION OF THE CEREBRAL CORTEX AT DEFINED CHARGE DENSITIES. D.McCreery and W.F.Agnew, Neurological Research Laboratory, Huntington Institute of Applied Medical Research, Pasadena, Ca.

In cats anesthetized with nitrous oxide and halothane, ion selective microelectrodes were used to monitor continuously the activity of potassium (a_K) in the extracellular compartment of the sensorimotor cortex during up to 4 hours of continuous electrical stimulation of the cortical surface above the ISMs. The electrical stimulus was a continuous train of biphasic constant current pulses through a disc electrode 1.1 mm in diameter. Charge densities correlated in previous studies in this animal with various degrees of neural damage were used. The compound action potential evoked by the surface stimulation was monitored continuously by means of an electrode in the ipsilateral pyramidal tract.

A stimulus charge density of 20 microcoulombs/cm^2. phase (20μc/cm^2.ph) (found in previous studies to produce only very minimal histological changes during 50 hours of continuous stimulation) evoked no or only a small transient increase in a_K within the grey matter, followed by return to the pre-stimulus base line activity within one minute. Higher charge densities produced larger initial increases in a_K and a slower return towards the pre-stimulus base line value. Continuous stimulation at a charge density of 100 μc/cm^2.ph (shown to produce severe neural damage if maintained for as little as 4 hours) at 20 pulses/sec produced an initial increase in a_K of 8-10 mM in the grey matter, followed by a gradual return within 1 to 2 hours to within 2 mM above the pre-stimulus value. After 2-3 hours of continuous stimulation a_K again began to increase. At a charge density of 100 μc/cm^2.ph and 50pps the magnitude and overall time course of a_K was very similar to that seen at 20 pps except that the initial increase was slightly larger and periodic "waves" of sequential increases and decreases of a_K were seen beginning one half to one hour after initiation of stimulation. These waves lagged slightly behind periodic increases and decreases in the amplitude of the earliest secondary (probably synaptically evoked) component of the compound action potential recorded from the pyramidal tract. In general, during continuous stimulation of the cortical surface, the time course of a_K followed that of the amplitude of the secondary component of the pyramidal tract potential. Although the local mechanisms for maintaining potassium homeostasis between brain compartments remains functional during several hours of intense stimulation, its inability to correct completely the increase in a_K may contribute to the neural damage occurring during such stimulation. Supported by NINCDS Contract # NO-1-NS-0-2275.

99.3 INTRACORTICAL CAPACITOR ELECTRODES: A PRELIMINARY EVALUATION. E.M. Schmidt, F.T. Hambrecht, and J.S. McIntosh. Lab. of Neural Control, NINCDS, NIH, Bethesda, MD 20205.

Electrical stimulation has long been used by neurophysiologists to modify and control the electrical activity of cortical neurons. When conventional noble metal electrodes are used (such as platinum, platinum alloys, or iridium), oxidation-reduction reactions can occur during current passage between the electrodes and the extracellular fluid surrounding them. Some of the products of these reactions are toxic to tissue and their generation is proportional to the charge density at the electrode-tissue interface. As intracortical electrodes are made smaller to increase their selectivity, charge density increases because the electrode surface area decreases faster than the total charge required to effectively stimulate neurons. The possibility of toxic reaction products accumulating is therefore increased and becomes even more significant if long-term stimulation is required. Capacitor electrodes can be used to stimulate nerve or muscle tissue without oxidation-reduction reactions because their insulating dielectric does not allow electrons to pass, even though ions in the tissue can be attracted or repelled by the charge on the electrode. The only capacitor electrodes available for neural tissue stimulation have been relatively large cylindrically shaped slugs (1mm diameter) obtained from capacitor manufacturers. These are suitable only for surface electrodes. However, some applications require long-term stimulation of cortical neurons at various depths with penetrating electrodes. For these we designed conically tipped tantalum/tantalum pentoxide capacitor electrodes with a maximum diameter of roughly 190 um and an exposed length of 500 um.

In vivo evaluation of these intracortical capacitor stimulating electrodes was carried out by chronically implanting 9 electrodes in the motor cortex of a monkey. The electrical characteristcs of each electrode were determined by passing a single 100 ua, 0.1 ms current pulse and measuring the resulting voltage between the electrode and a large indifferent platinum electrode located in the cerebrospinal fluid above the cortex. These measurements remained relatively stable over the 57 days of implantation. The effectiveness of the electrodes in stimulating cortical structures was evaluated by determining the minimum pulse train current required to produce a palpable forelimb muscle contraction. The threshold currents and muscles activated were determined during the period of implantation. These intracortical capacitor electrodes are effective for stimulation of small populations of neurons and are electrically stable during chronic implantaion without the hazards of oxidation-reduction reactions associated with noble metal electrodes.

99.4 SENSORY CODING IN NATURAL & ARTIFICIAL RECEPTORS, G. S. Wasserman. Sensory Coding Lab., Purdue Univ., West Lafayette, IN 47907.

There are two ways in which one can study the sensory codes used by receptors: One way is to compare the responses of natural receptors with the behavior of whole organisms when equivalent stimuli are delivered in both cases. Sensory codes are then extracted by searching for the features of the receptor responses that have the same formal properties as the behavior. Previous work along these lines suggested that the receptor code was task dependent and that different sensory codes mediated performance in detection and recognition tasks. For detection tasks, the initial transient peak of the receptor potential accounted for behavior; for recognition tasks, the integral of the receptor potential accounted for behavior. The weakness of this approach is twofold: It is correlational and it often compares receptor data taken from animals with behavioral data taken from humans. These weaknesses have led us to consider the advantages of studying the performance of artificial receptors implanted in human patients whose natural receptors have been destroyed. A review of the work done with these artificial receptors supports the suggestion we obtained from studying natural receptors: Adequate recognition occurs when artificial receptors use the recognition code; otherwise, sensation is limited to stimulus detection. However, coding variables have not been the explicit focus of this previous work with artificial receptors and hence this support is limited. We have therefore begun to study the effects of coding variables on recognition performance in patients fitted with artificial receptors. Preliminary results of these investigations will be presented.

99.5 Visual Prosthesis: Use by an Infant Macaque. E.R.Strelow* (SPON: A.H.Riesen).Dept. of Psychology, Univ. of California, Riverside, CA. 92521

An infant macaque monkey (M.arctoides) was born in the dark and subsequently reared for 35 days with its eyes covered, but using a head-mounted electronic prosthesis to assist space perception.The prosthesis (the Binaural Sensory Aid) provided distance, direction and object class information by an auditory display,and the head-worn portion of this device weighed 28 gm.

The animal was reared in a large cage with a cloth surrogate in place of its mother, and with a number of toys to interact with. The monkey showed a gradual increase in 'looking-type' head-turning behavior and after two weeks would reach for otherwise silent objects presented in a controlled setting. He thus showed a degree of behavioral plasticity in accepting artificial sensory input in place of normal visual stimuli, although the use of the prosthesis was rudimentary compared to visual skill, and compared to performance of the long-term blind who have used similar devices as aids to mobility.

Among issues raised for discussion are:1) the extent to which it is possible to create conditions which will encourage self-initiated learning of such novel sensory stimuli; 2) the adequacy of the sensory prosthesis as a source of spatial information; and 3) what would be the limits of this sensory substitution even with ideal prostheses and rearing conditions?

99.6 SENSORY THRESHOLDS ON TOES TRANSPLANTED TO THE HUMAN HAND. J. H. Solomon*, R. W. Dykes, D. C. Donderi* and R. K. Daniel. Depts. Psychology, Physiology, Surgery, Neurology and Neurosurgery, McGill University, Montreal, Quebec, H3A 1A1.

More than one year after surgery was performed to transfer the great toe to the hand to replace a missing thumb, cutaneous thresholds were measured in the transferred digit and compared to thresholds for normal thumbs. It has been known for many years that there is a correlation between tactile acuity on a particular body site and the density of cutaneous receptors in that region. This correlation has often been used to imply a causal relationship between the receptor density and tactile acuity. However, it has never been subjected to an empirical examination. The transplanted digits provide an opportunity to test this presumption of causality. Each patient underwent several hours of psychophysical tests designed to obtain pressure thresholds, two-point thresholds, and point localization thresholds. Thresholds were obtained on the normal thumb, transplanted toe, and normal toe. These were compared to equivalent data from 20 healthy, age-matched normal subjects. The results show that thresholds from the thumbs of normal subjects were not significantly different from thresholds on the normal thumb of the patients. Similarly, the thresholds on the normal toes of the subjects and patients did not differ significantly. The thresholds on the transferred toes were strikingly different from the thumbs of either subjects or patients. However, when compared to the thresholds on the normal toe, the values for the transplanted toes were within the normal range. Thus, despite the surgical manipulations, the transplanted toes functioned within the normal range for toes. This result suggests that the limiting factor in this series of sensory tests is the receptor density in the skin region from which the tissue originated. Further, it implies that there are limitations to the ability of regrowing axons to induce new sensory endings in glabrous skin.

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100.1 SACCADIC INTRUSIONS IN TRACKING EYE MOVEMENTS OF SCHIZOPHRENICS. S. Levin, A. Jones*, L. Stark, P.S. Holzman, E. Merrin*. Mailman Research Center, McLean Hospital and Harvard University, Belmont, MA 02178.

Schizophrenic patients have abnormal smooth-pursuit eye movements (SPEM) (Diefendorf & Dodge, 1908; Holzman, et al, 1974). These abnormalities are not due to drug medication and probably reflect a stable trait under genetic influence, as suggested by longitudinal, family, and twin studies. (Holzman, et al., 1977, 1980; Iacono & Lykken, 1979, 1980). The precise nature of the SPEM impairment must be determined with high-precision reflected light technique. We tested 6 chronic schizophrenics using an infra-red limbus-tracking technique with a total system bandwidth of 1 kHZ. In 4 of the patients we tested so far (67%), the main characteristics of the eye movement abnormalities appeared to be saccadic intrusions which are small amplitude eye movements in opposite directions. These saccadic intrusions were seen during smooth pursuit, visual fixation, vergence eye movements, and saccadic fixation rests, but not in VOR in the dark or during head-eye coordination. The dynamic characteristics of the saccadic intrusions were on the main sequence (Bahill & Stark, 1979) and cannot be distinguished from the saccadic intrusions which appear sometimes in normal fixation or pursuit.

In contrast to the impairement of SPEM and other fixation eye movements, saccadic eye movements, following a predictable or unpredictable target, had normal latencies and normal dynamic characteristics, confirming the results of a previous investigation using EOG recordings with a larger sample of patients (Levin, et al., 1981).

Although these results are preliminary, they clarify the nature of the primary irregularity previously seen during SPEM, and confirm that it consists of eye movements rather than bioelectric noise. Tracings of these eye movements are quite different from saccadic smooth pursuit seen often in a variety of benign and serious eye movement control disorders.

100.2 DISTURBED OCULAR MOTOR CONTROL IN HUNTINGTON'S DISEASE (HD). R. J. Leigh, S. A. Newman*, S. E. Folstein* and A. G. Lasker*. Depts. of Neurology, Ophthalmology and Psychiatry, The Johns Hopkins Univ. Sch. of Med., Baltimore, Maryland 21205

We have studied the eye and head movements of 37 patients with HD using video, D.C. electro-oculography and magnetic search coil methods. Fixation was abnormal in 20/27 patients because of small extraneous saccades which were most evident when a rich visual background surrounded the fixation target. Saccades were slow to clinical examination, in 19/35 patients. In some early-affected patients whose eye movements looked clinically normal, recordings showed disturbed peak-velocity/amplitude relationships, particularly in the vertical plane. Difficulty in initiating saccades was manifest by prolonged latency in 24/32, "mandatory" blinks in 12/34 and obligatory head movements in 33/36. Smooth pursuit was clinically abnormal in 14/29 patients; eye movement records showed that this was sometimes due to decreased pursuit gain but more often the abnormality was one of extraneous saccades such as those that interfered with fixation. Similarly, vergence was abnormal in 9/26 patients due to difficulties in fixating a stimulus target. The vestibulo-ocular reflex, assessed by eye movement recording and a bedside ophthalmoscopic test appeared normal in 36/37 patients. Gaze-holding ability was normal in 33/37 patients. Eye movements in patients who were receiving psychotropic medication did not appear to differ significantly from those receiving no treatment.

We postulated that the disturbances of fixation and saccadic initiation were due to frontal lobe involvement in HD since patients with frontal lobe lesions make saccades to behaviorally-inappropriate but visually-attractive stimuli (Buchtel, H. A. and Guitton, D., Neurosci. Abstr., 6:316, 1980). To test this, we studied 6 early-affected patients. They were instructed to fixate a central light-emitting dioded (LED) in darkness and ignore the appearance of a peripheral distracting LED until the central fixation light went out. All 6 patients made inappropriate saccades to the distracting stimulus in more than 50% of trials, even though the instructions were clearly understood. Four normal subjects tested in the same way showed less than 5% of trial errors. Thus frontal lobe involvement in HD may be the substrate for fixation instability. An alternate explanation is that the diseased striatum causes impaired function of units in the pars reticulata of the substantia nigra (Hikosaka, O. and Wurtz, R. H., Neurosci. Abstr., 6:15, 1980) that inhibit superior collicular neurons.

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100.3 VESTIBULO-OCULAR REFLEX DYSFUNCTION IN PARKINSON'S DISEASE O.B. White*, J.A. Saint-Cyr and J.A. Sharpe, Playfair Neuroscience Unit, Division of Neurology, Toronto Western Hospital, University of Toronto, Toronto, Canada.

Horizontal pursuit and vestibular smooth eye movements, in response to predictable target motion and passive whole body rotation at frequencies from 0.3 to 1.0 Hz, were quantified in 9 patients aged 45-74 with idiopathic Parkinson's disease. One patient had had a previous thrombotic stroke without residua and one had had a previous unilateral thalamotomy. All patients were receiving pharmacotherapy (L-dopa with a peripheral decarboxylase inhibitor with or without anticholinergics). Eye movements were measured by infra-red oculography and d.c. electro-oculography. Data were analysed off-line with a digital computer. Results were compared to those of 16 control subjects.

Vestibulo-ocular reflex (VOR) gains measured in alert patients in darkness were significantly reduced at 0.3 and 0.5 Hz in mild to moderately affected patients. Gain reduction was more marked in patients with severe hypokinesia and rigidity and evident at higher rotational frequencies. Fixation of a stationary target significantly increased VOR gain. During attempted cancellation of the VOR by fixating a target moving with the head, VOR gain was higher than in darkness. Cancellation was defective. Voluntary (non-visual) enhancement and cancellation of the VOR by attempted fixation of imagined stationary and moving targets respectively, accompanied defective visual control of the VOR. Smooth pursuit velocities were significantly reduced.

Impaired visual modulation of the VOR may be explained by the smooth pursuit defect. Defective voluntary modulation of the VOR indicates that Parkinson's disease also involves circuits that mediate non-visual control of the VOR. Disparity between reduced VOR gains in darkness and higher gains during attempted VOR cancellation, indicates that an alternate mode of compensatory smooth eye movement generation is available during passive rotation and is inappropriately activated in Parkinson's disease.

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100.4 CHANNELS IN THE VESTIBULO-OCULAR REFLEX (VOR). S.G. Lisberger and F.A. Miles, Laboratory of Neurophysiology, National Institute of Mental Health, and Laboratory of Sensorimotor Research, National Eye Institute, Bethesda, MD 20205

It has been usual to represent the mechanism underlying adaptive gain control of the VOR as a single pure gain element. We now report observations that cannot be explained by such a minimal model; we suggest that the VOR operates as a series of parallel channels with differing dynamics, and that the adaptive process can adjust the gain of each channel separately.

Four rhesus monkeys were subjected to passive whole-body oscillation while viewing either a) the stationary surroundings through x2-telescopic spectacles or b) a visual scene that oscillated with them (equivalent to x0-spectacles). VOR gain was estimated as peak-to-peak smooth eye velocity divided by peak-to-peak head velocity during sinusoidal oscillations of $\pm$25 deg/s at frequencies ranging from 0.1 to 4.0 Hz in darkness.

The passive oscillation paradigm produced larger changes in VOR gain at the adapting frequency than at adjacent frequencies. For example, adaptation at 0.2 Hz produced larger changes in VOR gain when tested at 0.2 Hz (average for the 4 monkeys reaching high gains of 1.66 and low gains of 0.42) than when tested at 2.0 Hz (up to 1.35 and down to 0.63). Similarly, adaptation at 2.0 Hz produced larger changes in VOR gain at 2.0 Hz (up to 1.62 and down to 0.39) than at 0.2 Hz (up to 1.10 and down to 0.62). While eye velocity remained 180 deg out of phase with head velocity at the frequency of adaptation, consistent small changes in phase shift were seen at adjacent frequencies. At frequencies above that used for adaptation, increases in VOR gain caused eye velocity to lag a perfect VOR while decreases caused eye velocity to show phase lead. Conversely, at frequencies below that used for adaptation, increases in VOR gain caused eye velocity to lead a perfect VOR while decreases caused phase lag.

Such frequency-selective adaptation suggests the existence of temporal frequency channels in VOR pathways. The complex dependence of VOR phase on gain and frequency can be explained if individual channels are also differentiated on the basis of phase shift. In this scheme, higher frequencies would tend to activate channels carrying signals with greater phase lead; the extent of overlap between the channels excited by the adapting and test stimuli would determine the magnitude of the phase shift seen in the VOR. An adaptive mechanism that can operate selectively over narrow bandwidths constitutes a highly-flexible and tuneable interface between afferents and motoneurons. By compensating in narrow bandwidths for frequency-dependent variations in primary afferent responses, this interface could provide a means of establishing frequency-independent performance in the adult VOR.

100.5 OPTICALLY-INDUCED CHANGES IN THE NEURAL COUPLING BETWEEN VERGENCE EYE MOVEMENTS AND ACCOMMODATION IN HUMANS. F.A. Miles and S.J. Judge*. Laboratory of Sensorimotor Research, National Eye Institute, Bethesda, MD 20205, and University Laboratory of Physiology, Oxford University, Oxford OX1 3PT, England.

Transfer of fixation between targets at different viewing distances involves changes in vergence eye movements and accommodation that operate to eliminate disparity and blur, respectively. However, during monocular viewing, when disparity cues are absent, changes in accommodation due to blur also result in linear changes in vergence: Accommodation-vergence (A-V). Similarly, during binocular pinhole viewing, when blur cues are minimal, changes in vergence due to disparity also result in linear changes in accommodation: Vergence-accommodation (V-A). We now report that these open-loop responses undergo adaptive modification when the apparent separation of the eyes is more than doubled with laterally-displacing, periscopic spectacles.

A-V and V-A were measured in two human subjects using a haploscope incorporating a laser optometer with Badal lens viewing to determine the accommodative state of the right eye. For measurement of A-V, the right eye fixated cross-hairs through a mixing cube and Badal lens, providing accommodative stimuli of 0-4D with fixed size and brightness cues; the left eye viewed a blank screen with alignment markings (offset to avoid fusion) through a mirror galvanometer system that, when aligned by the subject, indicated the horizontal position of that eye. A-V was expressed as a dimensionless gain parameter equal to the measured change in vergence per unit change in accommodation divided by the required change in vergence to maintain correct alignment of the eyes during that same change in accommodation. Initial A-V gains were 0.86 and 1.16, and after wearing the periscopic spectacles for 30 min, these increased to 1.43 and 1.89, respectively. For measurement of V-A, each eye fixated cross-hairs in Maxwellian view; blur cues were minimized by using pinhole light sources (<0.5 mm diam) and vergence was varied systematically by rotating the optical system in the left limb of the haploscope about the center of rotation of the left eye. V-A was expressed as a dimensionless gain parameter equal to the measured change in accommodation per unit change in vergence divided by the required change in accommodation to maintain correct focus of the eyes during that same change in vergence. Initial V-A gains were 1.09 and 0.86, and after wearing the periscopic spectacles for 30 min, these decreased to 0.51 and 0.41, respectively. Thus, challenged with optical devices that increase the separation of the two lines of sight, A-V and V-A gains show increases and decreases, respectively, that are appropriate for improving the rapid alignment and focussing of the two eyes.

100.6 ON THE MECHANISM OF VISUAL-VESTIBULAR INTERACTION. O. Bock* (SPON: C.M. Oman). Inst. of Physiol., Free Univ., Berlin, Germany.

The visual and vestibular systems interact in the control of eye movements, induced by head rotation. We investigated the mechanism of this interaction by using a conflict between the two systems, concerning the appropriate direction of eye movements. 10 human subjects were rotated sinusoidally at different frequencies in a rotating chair. They were in total darkness except for a small stationary target light (LED), seen via a chair-fixed mirror. In this way, relative motion of the LED with respect to the subjects' head was reversed in direction, (i.e. in phase with the head.) Under these conditions, the smooth pursuit system, driven by visually perceived target motion, is expected to initiate eye movements in phase with the motion of the subjects. The vestibulo-ocular reflex (VOR), activated by head motion, is, however, expected to initiate eye movements 180 degree out of phase with the subjects' motion. We recorded eye position by DC-EOG and calculated phase and gain of the smooth components of eye movement with respect to the stimulus. A strong dependency of eye motion on stimulus frequency was found up to about 0.8 Hz. The eyes were, within a few degrees, in phase with the stimulus, and the gain was near unity, decreasing with frequency. This suggests that in this low-frequency range, eye movements are predominantly controlled by the pursuit system. At frequencies above 1.6 Hz, eye motion was, within a few degrees, by 180 degrees out of phase with the stimulus, and the gain was near 0.7, suggesting a predominance of the VOR in the high-frequency range. Of particular interest are findings at frequencies between 0.8 and 1.6 Hz. No gradual transition from visually to vestibularly dominated phase and gain could be demonstrated with increasing frequency. Rather, for any given frequency in this range, the eyes repeatedly switched between movement unequivocally in phase, and movement unequivocally by 180 degrees out of phase with respect to the stimulus. This finding can be best explained by assuming that the control of eye motion repeatedly switches between the pursuit system and the VOR. Our results demonstrate that the interaction of VOR and smooth pursuit is non-linear, since a linear relationship would require a smooth transition from visually to vestibularly dominated eye movements. Rather, the two systems appear to compete for the control of eye motion in a frequency range, (0.8 to 1.6 Hz), where no clear dominance of either system exists.

100.7 VESTIBULOOCULAR COMPENSATION DURING TRANSIENT HEAD ROTATION: IS IT ADAPTIVELY REVERSED BY LONG-TERM VISION REVERSAL? G. Melvill Jones and A. Berthoz*. Dept. of Physiol. McGill Univ., Montréal, Québec, Canada and Laboratoire de Physiologie Neurosensorielle, 75270 Paris, France.

Long-term vision reversal eventually leads to apparent reversal of the vestibuloocular reflex (VOR) as tested by low frequency (0.17 Hz) sinusoidal rotation in the dark. The present study examines the question: Does similar adaptive reversal occur in the reflex eye movements induced by rapid stepwise rotation of the head between 2 stationary targets?

METHODS: A human subject wore horizontally reversing dove prisms for 19 days, during which time head and eye movements were measured in the following dark-tested conditions: 1) Sudden, passively induced, rotation of the head between two fixed angular locations separated by roughly 10° in a horizontal plane. 2) Similar actively induced head rotation.

RESULTS: Control tests showed normal VOR in these circumstances (eg Barnes, J. Physiol. 287:127), yielding mean values of VOR gain (eye vel./head vel.) ranging from 0.75 (SE 0.03,n=20) to 1.01 (SE 0.03,n=18) with passive and active rotation respectively. During adaptation to vision reversal a variety of modified patterns of oculomotor behaviour developed. Thus with passive head rotation the VOR (identified by its fixed latency) was always either of reduced gain (mean values ranged from 0.27 to 0.48), or completely absent. Particularly noteworthy is the fact that all identified VOR responses occurred in the normal direction. With active head movement the VOR was also of reduced gain and normal direction, but its occurrence was relative -ly infrequent. Instead, there was an interesting variety of different response patterns, described and interpreted in another communication (Berthoz & Melvill Jones, European Neuroscience, 1981). One of these patterns might readily be misinterpreted as a successfully reversed VOR, of gain close to unity. However, owing to its highly variable, and often negative, latency, this functionally effective form of adapted activity must have derived from a source other than the VOR.

It is CONCLUDED that maintained vision reversal produced substantial attenuation of the VOR in both active and passive conditions, including the ability to effect its complete blockage. However, although the adaptive process also appears to have produced a functionally effective form of reversed ocular compensation manifest during the actively generated head movement, this reversed response cannot be attributed to the VOR.

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100.8

WITHDRAWN

100.9 ARE SACCADIC REACTION TIMES MODULAR IN STRUCTURE? AN ANSWER FROM A WAITING-TIME ANALYSIS. P.E. Hallett and H. Doma*, Dept. of Physiology, University of Toronto, Toronto M5S 1A8, Canada.

1. Saccadic latencies in several tasks from this laboratory and the literature have been interpreted as being composed of fixed and variable parts (e.g. Hallett and Adams 1980 Vision Res. 20, 329), where the fixed delay is about 120 msec. Thus the mean latency $\bar{N}$ in the normal single-step tracking task (or N-task) is supposedly the sum of one fixed and one variable delay, while the mean latency $\bar{A}$" of successful attempts at our "anti" task (or A-task), in which the subject makes a saccade opposite to the step, is supposedly one fixed delay plus about two variable delays.

2. On a plot of mean (y) versus standard deviation (x) of latency the y-intercept should be the fixed delay, and the square of the slope the number of stages of waiting that make up the variable delay, if the variable delay is a gamma-distributed waiting time. Plots for the 8 subjects of Hallett and Adams, plus 1 new subject, give a fixed delay of 136 ± 6 msec in the N-task (105 ± 22 msec in the A-task) and a variable delay of 8 ± 2 stages in the N task (22 ± 6 in the A task). So this new approach supports the idea that these tasks are based on a common fixed delay, plus one or more doses of variable delay.

3. We have repeated these experiments and plots for 5 new subjects. More importantly we have fitted the present model (fixed delay plus gamma-distributed waiting time) to the actual latency distributions for the N and A tasks, using the chi-squared criterion. The ratio for the two tasks (Anti/Normal) between the fixed delays was 1.00 ± .14, between the mean waiting times 2.04 ± .66, and between the numbers of stages of waiting 2.61 ± .33 (these data are mean ratios ± standard deviations across the 5 subjects). The value for the fixed delay was 129 ± 11 msec, with 8 ± 1 stages of waiting in the N-task and about twice as many (17-21) stages in the A-task. Thus the essential hypothesis is well supported by these new data for bright stimuli (100 x foveal threshold, subject otherwise dark-adapted).

4. At low light levels (down to 1/30 x foveal threshold) latencies become so protracted and dispersed that the full waiting-time analysis is impractical, but the fixed delay may well increase - as it should do if it contains a retinal delay as Hallett and Adams claimed.

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100.10 NEURAL SIGNAL ENVELOPE SHAPES AND OPTIMIZATION OF MOTOR PERFORMANCE

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Optimization was early applied to physical systems using Euler's calculus of variations. Bellman-Pontriagin optimization theory employs control signal variations to achieve optimal performance for a fixed plant and a particular optimality criterion. Dynamical behavior of saccadic eye movements when measured precisely achieves enough observability to define the controller signals by means of inverse modelling. Assuming a time optimality criterion, detailed studies of first, second and third order controller signals have been performed (Cook, Clark, Lehman, and Stark). New behavioral properties of saccades such as dynamical overshoots, have been explained. Unfortunately animal nerve impulse recordings have been both inadequately sampled and have not been oriented toward contributing to this problem. Again for optimization studies on human locomotion, there is only sparse controller signal data (Pedotti, Krishnan and Stark). Recent experiments on the 'saccadic' trajectories of head movements have provided us with considerable amounts of EMG, behavioral, and modelling data (Zangemeister et al). The controller signals for fast head movements appear to be fourth order. Ensemble averaging of pre-edited EMG signals provide direct evidence for this. In addition, confirming inverse modelling evidence has been obtained in a similar manner to that in the eye movement studies.

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100.11 LESIONS IN AVIAN ACCESSORY OPTIC SYSTEM SEVERELY DISRUPT OPTOKINETIC NYSTAGMUS IN NON-HORIZONTAL DIRECTIONS. Josh Wallman, Jose Velez* and Olivia C. McKenna. Biology Dept., City College, City University of N.Y., New York, N.Y. 10031.

A major role of the accessory optic system appears to be the signalling of information about the movement of the visual world (retinal slip) to the brain. Indeed, the accessory optic system seems to provide the major source of visual afference for non-horizontal optokinetic eye movements. These assertions are based on evidence of appropriate single unit characteristics and appropriate anatomical projections in both mammals and birds. We report here additional evidence resulting from the effects of lesions to the accessory optic system of birds.

Stereotaxic electrolytic lesions were made in the nucleus of the basal optic root (nBOR; also called ectomamillary nucleus) of chickens. The optokinetic eye movements were evaluated by measuring the average slow phase velocity of optokinetic nystagmus (OKN) to stimuli moving in each horizontal direction at a number of different stimulus speeds and to stimuli moving in various non-horizontal directions ranging from vertical to torsional.

The results were that complete lesions of nBOR proper and of its dorsal cap resulted in virtually complete loss of OKN to stimulus movement in vertical, torsional and intermediate directions. Partial lesions of nBOR proper caused changes in the relative sensitivity to stimulus movement in different directions. In most cases, even with the complete lesions, the deficits were accompanied by horizontal OKN within the normal range. (We are uncertain at this point about the effect of lesions of nBOR lateralis, which may be homologous to the mammalian dorsal terminal nucleus and which, along with a pretectal nucleus, probably signals horizontal retinal slip.)

These results support neurophysiological and anatomical evidence for the accessory optic system being the principal source of non-horizontal retinal slip signals to the oculomotor system; furthermore, they demonstrate the relative independence of the afferent subsystems concerned with different directions of retinal slip. (Supported by NIH EY-2937.)

100.12 VISUAL TRACKING IN CHICKENS. D. W. Pratt* (Spon: M. Romagnano) Dept. Biology, City College, CUNY, New York, N. Y. 10031.

The head and eye movements used by chickens to maintain fixation of moving objects were examined by cinematography. Chickens were filmed as they pecked at individual food pellets moving past them on a conveyor belt. Both linear horizontal and sinusoidal horizontal motion were used. Stimulus velocities from 3°/sec. to 130°/sec. were used. The photographic measurement technique has an angular resolution of better than 50' of arc and a bandwidth of up to 64 Hz. Pellets were tracked under all conditions as indicated by the fact that the chickens pecked accurately at them.

During tracking the head movements follow the pellet's movements. The angular change in the horizontal head position follows the angular change in the direction of the pellet from the head. Saccadic eye movements are used to change the direction of gaze. Between saccades the eyes compensate for angular head movements and maintain the direction of gaze in space. The pellet's image therefore moves across the retina. The chickens do not show an optokinetic response to this movement Smooth pursuit eye movements were not seen at stimulus. velocities down to 3°/sec.

101.1 DIFFERENTIAL BINDING OF LOTUS TETRAGONOLOBUS AND ULEX EUROPEUS LECTINS TO FUCOSYL-GLYCOCONJUGATES ON RAT CEREBELLAR CELL SURFACES. Lee N. Minier, Paul F. Erickson and Robert S. Lasher. Anatomy Dept., Univ. Colo. Hlth. Sci. Ctr., Denver, CO 80262

Several roles have been postulated for the various glycoconjugates (glycoproteins in particular) which are known to be components of the external neuronal cell surface. Among those functions suggested are involvement in the processes of cell recognition, cell-substrate adhesion, memory and synaptogenesis. During the course of our studies on the composition of the neuronal cell surface, we investigated the binding patterns of several plant lectins to rat cerebellar cells maintained in dispersed cell culture. With respect to the distribution of fucosyl residues on cerebellar neuronal and nonneuronal cell surfaces, two distinct binding patterns were observed for the lectins derived from Lotus tetragonolobus (Lotus A) and Ulex europeus (UEA), both of which have been shown to have binding specificities for α-L-fucose. Dispersed cell cultures prepared from 2-day old rat cerebellums were grown on glass coverslips for periods between 4 and 28 days. Selected cultures of various ages were washed with Saline G and were incubated for 15 min in solutions of Lotus A or UEA that had been radiolabeled with NaI^{125}. Control incubations included the presence of 0.1M α-L-fucose in the wash and incubation steps. The cells were washed, fixed in 2% paraformaldehyde, and then were processed for light microscope radioautography. The resultant radioautographs indicated that Lotus A bound almost exclusively to the neuronal cell aggregates in the cultures at all time points investigated. In particular, the grain density appeared to be greatest over the interconnecting fiber fascicles and neuropil comprising the central portion of the aggregates. Most nonneuronal cells exhibited surface labeling only marginally greater than background. The presence of α-L-fucose in the reaction medium completely inhibited binding of the radiolabeled lectin. Conversely, parallel experiments indicated that UEA did not bind to any appreciable extent to the surfaces of either neuronal or nonneuronal cells. Allied experiments showed conclusively that radiolabeling had not altered the biological activity of either lectin. In efforts to determine the nature of the fucosyl-conjugates detected by Lotus A, cerebellar cells at different ages were incubated in medium containing H^3-fucose. Radioautographs of these cells prepared for light microscopy revealed that fucose was preferentially incorporated into the processes and neuropil of the neuronal cells; little carbohydrate was taken up by nonneuronal cells. Preliminary studies using polyacrylamide gel electrophoretic methods have indicated that the H^3-fucose was incorporated primarily into 4-5 major polypeptide groups. (Supported by NIH Grants NS-13133 and NS-09199 (RSL)).

101.2 LECTIN RECEPTORS IN COCKROACH MUSCLES: DISTRIBUTION AMONG VARIOUS MUSCLES AND CHANGES AFTER DENERVATION. J. L. Eastburn*, R. T. Caldwell*, and J. L. Denburg. Zoology Department, Univ. of Iowa, Iowa City, Iowa 52242.

We have been studying the reformation of connections between axotomized, identified motor neurons and leg muscles in the cockroach, Periplaneta americana, the end result of which is the original innervation pattern. A role for cell surface glycoproteins in this possible intercellular recognition is examined by determining whether the various muscles possess different glycoproteins on their surfaces and whether there are any changes in these glycoproteins upon denervation. The presence of glycoproteins is detected by the binding of fluorescent derivatives of an array of plant lectins to frozen sections of muscles and to muscle proteins in SDS polyacrylamide gels after fractionation by electrophoresis.

The set of six coxal depressor muscles (CDMs) were studied in detail. It is composed of muscles 178 and 179 innervated by a single motor neuron, D_f; muscles 177d and e innervated by motor neuron D_s; muscles 177d' and e' in which every fiber is innervated by both D_f and D_s. These muscles are readily identified in frozen sections of the coxal segment of the leg. Fluorescent derivatives of all lectins which bound to these sections did so only to the cell surfaces and extracellular matrix. Lectins with binding sites for α-D-mannosyl and α-D-glucosyl residues and wheat germ agglutinin with a specificity for N-acetylglucosamine bind uniformly to all CDMs. Lectins from peanut, soybean and Bandieraea simplicifolia which recognize terminal α-D-galactose also bind to each of the CDMs but in different amounts. Binding to 177d',e'> 177d,e > 178,179. Binding of all lectins except WGA was totally eliminated in the presence of 0.5M sugars that are specific for each site. No binding was detected of the lectins from Dolichos biflorus, Ricinus communis and Ulex europaeus. These results of the histochemical examination are confirmed by the biochemical analysis of lectin receptors after fractionation by SDS polyacrylamide gel electrophoresis. However, it is additionally demonstrated that for lectins like ConA, which bind uniformly to all muscles, there are qualitative and quantitative differences in the population of glycoprotein receptors among the CDMs. These receptors may be classified according to their distribution among the CDMs and by the various effects which denervation has on their levels. Some of the characteristics found in these receptors might be expected to be expressed by macromolecules either involved in cell adhesion or cell recognition or affected by trophic interactions between the cells. (Funded by PHS research grants NS 14295 and NS 15350)

101.3 LECTIN BINDING OF DEVELOPING MOUSE RETINA. J.C. Blanks and L.V. Johnson*. Doheny Eye Foundation and Departments of Ophthalmology and Anatomy, University of Southern California, Los Angeles, CA.

Glycosylated cell surface macromolecules are likely to be of importance in the establishment of synaptic junctions. Consistent with such a role are reports of age-related modifications in cellular glycoprotein composition during development of the central nervous system. The neural retina provides an ideal model for the study of cell differentiation and cell-cell interactions in development as it is comprised of a relatively small number of identifiable cell types arranged in discrete layers. In these studies, the carbohydrate composition of the early postnatal and adult mouse retina has been examined by the use of carbohydrate-specific lectins. A battery of 8 lectins of differing carbohydrate sequence specificities was employed; these included concanavalin A (Con A), wheat germ agglutinin (WGA), soybean agglutinin (SBA), peanut agglutinin (PNA), Ulex uropeaus agglutinin (UEA), Ricinus communis agglutinin I (RCA), Dolichos biflorus agglutinin (DBA) and Limulus polyhemus agglutinin (LPA). Unfixed frozen sections (8-10 μm) of adult and early postnatal (days 0-18) mouse retina were treated with fluorescin isothiocyanate conjugated lectins and examined by fluorescence microscopy.

The results obtained reveal selective lectin binding by all retinal layers in the adult and throughout postnatal development. In general, an increase in the intensity of fluorescent staining by Con A (α-D-Glu, α-D-Man), WGA (β-D-NAcGlc), DBA (α-NAcGal), LPA (sialic acid) and UEA (α-L-fucose) is observed in association with retinal development, suggesting an increase in the expression or accessibility of complex carbohydrates. SBA (α or β-D-Gal, α-NAcGal) and PNA (Gal-GalNAc) show little to no binding to retinal layers but are bound by scleral components. All lectins binding to retinal layers show some degree of reactivity with rod inner segment regions. However, only Con A and WGA bind to rod outer segments, suggesting a significant alteration in the glycosylated components of outer segment membrane. Another striking observation is the appearance of intense fluorescent staining of the ganglion cell and inner nuclear layers by LPA during development. Concomitantly, an almost complete lack of detectable LPA binding is observed on establishment of the inner synaptic layer. These results indicate that sialic acid moieties may be of importance in establishing the structural and functional integrity of this portion of the retina. Current studies are analyzing the relationship between membrane density and the intensity of fluorescent lectin staining in the various layers of the developing retina. Retinal degenerative mutants are also being examined to determine if abnormalities in carbohydrate composition are detectable.

101.4 BRAIN GANGLIOSIDES: CONTENT AND PATTERN IN FROG, TOAD, AND SALAMANDER. L.N. Irwin. Dept. Biol., Simmons College, Boston MA 02115.

Gangliosides are cell-surface macromolecules of unknown function concentrated in brain tissue. Their content and pattern are known to vary phylogenetically but the extent of this variation has not been systematically studied below the class level; therefore, I have analyzed the content and pattern of brain gangliosides from five amphibian species. Wole brains were collected from three anurans -- Rana pipiens, Bufo marinus, Xenopus laevis -- and two salamanders -- Ambystoma tigrinum (At) and Necturus maculosa (Nm). Gangliosides were isolated from a total lipid extract (Irwin & Irwin. Anal. Biochem. 94:335) and quantified by a thiobarbituric acid assay for sialic acid. Ganglioside patterns were developed by thin-layer chromatography and quantified by scanning densitometry. Total ganglioside content was similar in all five species, with the two salamanders yielding the highest (362 μg/g wet weight for At) and lowest (176 μg/g wet weight for Nm) values. The chromatographic separations for gangliosides showed two distinct qualitative patterns. For the three anurans, the ganglioside pattern was dominated by three bands migrating at the rate of the mammalian disialoganglioside D1b or slower, as previously observed by several workers. However, both salamanders showed a distinctly more mammalian-like pattern, with 30% (At) and 47% (Nm) of total ganglioside sialic acid comigrating with the mammalian disialoganglioside D1a. This suggests that the preponderance of slowly-migrating gangliosides usually reported for frogs may not be typical of amphibian gangliosides generally. Among the three anurans, and between the two salamanders, quantitative differences in ganglioside pattern were also noted, suggesting that ganglioside patterns may be more variable, and hence of greater taxonomic significance, at the infraclass level than previously assumed. It also follows that a systematic study of ganglioside diversity as a function of phylogenetic, ontogenetic, and ecological variables may shed light on the biological role of these molecules. (Supported by grants from NIH and the Simmons College Fund for Research.)

101.5 THE STRUCTURE OF THE EXTRACELLULAR MATRIX ASSOCIATED WITH AXONS DIFFERS IN NERVE AND MUSCLE. D.P. Kuffler, Dept of Neurobiology Stanford Univ. Sch. of Med., Stanford, Calif. 94305

The extracellular matrix (ECM) of nerve and muscle plays an important role in the regeneration of the neuromuscular junction after trauma. To learn more about the ECM of axons I examined the organization of the ECM associated with different types of axons that innervate frog cutaneous pectoris muscle. I found that axons, regardless of type, have a different sheath of ECM when they are in nerve bundles than when they are free within muscle.

Frog peripheral nerves are enclosed by perineurium and each axon is ensheathed by Schwann cell processes. Surrounding each Schwann cell is a 20 nm thick basal lamina, an anatomically well defined component of the ECM. Within muscles the axons exit from the perineurium to run free for varying distances before terminating. At the point of exit from the perineurium the myelinated motor axons lose their myelin, but retain a Schwann cell sheath as do the unmyelinated autonomic axons. I found that at points of exit from the perineurium the Schwann cells of both the myelinated and unmyelinated axons acquire a dense particulate layer of extracellular matrix that extends the entire length of the axon and ranges up to 2 um thick. At the neuromuscular junction this thick particulate layer blankets the Schwann cell cap of the axon terminal. The particulate material is sparsely invested with collagen fibrils except at its perimeter; thus it forms a well-defined extracellular structure around each free axon.

Additional observations include: 1) A thick coat of material similar to that of free axons surrounds capillaries and certain other cells within the muscle, but is not found in extrajunctional regions of myofibers which have a basal lamina a few 10's of nm thick. 2) A basal lamina-like structure around free axons was best and most consistently seen in preparations where the ECM was poorly fixed and much of the thick particulate coat had "washed out"; perhaps the thick ECM coat of free axons is an expansion of the basal lamina characteristic of axons in nerves. 3) The thick ECM coat is stable in the absence of cells; it persists for months after removal of myofibers, axon terminals and Schwann cells from the neuromuscular junctions.

One explanation of these findings is that the characteristics of extracellular matrix around axons and their Schwann cells is influenced by the axon's environment which differs inside and outside a nerve. Alternately, the Schwann cells inside and outside a nerve may be of different types. (Supported by a USPHS NRSA Postdoctoral fellowship, and USPHS Grant NS14506.)

101.6 STUDIES ON THE SYNAPTIC BASAL LAMINA FROM THE ELECTRIC ORGAN. Bernardita Mendez* and Nibaldo C. Inestrosa*.(SPON.Ralph J. Greenspan). Division of Neurobiology, University of California, San Francisco, CA 94143, USA and Lab. Neurophysiology, Catholic University, P.O.Box 114-D, Santiago, CHILE .

Recent evidence suggests that the synaptic basal lamina (BL) may contain specific sites for the anchorage of the acetylcholinesterase (AChE) and some of its components may serve important regulatory roles in the pre- and postsynaptic differentiation of previously formed neuromuscular structures. However, no direct biochemical demonstration has been provided for the association of the collagen tailed forms of AChE with the BL,nor has formal characterization of the molecules involved in these functions been achieved. We report here experiments on the relationship between the asymmetric AChE forms and BL components, as well as a preliminary characterization of the polypeptide composition of the BL from the electric organ, a rich source of synaptic macromolecules.

BL was purified from Electrophorus electricus, Torpedo californica and Discopyge tschudi. The AChE molecular forms were characterized by sucrose gradient analysis.

Results indicate that the asymmetric AChE form (A_{12}) copurifies with a crude BL fraction obtained by subcellular fractionation followed by discontinuous sucrose gradient (17-60%). Eighty percent of the AChE activity remains in this fraction after treatment with Triton X-100. Thus, these results provide direct biochemical evidence for the association of the A_{12} AChE form with the synaptic BL.

BL from Torpedo and Discopyge are closely related, containing fifteen percent of total AChE activity, the A_{12} AChE form and a similar pattern of peptides as visualized by SDS-polyacrylamide gel electrophoresis. Differences are observed with respect to Electrophorus BL, both in total AChE activity (30%) and in peptide composition. However, in BL from all three at least nine polypeptides are common and the three most prominent ones show similar molecular weights (200 K, 55 K and 47 K daltons) and could represent, together with A_{12} AChE, basic components of the postsynaptic BL. Partial purification of some of these peptides can be obtained by succesive salt treatments with 1M NaCl, 1M $MgCl_2$ and 4M Guanidine-HCl.

Current studies are directed to establish the collagenous and non-collagenous domain of the BL as well as to determine the identity of some of the peptides present in it.

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101.7 GENERATION OF MONOCLONAL ANTIBODIES TO GALACTOCEREBROSIDE. A. Rostami*, P.A. Eccleston*, R.P. Lisak and D.H. Silberberg. Dept. of Neurology, Univ. of Pa. Med. Sch., Philadelphia, PA 19104.

Galactocerebroside (GalC) is a membrane glycolipid of glial cells. Rabbit anti-GalC has been used to identify oligodendroglia and Schwann cells in culture and to produce experimental demyelination in vivo and in vitro. These studies have been limited by the nature of polyclonal antisera which contain a family of antibodies, and by the inconsistency of titers of rabbit antisera. To circumvent these problems, we have produced monoclonal antibodies to GalC. BALB/c mice were repeatedly sensitized with bovine GalC in bovine serum albumin and complete Freund's adjuvant. Spleen cells from the sensitized animals were fused with a non-secreting mouse myeloma line (SP_2) using polyethylene glycol. Supernatants from the successful fusions were screened for the presence of anti-GalC antibody by a solid phase radioimmunoassay (RIA) which we developed, using ^{125}I-rabbit anti-mouse $F(ab')_2$ as second antibody. Four anti-GalC secreting hybrids were detected and one successfully cloned. The supernatants and ascitic fluids produced by injecting clones into BALB/c mice have high titer, consistent, and reproducible anti-GalC activity. Using immunofluorescence, the antibody binds only oligodendrocytes in rat central nervous system (CNS) culture as assessed with immunofluorescence. Double staining with rat anti-glial fibrillary acidic protein and anti-large external transforming substance antisera reveals no binding of monoclonal anti-GalC to astrocytes or fibroblasts. Schwann cells, but not fibroblasts, are stained in short-term cultures of rat Schwann cells. Similar percentages of oligodendrocytes were stained in 1, 3 and 7 day old rat CNS cultures with either monoclonal antibody or rabbit anti-GalC. Monoclonal anti-GalC binds strongly to GalC and monogalactosyldiglyceride, but not to ceramide, ganglioside and glucocerebroside in the RIA. These "immortal clones" are a supply of high titer, monospecific antibodies with constant biological properties. This monoclonal antibody should prove useful for highly specific cell surface identification, sorting, and studies of demyelination and related cytotoxicity.

101.8 ANTI-N6, AN ANTISERUM THAT SPECIFICALLY BINDS TO MANY TYPES OF NEURONS. William Stallcup*, Leslie Arner* and Joel Levine* (SPON: P. Brehm) Neurobiology Lab., Salk Institute, San Deigo, CA 92138

We have prepared antisera against PC12 cells in order to define cell surface antigens specific to neurons. The cellular specificity of the antisera was assessed by immunofluorescent staining of primary cultures of embryonic rat brain, cerebellum, and spinal cord and neonatal dorsal root (DRG) and superior cervical ganglia (SCG). Double label experiments using cell type specific markers [tetanus toxin for neurons (TT), anti-galactocerebroside for oligodendrocytes (GC), anti-glial fibrillary acidic protein for astrocytes (GFAP) and anti-fibronectin for fibroblasts] demonstrated that anti-PC12 specifically stains different types of neurons but does not react with astrocytes, oligodendrocytes or fibroblasts.

We immunized rabbits, mice, and guinea pigs with PC12 cells and absorbed the antisera with 2 glial cell lines, B9 and B92, and with the PC-G2 pheochromocytoma cell line. The resulting antisera, which we call anti-N6, react with both differentiated and undifferentiated PC12 cells as well as with process-bearing cells in primary cultures of embryonic cerebellum and brain. These N6 positive cells could not be labeled with anti-GC or anti-GFAP but were labeled with TT demonstrating that they are neurons. Anti-N6 binds to neurons in primary cultures of neonatal DRGs and SCGs and also stains adrenal chromaffin cells in culture. When anti-N6 was absorbed with adrenal medullary tissue, it no longer stained chromaffin cells but continued to stain central and peripheral neurons. Conversely, anti-N6 absorbed with particulate fractions of brain stained chromaffin cells but no longer stained neurons. Thus, anti-N6 defines at least 2 cell surface specificities; one found on neurons and one found on adrenal chromaffin cells.

To determine whether anti-N6 recognizes any proteins or glycoproteins on the cell surface, we prepared detergent extracts of 125-I labeled PC12 cells and subjected the extracts to immune precipitation. Autoradiographic analysis of SDS gels of the immune precipitates indicated that anti-N6 recognizes polypeptides with molecular weights of 230,000, 180,000, 170,000 and 140,000 daltons. The 230K component is likely to be a glycoprotein since it can be biosynthetically labeled with 14-C galactose. Anti-N6 precipitated polypeptides of similar molecular weights from extracts of labeled cerebellar, spinal and DRG neurons. Absorption with brain but not adrenal medulla removed the ability of anti-N6 to precipitate the 230K polypeptide. These results demonstrate that anti-N6 is a general surface marker for several different types of neurons. It is likely that a 230K glycoprotein is present on the surface of these neurons.

101.9 IMMUNOLOGICAL ANALYSIS OF HUMAN NEURONS. Lois A. Lampson* (SPON: E. Stellar). Dept. of Anatomy, Univ. of Penn. Sch. of Med., Philadelphia, PA 19104.

A panel of monoclonal antibodies that react with human neuroblastoma-derived cell lines has been raised. The majority also react with normal human brain, as well as normal nervous tissue of mice, rats, and cats. The majority retain their antibody activity after the target tissue has been fixed with 4% aldehydes. These properties have been exploited in two ways. (1) The antibodies have been used to visualize their antigens in well-fixed cat tissue at the light and EM level, using the unlabeled antibody peroxidase-antiperoxidase method of Sternberger. For example, antibodies that react with human neural tissue have been shown to label different parts of neurons, or selective populations of cells by using cat retina as the target tissue. (2) The antibodies have been used to characterize their antigens biochemically, using cell lines as a source of homogeneous material. This includes comparisons of the amount of antigen carried by different cell types, as well as gel analysis of the molecular weight and chain structures of the molecules. For example, the expression of the major histocompatibility antigens on cells of neuronal, glial and lymphoid origin have been compared.

Major obstacles to the systematic study of human neurons have been the difficulty of obtaining optimally preserved tissue, coupled to the more general problems of the heterogeneity of normal nervous tissue and the lack of mono-specific reagents. The abundance of monoclonal antibodies that react with well-fixed tissue of small animals as well as with human tissue and cell lines makes it possible to circumvent these problems, allowing both anatomical and biochemical analysis of normal human neuronal antigens.

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101.10 EXPRESSION OF THE THY 1 ANTIGEN IN LONG-TERM CULTURES OF EMBRYONIC MOUSE SPINAL CORD R. H. Brown, Jr.,* J. S. Schweitzer,* and M. A. Dichter* (SPON: W. F. White). Mental Retardation Center, Children's Hospital, Boston, Ma. 02115

The theta or Thy 1 antigen is a cell surface differentiation molecule present on certain cell types including thymocytes, T-lymphocytes and neurons. It has been suggested that this molecule may facilitate cell-cell interactions. Several authors have reported its presence on neurons in short-term cultures and it has been detected transiently on cultured myoblasts prior to fusion. We have studied the time course of appearance of the theta antigen in long-term cultures of thirteen day fetal mouse spinal cord. We used indirect immunofluorescence with a monoclonal IgM antibody to Thy 1.2 and two cell markers, tetanus toxin and rabbit anti-glial fibrillary acidic protein (GFAP) which label neurons and astrocytes respectively. We observed:

(1) Neuronal Thy 1.2 antigen appears at seven days and is maximally evident (30% of neurons positive) at three weeks. This extent of neuronal positivity does not differ significantly from that seen after up to eight months. The positive neurons include several morphologies but most typical are large, multipolar cells, round cells resembling neurons in dorsal root ganglia and small, bipolar cells.

(2) Astrocytes, as defined by double labelling with anti-GFAP and anti-Thy 1.2, first express the Thy 1.2 antigen at four or five days. Maximum positivity (30 - 50% of astrocytes) occurs at three weeks and persists up to eight months or more.

(3) Within the first five days two cell types were Thy 1.2 positive: (a) flat, polygonal cells, often clustered and closely resembling those which eventually become GFAP positive, and (b) a smaller population of elongate, sometimes fusiform cells typically appearing at the periphery of the polygonal cell clusters.

(4) In general, neuronal growth is most dense around islands or clusters of astrocytes. In these regions the Thy 1.2 antigen is most abundant.

101.11 IMMUNOCHEMICAL STUDIES OF THE NERVE GROWTH FACTOR-INDUCIBLE LARGE EXTERNAL GLYCOPROTEIN (NILE). S.R.J. Salton*, L.A. Greene*, C. Richter-Landsberg* and M.L. Shelanski*(SPON: R. Margolis). NYU Sch. of Med., New York, NY 10016.

In the current studies, NGF-treated PC12 rat pheochromocytoma cells grown in monolayers were extracted with sodium deoxycholate and the extract enriched for glycoproteins by chromatography on wheat germ agglutinin columns. The resultant glycoproteins were further purified by SDS-polyacrylamide gel electrophoresis. The NILE (M=230kD) band was excised and used to raise an anti-serum. This antiserum (anti-230) selectively precipitated the NILE glycoprotein from a 2.5% Triton extract of PC12 cells which had been metabolically labeled for 72 hrs with ^{3}H-fucose. Rocket immunoelectrophoresis of PC12 cells grown for two weeks with NGF reveals a 3-4 fold increase in NILE per mg protein as compared to untreated cells. Triton extracts of cultured N115 neuroblastoma, rat sympathetic neurons and rat cerebellum all showed the presence of a cross-reactive immunoprecipitable glycoprotein of similar or slightly lower molecular weight. A soluble glycoprotein (M=210Kd) released into the medium by PC12 cells was also selectively precipitated by this serum. By indirect immunofluorescence, anti-NILE was localized on the cell surface of NGF-treated and -untreated PC12 cells in a widespread, somewhat granular pattern. N115 neuroblastoma, cultured rat sympathetic neurons and cultured rat cerebellum cells also stained positively for NILE. In cerebellum, the cells stained by anti-NILE were distinct from those labeled with anti-GFA in double-label studies. Cultured rat fibroblasts, gerbil fibroma cells and C_6 glioma cells lack surface NILE. Stretch preparations of rat iris show staining in a similar distribution as that found for formaldehyde-induced catecholamine fluorescence. Trypsinized PC12 cells show NILE staining. This is consistent with the precipitation of an immunoreactive 100,000 dalton carbohydrate-containing fragment from Triton-extracts of trypsinized cells. These data suggest that NILE is a widely distributed component of neuronal surfaces which is, as yet, functionally undefined. (Supported by grants NS15076, NS16036 from the NIH, the March of Dimes and the McKnight Foundation. S.R.J.S. is a MSTP Fellow.)

101.12 CHARACTERISTICS OF AN A_{12} FORM OF ACETYLCHOLINESTERASE IN C_2, A MOUSE MUSCLE CELL LINE. Nibaldo C. Inestrosa* and Zach W. Hall (SPON. Pamela D.Gorin).Division of Neurobiology, Univ. of Calif., San Francisco, CA 94143

In vertebrate muscles, a particular form (A_{12}) of acetylcholinesterase (AChE) is concentrated at the neuromuscular junction. The characteristic feature of this form is a long, collagen-like tail. Although circumstantial evidence suggests that A_{12} is associated with the basal lamina, there has been no direct demonstration of its cellular localization. We have found that C_2, a cell line derived from adult mouse skeletal muscle, accumulates the A_{12} AChE form in culture after myoblast fusion. We report here experiments on the localization of the enzyme and on its regulation.

The A_{12} AChE form is at the cell surface as evidenced by protection from DFP inactivation by the impermeable inhibitor BW-284c51, and solubilization by collagenase digestion. The A_{12} enzyme is extracted by high salt, but not by Triton X-100; thus, it is probably associated with the extracellular matrix. Histochemical localization of AChE reveals a patchy distribution of the surface enzyme. Collagenase digestion, which removes only the A_{12} form, abolishes this staining pattern, indicating that the patches consist of the A_{12} enzyme.

Muscle activity has been reported to be essential for the expression of AChE at developing nerve-muscle contacts and for the synthesis of the A_{12} form in rat primary muscle cultures. We examined the effect of muscle activity on A_{12} synthesis by C_2 cells. Spontaneous contractions were suppressed by blocking action potentials with 1uM TTX. This treatment resulted in a 40% decrease in total AChE activity, but all forms were equally affected. Thus the A_{12} AChE on C_2 myotubes is not selectively regulated by muscle activity, and does not require muscle activity for its expression.

We conclude that C_2 cells produce and secrete, or assemble in the extracellular matrix, an A_{12} AChE that resembles that found at adult endplates, although its mode of regulation may be different. We have reported elsewhere(Miller & Hall, these Abstracts) that these cells produce clusters of AChRs and also three antigens that are found in the basal lamina of synapses in adult muscle fibers (Inestrosa et al. (1981) J. Supra. Mol. Struct. 5:297). Thus, C_2 cells, in the absence of nerves produces several specialized postsynaptic components of the neuromuscular junction.

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101.13 REGULATION OF ACETYLCHOLINESTERASE (ACHE) FORMS IN QUAIL MUSCLE CULTURES. J.E. Bulger*(1), G.T. Patterson, W.R. Randall* and B.W. Wilson. Departments of Avian Sciences and Biochemistry and Biophysics(1), University of California, Davis, CA 95616.

Cultured Japanese quail muscle cells are one of the few systems reported to possess a high molecular weight form of ACHE (Emmerling et al. Soc. Neurosci. Abs. 5,479, 1970). This study confirms the presence of a 20S form of ACHE in cultured quail muscle, suggests that it is at the surface of the cells and shows its level can be regulated by factors in the sera used to grow the cells. Nine day old quail embryo pectoral muscle was dissociated into single cells and grown on collagen coated dishes in a medium of 10% horse serum, 2% quail embryo extract and 88% MEM. ACHE activity was determined by a radiometric method after extraction of the cells with a buffer containing 1M NaCl, 0.5% Triton X 100 and 0.2mM EDTA. When cultures grown 11-12 days in vitro were incubated for 24 hours in a medium lacking serum, extractable cell ACHE fell 21%, ACHE released to the medium fell 50% and incorporation of leucine into protein fell 43% compared to cells maintained in complete medium. Sucrose gradient centrifugation of control cell extracts showed three major peaks of ACHE activity with S values of approximately 7S, 12S and 20S. The amounts but not the sedimentation coefficients of the ACHE forms changed when the cells were incubated in serumless medium. Although the total amount of ACHE activity decreased, that portion attributable to the 20S ACHE form increased from an average value of 1.0×10^{-2} to 1.3×10^{-2} IU/dish. Activities of the smaller ($7S_2$ and 12S) ACHE forms decreased from 5.3×10^{-2} to 3.6×10^{-2} IU/dish in the same 3 experiments. 7S and 12S, but not 20S ACHE was released into the serumless media. Measuring the hydrolysis of acetylcholine by intact cells (Rotundo and Fambrough, Cell, 22, 583, 1980) provided evidence that the increase of 20S ACHE in cells kept in serumless media was due to increased accumulation of enzyme on the cell surface. Surface ACHE activity was 1.15±0.05 (SD) higher in cells incubated in serumless than in control medium. The results confirm the presence of a 20S ACHE form in quail mucle cultures and raise the possibility that factors in the sera are involved in the regulation of the synthesis and assembly of ACHE on the muscle cell surface during development. (Supported by NIH grant ES 00202 and the MDA).

101.14 COMPARISON OF PNS MYELIN SPECIFIC PROTEINS AND CELL SURFACE POLYPEPTIDES OF CULTURED SCHWANN CELLS. Jun E. Yoshino* and George H. DeVries (SPON: R. Krieg), Department of Biochemistry, Medical College of Virginia, Richmond, Virginia 23298

Our laboratory previously reported the ability of axolemma-enriched fractions isolated from both CNS and PNS tissue to stimulate the proliferation of quiescent cultures of Schwann cells (Minier and DeVries, Soc. Neurosci. Abstr. 7, 550, 1980; Yoshino and DeVries, Trans. Am. Soc. Neurochem. 12, 200, 1981). In order to study the molecular changes which the surface of the Schwann cells undergoes during proliferation, the polypeptide composition of quiescent Schwann cell populations was determined by radiolabeling with ^{125}I. Primary cultures of Schwann cells were prepared from dissociated sciatic nerves of newborn rat pups using a modification of the procedures of Brockes et al. (Br. Res. 165, 105, 1979). The polypeptides on the surface of the Schwann cells were labeled by both lactoperoxidase and Iodogen catalyzed iodination. Analysis of the iodinated proteins by autoradiography after SDS-polyacrylamide gel electrophoresis (PAGE) revealed no difference in the polypeptides which were labeled by the two techniques. Eight polypeptides of molecular weights 160K, 82K, 70K, 45K, 40K, 32K, 28K and 15K were identified on the surface of the Schwann cells. However, after a week in culture the 160K protein was no longer accessible to labeling with ^{125}I, and the ^{125}I labeling of the 82K polypeptide band was also diminished. The two most rapidly migrating polypeptides possessed molecular weights similar to the peripheral myelin proteins, P_o and P_r. In order to determine whether the 28K and 15K polypeptides were related to either P_o or P_r, the iodinated peptide maps generated by trypsin digestion were examined using the procedures of Elder et al. (JBC 252, 6510, 1977). The location of the two labeled Schwann cell polypeptides was determined by radioautography, and the proteins were excised from the gel and re-iodinated. P_r, obtained from peripheral myelin after SDS-PAGE, and purified P_o were also iodinated as standards. Examination of the two dimentional peptide maps generated by P_o and the 28K polypeptide as well as P_r and the 15K protein did not reveal any significant homology between the myelin specific proteins and the polypeptides present on Schwann cell membranes. The inability to detect myelin proteins on the surface of quiescent cultures of Schwann cells by labeling with ^{125}I is in agreement with the results obtained by Brockes et al., who reported a lack of immunoreactive antigens on cultured Schwann cells using specific antibodies directed against P_o and P_r (J. Neurocyto. 9, 67, 1980). (Supported by NIH grant NS 15408-02)

101.15 FIBRONECTIN AND SURFACE SPECIALIZATIONS ON CULTURED CHICK MYOTUBES. Thomas G. Burrage and Thomas L. Lentz. Section of Cell Biology, Yale University School of Medicine, New Haven, CT 06510.

Surface specializations or patches consisting of a thickened plasmalemma, subsarcolemmal density, and amorphous surface coat occur on intact and cultured myotubes and contain a high density of acetylcholine receptors (Burrage and Lentz, 1981, Dev. Biol., in press). To characterize these patches further, we have studied the distribution of fibronectin, a cell surface glycoprotein involved in cell adhesion. An antibody to fibronectin was incubated with chick myotubes cultured for 6 days and localized at the electron microscope level with a ferritin-labeled antibody. Fibronectin was localized in a discontinuous fashion on the myotube surface. It occurred in the heaviest concentration in association with the well developed extraneous matrix where the cells were in close proximity to the substrate. Ferritin label also occurred in high density in the extraneous material of the specialized patches located elsewhere on the surface. It was not restricted to patches and occurred less densely in small, discontinuous clusters along the plasmalemma of the entire cell surface. Double labeling of the myotubes with a horseradish peroxidase-α-bungarotoxin conjugate (HRP-α-BTX) and fibronectin antibody revealed a co-localization of regions of high density acetylcholine receptors and fibronectin. Patches binding the HRP-α-BTX conjugate also bound fibronectin antibody, but the latter had a wider distribution and occurred in patches and on unspecialized cell surface which did not bind the conjugate. The presence of the greatest amounts of fibronectin in specialized patches of the myotube surface indicates these regions may be preferential sites of adhesion, either to the substrate or an advancing nerve. However, the ubiquity of the fibronectin on the surface indicates that other factors are involved in the localization of a high density of receptors and in specifying the site of innervation. (Supported by NIH fellowship F32-NS06144 and NSF grant BNS 80-18520).

101.16 SURFACE REPLICA TOPOGRAPHY OF RETINAL PIGMENT EPITHELIUM. Barbara J. McLaughlin and Lou G. Boykins*. Department of Anatomy. University of Tennessee Center for the Health Sciences, Memphis, TN 38163

Retinal pigment epithelial cells (PE) are phagocytic cells which continually phagocytize specific particles, namely photoreceptor outer segments (OS). The initial phagocytic step is thought to be mediated by complementary recognition molecules on the PE microvillous and OS membrane surfaces, which may also display a characteristic surface morphology. In order to explore this possibility we have studied surface replicas of the PE apical membrane surface and microvillous membranes from retinas of Long-Evans rats. Retinas are dissected in RPMI 1640 medium with Hepes buffer and the PE cell layer is detached from the neural retina and glued to a cover slip (microvillous border up) and fixed for 1 hr in 2% glutaraldehyde and 0.5% osmium in 0.1M sodium cacodylate buffer, dehydrated in graded methanol and critically point dried. The tissue is replicated in a Balzer's freeze-fracture apparatus using either rotary or unidirectional platinum coating and then digested and the surface replicas examined in the electron microscope. Corresponding blocks of the PE cell layer are also processed for EM thin sectioning and stained in heated uranyl acetate to enhance membrane surfaces for comparison with surface replica topography. The replicated PE topography is characterized by circular bumps (35 nM in diameter) irregularly dispersed on the apical membrane surface. The PE microvilli exhibit more numerous and densely packed bumps along their entire membrane surfaces, giving a "cobblestone" appearance to the surface topography. When viewed on their surface these bumps measure approximately 50 nM in diameter and when viewed from a side angle the bumps measure approximately 20 nM. Thin sections of the replicated PE cell layer show periodic tufts of electron-dense material along the microvillous membranes which may correspond to the microvillous cobblestones observed in surface replicas. The tufts measure between 30-35 nM wide and protrude 20 nM from the membrane surface. Previous studies in our lab using lectin cytochemistry have demonstrated periodic labeling of these tufts along retinal PE microvilli (Wood and McLaughlin, Invest. Ophthal. Vis. Sci. 19:728-742, 1980) which suggests that they are glycoconjugates. Future studies will be aimed at defining the nature and function of these PE surface features and their relation to phagocytic recognition in the retina. Supported by USPHS EY 02853.

101.17

WITHDRAWN

101.18 EXFOLIATION AND REPLACEMENT OF PLASMA MEMBRANE ECTO-5'-NUCLEOTIDASE IN CULTURED BRAIN CELLS. Norman Salem, Jr.* and Eberhard G. Trams* (SPON: M. Goldstein). NINCDS, NIH, Bethesda, MD 20205

C6 glioblastoma cells express about half of their 5-nucleotidase activity as an ecto-enzyme. Membrane derived microvesicles or "exosomes" are released from cells in culture containing ecto-enzymes. The membrane impermeant trinitrobenzenesulfonic acid (TNBS) irreversibly reacts with and inactivates ecto-5'-nucleotidase and was used to study 5'-nucleotidase metabolism and its regulation. C6 (rat brain) glioblastoma monolayer cultures were washed and treated with 0.5 mM TNBS for 15 min at 37°C and pH 8.1. Reagent was removed, cells washed, and the usual growth media was added for 0-72 hrs before enzyme assay. Monolayer ecto-5'-nucleotidase activity was assayed by measurement of ^{32}P released from ^{32}P-AMP. We found that 70-80% of ecto-5'-nucleotidase activity was lost after this treatment but that the cells were viable as measured by trypan blue staining, containment of intracellular LDH, incorporation of ^{3}H-thymidine into DNA, and cell growth. This inactivation was selective because ecto-ATPase was only slightly inactivated and acetylcholinesterase remained unaffected. After 24 hrs of incubation with growth medium C6 cell ecto-5'-nucleotidase activity had nearly recovered (88±4% of control). Fetal calf serum was not necessary for recovery as cells deprived of serum for three days also recovered activity. The rate of increase in ecto-5'-nucleotidase activity of TNBS treated cells exceeded that of control cells but there was no general increase in membrane biosynthesis as evidenced by normal specific binding of ^{125}I-cholera toxin to GM_1. There was no recovery of surface activity in the presence of the protein synthesis inhibitor cycloheximide (0.2 ug/ml) but recovery began when cycloheximide was removed. TNBS treatment causes a concomittant loss in exosomal 5'-nucleotidase during the active recovery period but exfoliated enzyme activity approaches normal levels as the membrane enzyme recovers. Exosome production is normal during the recovery period as measured by GM_1 and protein content. Our data indicate that ecto-5'-nucleotidase replacement occurs via de novo synthesis rather than by insertion of preformed enzyme. Decrease in exfoliated 5'-nucleotidase activity indicates either release of inactivated plasma membrane enzyme or a selective conservation of the enzyme in the exfoliative process.

101.19 "SEROTONECTIN": EVIDENCE FOR THE LOCALIZATION OF THIS SEROTONIN-BINDING GLYCOPROTEIN TO PLATELET PLASMA MEMBRANE AND ITS INVOLVEMENT IN SEROTONIN UPTAKE. W.J. Kupsky*, M.D. Gershon, Y.L. Huang* and H. Tamir, Dept. of Anat., Columbia Univ., Coll. of P&S and N.Y. State Psych. Inst., New York, NY 10032.

Cells that store serotonin have been found to contain serotonin-binding proteins. These proteins differ and can be categorized according to the developmental origin of the serotonin-containing cell; thus, the neuroectodermal serotonin-binding protein, found in central and enteric serotonergic neurons, differs from the serotonin-binding proteins of platelets, mast cells, and enterochromaffin cells, and these, in turn, differ from one another. Platelets, because of their ability to take up serotonin, are often considered models of neurons. One of the two platelet proteins that bind serotonin is a glycoprotein (K_D = 10nM). We have purified this protein by molecular sieve chromatography, affinity chromatography (using a Con-A-sepharose column) and gel electrophoresis. Rabbit antibodies were raised against this protein and were shown to be specific by testing on Ouchterlony plates and by rocket immunoelectrophoresis. We propose to call this protein serotonectin. Serotonectin-like material was localized immunocytochemically in rat platelets using the unlabeled antibody PAP bridge technique. Platelets were examined by light and electron microscopy. Serotonectin immunoreactivity was found to be associated with the platelet plasma membrane. Gentle washing of platelets with salt solution (Krebs) removed up to 76% of the extractable serotonectin present in platelet suspensions. Washing, in contrast, released no detectable lactic dehydrogenase and removed less than 25% of the intracellular serotonin-binding platelet albumin. Antibody to serotonectin (4.5 mg/ml of the gamma globulin fraction of immune sera) inhibited the uptake of ^{3}H-serotonin (0.2 uM) by rat platelets. These data suggest that serotonectin is present on the external surface of platelet plasma membranes; however, it is probably not an integral membrane protein. Serotonectin seems, nevertheless, to be involved in platelet uptake of serotonin. Supported by grants, NSF 09335, NS07062, NS12969.

102.1 CHOLINE UPTAKE IN CULTURED Y79 RETINOBLASTOMA CELLS:EFFECT OF MEMBRANE POLYUNSATURATED FATTY ACID COMPOSITIONAL CHANGES.
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Neural tissue membranes are highly enriched in essential polyunsaturated fatty acids, especially arachidonic acid (20:4 n-6) and docosahexaenoic acid (22:6 n-3). However, the functional significance of these polyunsaturates is unknown. In an attempt to elucidate the functional significance of the high polyunsaturated fatty acid content on membrane-related processes in neural tissues, we have examined the effect of changes in polyunsaturated fatty acid content on the transport of choline in cultured human Y79 retinoblastoma cells. When grown in fetal calf serum, the phospholipid composition of these cells of neural origin is low in polyunsaturates. By contrast, when grown in a medium supplemented with polyunsaturated fatty acids, the phospholipid composition of the cells can be enriched with varying amounts of the unsaturates of the n-9, n-6, and n-3 families. In this way, the average number of double bonds of the fatty acids present in a membrane-enriched microsomal preparation can be increased by over 50%. Choline uptake in these cells occurs by at least two kinetically distinguishable processes, classified as high affinity (K_m' = 2.2±0.13 μM, V_{max}' = 27.0±2.9 pmol $min^{-1}mg^{-1}$) and low affinity (K_m' = 20.4±1.2 μM, V_{max}' = 402±49.1 pmol $min^{-1}mg^{-1}$) (mean ± SE; n = 4). The kinetic parameters for high affinity choline uptake were altered when the cells were enriched with essential polyunsaturates of the n-6 or n-3 classes, but they were not different from control values when cells were enriched with the nonessential n-9 class of unsaturated fatty acids. The K_m' of the high affinity system decreased to 0.89±0.05 μM when the cells were grown in a medium supplemented with an n-3 precursor, linolenic acid (18:3 n-3), to 1.01±0.19 μM with docosahexaenoic acid, and to 1.25±0.08 μM with arachidonic acid. The V_{max}' also decreased in cells which had been enriched in essential polyunsaturates, but the decrease was significant only in the case of arachidonic acid supplementation (15.8±1.0 pmol min^{-1} mg^{-1}). Supplementation with oleic acid (18:1 n-9) produced no change in either the average number of double bonds per fatty acid or in these kinetic parameters. These findings suggest that one effect of the large amount of essential polyunsaturates in neural tissue membranes if the facilitation of choline uptake at low concentrations. Further this facilitation is a property of both the n-3 and n-6 classes of essential polyunsaturated fatty acids. (Supported by research grants HL14,781 and GM07331).

102.2 MEMBRANE SUBFRACTIONS FROM PURIFIED OLIGODENDROGLIA IN CULTURE.
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Oligodendroglia, isolated in bulk from subcortical white matter from adult bovine brain, can be maintained as suspension cultures. While in culture they produce whorls of membrane lamellae adjacent to the cell soma. The cells remain in good health during this event, are metabolically very active, and synthesize a variety of lipids and proteins, especially lipids found in myelin. When subcellular fractions are prepared from the cells in culture, three membrane subfractions are obtained. These are a glial light fraction (at 0.32 M sucrose) consisting of large vesicles; an intermediate fraction (at 0.5-0.6 M sucrose) that contains whorls of membrane lamellae; and a plasma membrane fraction (at 1.0 M sucrose) consisting primarily of small vesicles. The intermediate subfraction is enriched in 2',3'-cyclic nucleotide 3'-phosphodiesterase while the plasma membrane subfraction is enriched in Na^+, K^+-ATPase and 5'-nucleotidase.

Gradient slab gel electrophoresis was used for analysis of the proteins in the plasma membranes and the intermediate subfractions. Coomassie stained gel patterns of the plasma membranes show a minimum of 40-50 proteins with average approximate molecular weights ranging from >200,000 to 12,500. Some 15 proteins are more prominently stained in this subfraction. The membrane lamellae subfraction has fewer prominently stained bands. Autoradiography was used to detect binding of radioiodinated lectins to glycoproteins after electrophoretic separation on the gradient slab gels. Multiple glycoproteins were observed. Thus both membrane subfractions are complex structures composed of a heterogeneous mixture of proteins and glycoproteins. In general the membrane lamellae produced in culture tend to have a somewhat less complicated pattern with fewer proteins and glycoproteins than the plasma membranes. (Supported by funds from the Kroc Foundation, the Multiple Sclerosis Society and NS-14577)

102.3 DOES TRANSMETHYLATION INCREASE FLUIDITY OF ONE LEAFLET WHILE INCREASING THE VISCOSITY OF THE OTHER? Rodman G. Miller
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Hirata and Axelrod (see Nature 275:219,1978 and 288:278,1980) have correlated adrenergic receptor binding, phosphatidylethanolamine methylation and translocation (transmethylation, TM)across the membrane with a decrease in RBC ghost membrane viscosity (measured with diphenyl hexatrine, DPH). They propose changes in membrane viscosity are responsible for subsequent effects of receptor activation. Vance and Kruijff (Nature 228:277,1980) have questioned whether the change in viscosity (1.6 to 1.1 poise) could be brought about by the observed degree of TM (less than .01% of membrane lipids). This report indicates how even a small ammount of TM could produce a large effect on the apparent membrane viscosity as measured with DPH.

At the center of the controversy is the use of DPH to measure viscosity. DPH is shorter than palmitic acid and thus a DPH molecule will reflect the microviscosity within a single leaflet. DPH should partition into the leaflet with the lowest surface tension. In general, this leaflet will have the greater fluidity.

TM will alter the surface tension of the two leaflets. The effect of translocation of less than .01% of the total surface area from one leaflet to the other is difficult to assess precisely. However, the proportion of the membrane which is reactive to changes in surface tension (i.e.can stretch to fill in) and accessible to DPH can be estimated at much less than 28%: 50% of the RBC ghost surface area is protein and 22% is occupied by nonsterol lipids bound to protein (figures computed from freeze fracture morphometry assuming that all proteins observed as particles traverse the membrane). Thus 72% of the surface should not react to changes in surface tension and should exclude DPH. In the remaining 28% of the membrane, slightly over half of the lipid is cholesterol (.35nm^2) which binds to phospholipids (.63nm^2) to form a complex (.82nm^2). This large condensing effect of cholesterol suggests that the complex should be rather rigid, not very reactive to surface tension changes and, again,will exclude DPH.

If the RBC membrane is composed of units which are as rigid as this would suggest, small ammounts of TM could have a profound effect on microviscosity as measured by DPH. One leaflet will become more viscous while the other more fluid. DPH, however, allows us to observe only the latter leaflet. This analysis suggests that DPH may a general probe for detection of alterations in membrane lipid symmetry.

102.4 SUBSTRUCTURE IN ASTROCYTIC ASSEMBLIES DEMONSTRATED BY RAPID FREEZING AND LOW-TEMPERATURE FREEZE FRACTURING.
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Freeze fracture techniques reveal that mammalian astrocytic plasmalemma contains rectangular or square aggregates of uniform, small particles packed in orthogonal array. We have examined the structure of these aggregates, termed "assemblies," in cerebellar tissue rapidly frozen by contact against a copper block cooled by liquid helium. Frozen tissue was further cooled to 10-85°K on the stage of a modified Balzers freeze-fracture apparatus helium-cryopumped to a vacuum of approximately 10^{-8} torr. The tissue was then fractured once with a cold sapphire knife, and then replicated with Pt/Ir/Ta alloy to produce a high resolution replica (grain size less than 10 angstroms). In such rapidly frozen, cryo-fractured tissue, the particles of assemblies appear to fracture at two levels within the membrane. Plane I fractures reveal bullet-shaped particles associated with the cytoplasmic membrane half, packed in orthogonal order, similar to the particles seen in fixed or conventionally fractured material. Plane II fractures appear to break into the particle structure close to the cytoplasmic membrane half, such that the aggregate constituting the assembly appears as a flat mesa elevated only slightly above the cytoplasmic membrane half fracture face. With low angles of shadowing, the mesa exhibits a regular pattern of pits. Both planes of fracture may be evident in a single assembly, revealing hemispherical particles distributed over the mesa-like surface, aligned between the rows of pits in the mesa. It seems probable that the mesa actually consists of closely-packed disc-like particles, and that hemispherical particles cap such discs to form the bullet-shaped particle of the fracture plane I image. Prolonged etching does not alter the appearance of the particle arrays, but does reveal a subtle pattern of particles on the true outer surface of the membrane in register with the plane I particles. We are still uncertain whether the particles on the true outer surface of the membrane are the tops of the particles seen with fracture plane I, or whether the plane I fracture breaks the particles somewhat deeper in the membrane. We have not recognized filaments or other structures inserting into the true outer surface of the membrane at sites of assemblies. Thus the (presumed) protein represented by the particles of the assemblies extends from the outer surface of the membrane into the hydrophobic interior. It is possible that the two planes of fracture within the particles reflect substructure in the protein.

102.5 GOLGI APPARATUS AND COATED VESICLES IN MEMBRANE CIRCULATION IN RANA PIPIENS RETINAS: STUDIES WITH CHOLERA TOXIN AND MONENSIN. Mary Lou Matheke* and Eric Holtzman. Dept. of Biological Sciences, Columbia University, New York 10027.

Several aspects of membrane genesis and cycling have been studied in frog photoreceptors, a favorable system for the study of such processes since they are compact and show a highly polarized distribution of specialized membranes.

Retinas incubated in cholera toxin-HRP (gift of N. Gonatas) show accumulation of the toxin in Golgi associated sacs and tubules and in small vesicles in the cell body, axon and terminal. This suggests that Golgi associated membrane systems of the photoreceptor participate in the degradation and/or reutilization of membrane retrieved from the cell surface by endocytic processes. As in Gonatas' work on conventional neurons, some of the toxin-labeled structures are of the sort that show acid phosphatase activity.

Monensin, a sodium ionophore,blocks the exit of newly synthesized proteins from the Golgi region of some secretory cells. We have used this agent to manipulate the migration of proteins in photoreceptors without abolishing protein synthesis. In monensin-treated isolated retinas, the Golgi region of the photoreceptor myoid shows numerous large vacuoles. Smaller numbers of similar vacuoles are present in the terminals and at the tip of the ellipsoid. The agranular reticulum normally present in the rod axon is diminished or absent. Instead, the axon contains many vesicles similar in size to those seen at synapses. In autoradiographic studies with ^{3}H-leucine, retinas incubated in monensin show inhibition of the formation of bands at the base of the rod outer segment (ROS) and fewer than normal numbers of grains over the remainder of the outer segment. Most of the grains are located in the myoid region, especially in the supranuclear zone. These findings are consistent with the central role postulated for the Golgi apparatus in the biogenesis of outer segment membranes and suggest that monensin blocks the migration of this membrane.

Coated vesicles are seen attached to the plasma membrane at the apical region of the rod inner segment. In HRP preparations, endocytic vesicles are found in this region even after incubations of only a few minutes and under conditions that depress encytosis at the terminal (light; presence of cobalt). These vesicles might participate in membrane recycling phenomena linked to the genesis of outer segment membranes and/or in specific endocytic uptake processes occurring in the apical portion of the outer segment.

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102.6 CHANGES IN MEMBRANE SPECIALIZATIONS AT THE NODE OF RANVIER DURING TELLURIUM-INDUCED DEMYELINATION AND REMYELINATION. Clayton A. Wiley-Livingston and Mark H. Ellisman, Department of Neurosciences, University of California, San Diego, School of Medicine, La Jolla, CA 92093.

We have studied the effects of tellurium-induced demyelination and subsequent remyelination on the thin section and freeze-fracture morphology of rat peripheral nerves. Early demyelinative changes include: increased concentrations of neurofilaments and mitochondria in axons; fragmentation of the annulus of particles composing the E-face nodal specialization; and an increase in Schwann cell cytoplasm. Demyelination was characterized by vesicular and lamellar disruption of entire sheath segments, maintenance of basement membranes but little inflammatory response. Junctional specializations at the paranode disappeared only after destruction of the sheath had led to retraction of glial loops. Large denuded axons displayed fracture faces with a uniform distribution of particles except for infrequent small patches of particles in the axon E-face. The demyelinated axons underwent a rapid remyelination after removal of the 1% tellurium diet proceeding as described in normal developing animals. Early myelin wrappings were found adjacent to patches of particles in the axonal E-face at sites destined to become nodes of Ranvier. As new wrappings were laid down, terminal loops of myelin, along with associated rows of dimeric particles in the axonal P-face, were wound into their paranodal location. The size of the axonal E-face particle patch increased along with the number of paranodal windings until an annulus of particles was obtained. The thin section and freeeze-fracture morphology of remyelinated fibers was indistinguishable from the morphology of control fibers. This sequence of events and correlated physiology may help elucidate functions of membrane specializations in myelination and nerve conduction.

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102.7 NORMAL FUNCTION IN SARCOPLASMIC RETICULUM FROM MICE WITH MUSCULAR DYSTROPHY. R. E. Mrak* and S. Fleischer* (SPON: F.R. Freemon). Depts. of Molecular Biology and Pathology, Vanderbilt Univ.; and Research and Laboratory Services, Veteran's Administration Medical Center; Nashville, TN 37235.

A new technique, which is rapid and gentle, has been developed for the isolation of sarcoplasmic reticulum (SR) from small amounts of muscle tissue. This involves gentle homogenization (two minutes at 1140 RPM) in a Waring Mini-Sample Container, two differential centrifugations followed by KCl extraction and a three-step discontinuous sucrose density gradient, with recovery of SR at the 29/41% sucrose (w/w) interface.

Normal and dystrophic SR were indistinguishable by electron microscopy (EM). Thin sections of full-thickness pellets of normal and dystrophic SR showed typical small vesicles with membrane asymmetry as demonstrated by tannic acid fixation. Occasional tubular and triad forms are present and more rarely mitochondria. Freeze-fracture EM showed typical asymmetric particle distribution. Assays for surface membrane markers (acetylcholinesterase, 5'-nucleotidase, adenylate cyclase) and mitochondrial markers (succinate-cytochrome c reductase) demonstrated only low levels of contamination by these organelles.

Isolated SR from normal mice had the following characteristics: Calcium loading rate (oxalate-assisted) 2.5 µmoles/mg-min and capacity 5.6 µmoles/mg, calcium-stimulated ATPase 1.9 µmoles/mg-min, calcium pump content 65% (as measured by densitometry of Coomassie-blue-stained sodium dodecylsulfate polyacrylamide electrophoretic gels). Corresponding numbers for dystrophic SR were 2.1, 4.7, 1.5, and 56. Thus the dystrophic/normal ratios were 0.84, 0.84, 0.79, and 0.86.

The kinetics of calcium removal and the final limiting free calcium concentration achieved were the same in experiments in which the total SR capacity exceeded the total free calcium available.

Calcium-insensitive ("basal") ATPase was 3.5-fold higher in dystrophic as compared with normal SR (0.7 vs 0.2 µmoles/mg-min). This activity correlated with the surface membrane markers 5'-nucleotidase and adenylate cyclase both in the two SR fractions and in a light membrane fraction (isopycnic density 10-29% sucrose [w/w]) enriched in surface membranes.

We conclude that the small differences in calcium pumping ability and calcium-stimulated ATPase are due to small differences in purity of the two isolated fractions, and that the calcium-insensitive ("basal") ATPase activity is attributable to contaminating surface membranes. [This work was supported by MDA (S.F) and during the tenures of an MDA Fellowship (1978-80) and a V.A. Career Development Award (1980-81) to R.E.M.]

102.8 REVERSIBLE LOSS OF HIGH-AFFINITY, SODIUM-DEPENDENT γ-AMINOBUTYRIC ACID UPTAKE BY SYNAPTIC PLASMA MEMBRANES DEPLETED OF CHOLESTEROL. Paula North* and Sidney Fleischer* (SPON: L. Aulsebrook). Dept. of Molecular Biology, Vanderbilt University, Nashville, TN 37235.

The cholesterol/phospholipid (Ch/PL) ratio of synaptic plasma membranes from rat forebrain was varied using a general lipid exchange protein [Biochemistry 19:1433 (1980)] purified from beef liver. Ch/PL ratios from 0.18 to 1.12 were produced, from a normal value of 0.51, by incubation of the membranes with exchange protein and an excess of either egg phosphatidylcholine (PC) or cholesterol/egg PC liposomes under mild conditions (60 min at 32°C). The degree of cholesterol loading and depletion, which was minimal without exchange protein, was controlled by varying the amount of exchange activity present. Liposome sticking, which represented 5-15% of total phospholipid, was corrected for using a non-exchangable marker. Cholesterol depletion or loading was accompanied by an increase or decrease, respectively, in the phospholipid/protein ratio, and changes in the cholesterol and phospholipid content (per mg protein) were reversible. Cholesterol which had been incorporated into the membranes exchanged back out into egg PC liposomes in the presence of exchange protein at the same rate as the bulk pool of synaptic membrane cholesterol. The normal and cholesterol modified membrane vesicles were morphologically alike by freeze-fracture.

The ability of synaptic plasma membrane vesicles to take up γ-aminobutyric acid (GABA) by a Na^+ gradient-dependent mechanism was studied using the method of Kanner [Biochemistry 17:1207 (1978)] in which the vesicles were soaked in K phosphate and then diluted into NaCl containing 0.15µM 3H-GABA, followed by filtration. Cholesterol loaded vesicles took up GABA at the same rate as controls (29.7 pmoles/min/mg). However uptake was progressively lost with cholesterol depletion, reaching 70-100% loss at Ch/PL ratios near 0.2. Uptake was largely restored by reloading the vesicles with cholesterol. The passive permeability of the cholesterol depleted membranes to various ions and GABA was not altered relative to controls as measured by light scattering and isotope dilution. These observations indicate that the Na^+-dependent GABA uptake mechanism is directly influenced by membrane cholesterol content.

In summary, a general lipid exchange protein from liver can be used under minimally perturbing conditions to reversibly vary the Ch/PL ratio of synaptic plasma membranes over a wide range. This allows a definitive study of the influence of cholesterol content on membrane function. We find that Na^+-dependent GABA uptake by synaptic plasma membrane vesicles is reversibly lost upon depletion of membrane cholesterol.(Supported, NIH AM 14632).

102.9 HUMAN CNS MEMBRANE PROTEINS RESOLVED BY TWO-DIMENSIONAL GEL ELECTROPHORESIS WITH SILVER STAINING. K.S. Kosik*, D.J. Selkoe, J.M. Gilbert and P. Strocchi*. Mailman Research Center, McLean Hospital, Belmont, MA 02178. (SPON: J.M. Gilbert)

Fractions enriched in synaptic membranes, neuronal cell bodies, rough endoplasmic reticulum (RER) or smooth endoplasmic reticulum (SER) were isolated from frontal cortex of brain obtained 2-15 hours post-mortem. Enrichment of organelles in each fraction was verified by electron microscopy. The neuronal cell bodies were further fractionated into a soluble and insoluble fraction by centrifugation at 100,000 g. Protein determinations revealed that greater than 75% of the protein in these neuronal fractions remained insoluble. The proteins in each fraction were analyzed by two-dimensional gel electrophoresis (2DGE) using a first-dimensional pH gradient of 5.1 to 6.4 by a modification of the O'Farrell procedure. Proteins were detected by the method of Oakley (Ann Biochem 105:361-363, 1980) using the ultrasensitive silver stain. Comparison was made to protein staining of the electrophoretograms with Coomassie brilliant blue. With equal loading of protein the number of spots visualized could be increased five-fold. In all fractions examined all proteins which stained with Coomassie also appeared on the silver stained gels. Furthermore, Coomassie staining of a silver stained gel revealed no new spots. However, some spots clearly of high density on the silver stained gels did not appear on gels stained with Coomassie. Since it is accepted that the intensity of Coomassie staining reflects relative protein concentration, densitometric analysis of silver stained gels may not reflect the relative concentrations of component proteins.

The isolated human fractions were analyzed in comparison with each other. It was apparent that each fraction by 2DGE showed a unique protein array, hence permitting identification of a subcellular fraction by its own two-dimensional pattern. Specific differences in the polypeptide composition of the various fractions will be described. Among other changes, the configuration of proteins in the tubulin region showed differences among fractions. These overall patterns were highly reproducible in samples from different human brains, although minor individual differences occurred. The fractions also bear a great similarity to identical fractions isolated and analyzed by 2DGE in the rat (Strocchi et al. Trans Am Soc Neurochem 12:113, 1981). We are pursuing these results by determining the effect of post-mortem interval on the protein arrays as well as assessing individual phenotypic differences and selective protein alterations in human disease states. (Supported by National Huntington's Disease Fellowship Award (KSK) and NIH grants AG 02126 (DJS, JMG) and MH 70713 (JMG).)

102.10 VARIATIONS IN VESICULAR MORPHOLOGY IN THE FROG PERINEURIUM DEMONSTRATED WITH LANTHANUM AND STEREOSCOPIC ELECTRON MICROSCOPY N.L. Shinowara, M.E. Michel and S.I. Rapoport. Laboratory of Neurosciences, GRC, Natl. Inst. Aging, Baltimore, MD 21224.

The perineurium, an extension of the pia arachnoid sheath, enwraps all peripheral nerves to form the blood nerve barrier. EM studies of the frog perineurium have shown that the thin layers of flat cells are characterized by the presence of numerous pits and vesicles at or near the surface membranes. Recent papers have discussed the formation of channels by vesicular fusion as a mechanism of transcellular transport (Bundgaard et al., Proc. Natl. Acad. Sci. USA 76: 6439, 1979; Frokjaer-Jensen, J. Ultrastruc. Res. 73: 9, 1980). In order to better visualize the various vesicular patterns, we used colloidal and ionic forms of lanthanum as tracers. Sciatic nerves of adult Rana pipiens were fixed in situ or removed and placed into buffered fixative (2-4% glutaraldhyde for 30 min or changes of 0.05% OsO_4/1.5% glut. for 15 min) and then left in fresh buffered glutaraldhyde with tracer for 3-5 hr. The tied cut ends of the nerve were first secured above the fixative solution to prevent leakage of tracer into the nerve. Tracer was in all solutions up to dehydration. Plastic embedded sections (50-100nm) were viewed on a JEOL-TEM equipped with a goniometer and tilting stage. Each area of interest was examined at a wide range of tilting angles (-30^{o} to $+30^{o}$) to obtain a 3-dimensional picture of each vesicular profile. Lanthanum was seen intermittently in all layers of the perineurium, down to the inner "barrier" layers, but never within the endoneurium. In areas of lanthanum penetration, tracer was observed in the extracellular space laterally for long distances, between unconnected ends of cellular processes, and within vesicles attached to surface membranes. In general, the vesicles appeared as small round bottomed or oval flasks extending from both cell surfaces. Occasionally a filled vesicle appeared free within the cytoplasm. When specimens were tilted, several variations in vesicle shape were noted. Flasks which had appeared to fuse with the opposite membrane to form a channel were actually unattached. "Free" vesicles were often either attached by necks to the cell surface or formed clusters with other flasks. Although some chains or clusters of vesicles were observed, they were attached to only one surface membrane. These results indicate that under these experimental conditions, a large number of vesicles, connected to the surface membranes, appear at the inner cellular layer of the perineurium. However, vesicle fusion and transcellular channels do not signi cantly contribute to the passage of material between perineurial layers.

102.11 REACTIVATION OF MYOTOXIC PHOSPHOLIPASE A_2-INACTIVATED SARCOPLASMIC RETICULUM. S. Helmke*, J.W. Morrow and B.D. Howard. Dept. of Biol. Chem., UCLA, Los Angeles, CA 90024.

Certain snake venoms contain phospholipase A_2 (PLA) neurotoxins that inhibit acetylcholine release from nerve terminals. Some of these neurotoxic PLAs are also myotoxic in that they cause a degeneration of muscle independent of their effects on neurons. The PLA activity appears to be necessary for their toxicity, but it is not sufficient to account for their toxicity. Indeed, most known PLAs are not neurotoxic or myotoxic in spite of having a specific enzyme activity that is greater than that of the toxic enzymes. There is good evidence that these toxins act by altering ion fluxes across neuronal and muscle membranes, most likely by degrading lipids near an ion channel or pump. The myotoxic PLAs inhibit Ca^{2+} uptake into sarcoplasmic reticulum (SR) vesicles from skeletal muscle, and this effect has been shown to be a relevant correlate of their toxicity. Notexin, which is a myotoxic PLA, and IVa PLA, which is nontoxic but has greater enzyme activity than notexin, were compared with respect to ability to inhibit Ca^{2+} uptake into SR vesicles. The efficacy of notexin, but not that of IVa PLA, was markedly dependent on SR concentration. With dilute SR, notexin was more effective than IVa PLA; however with concentrated SR, notexin was very much less effective than IVa PLA. These results are interpreted to indicate that SR has a site to which notexin, but not IVa PLA, can bind with high affinity. Reconstitution studies have shown that this site is not a lipid unique to SR, but more likely the site is a protein in the SR membrane. Native SR vesicles were completely inactivated by treatment with notexin, then solubilized with detergent and reconstituted by detergent removal, in some cases with soybean phospholipids added. The reconstituted SR vesicles regained the ability to take up Ca^{2+}. Supported by grants from USPHS and Muscular Dystrophy Association.

102.12 AFFINITY PARTITIONING AND CHARACTERIZATION OF POSTSYNAPTIC MEMBRANES FROM MAMMALIAN BRAIN. S.D. Flanagan, B. Yost* and G. Crawford*. Division of Neurosciences, City of Hope Res. Institute, Duarte, CA 91010.

Asymmetric or Gray's Type I synapses contain a prominent postsynaptic density (PSD) structure, whose functional role is largely unknown. The morphology of the PSD is largely unaffected by moderate amounts of nonionic detergents, and this fact has been successfully utilized to prepare fractions highly enriched in the PSD structure. Unfortunately, the use of nonionic detergents results in the extraction of most of the lipid from the membrane bilayer and is likely to substantially alter the structure of the overlying postsynaptic membrane.

Crawford, Osborne and Potter (J. Neurocytol., in press) developed an extraction procedure, not dependent upon detergents, for rendering postsynaptic membranes detached from presynaptic structures. Ratner and Mahler (JCB 83: 271a) utilized this extraction procedure followed by intense homogenization and discontinuous sucrose density centrifugation to prepare fractions enriched in postsynaptic membranes with attached PSD structures. Their fraction, from the 1.5 M / 2.0 M sucrose interface, contains only a portion of the total PSD structures. We analyzed the basis for this purification procedure by utilizing continuous sucrose density gradients in a Z-60 zonal rotor. In order to analyze the relative content of PSD in the various fractions, we developed a novel gel electrophoresis method for analyzing the PSD content of the fractions by quantitative densitometry of electrophoretic bands in deoxycholate insoluble residues. It is apparent that a major fraction of the PSD structures overlap with mitochondria remaining from the synaptic plasma membrane preparation. We have developed a convenient aqueous polymer two-phase extraction procedure for largely removing mitochondrial membranes from the postsynaptic membranes containing PSD structures. We achieved further purification of PSD enriched structures by utilizing affinity partitioning to purify membranes on the basis of their sulfhydryl content. Tubulin and the PSD protein, major components of the PSD structure based upon previous reports, are also the major components of our postsynaptic membrane fraction and contain the predominant content of reduced sulfhydryl groups. We are examining the efficacy of our separation procedures by morphological analysis at the electron microscopic level. Preliminary results suggest that our highly enriched fractions contain the PSD structure and neurotransmitter receptor binding sites. Since this procedure obviates the requirement for detergent treatment, we expect to observe enzymes characteristic of the native synapse. (Supported by NS-14281 and NS-12116.)

102.13 SUBCELLULAR FRACTIONATION OF THE CHICK RETINA. Nigel G.F. Cooper*, Hue-ly Guan* and Barbara J. McLaughlin. Department of Anatomy. University of Tennessee Center for the Health Sciences, Memphis, TN 38163

We report here on the isolation of synaptosomes (SS), synaptic plasma membranes (SPM) and postsynaptic densities (PSD) from fractions rich in photoreceptor synaptic terminals and their characterization by electron microscopy (EM) and polyacrylamide gel electrophoresis (PAGE). By EM analysis, minimal hand homogenization (5-10 strokes) of chick retinas (8-20 retinas) in 0.32M sucrose does not lead to a significant release of SS, most of which remain with the pre-P_1 or nuclear pellet (150g-10 min). In our hands, a large yield of SS into the P_1 pellet (800g-10 min) requires more extensive homogenization of this pre-P_1 pellet (3x20 strokes). Fractionation of the SS rich P_1 pellet on a sucrose gradient for 2 hrs at 82,500g yields 4 fractions of photoreceptors. By EM, two of the fractions (1.6M/1.8M and 1.8M/2.0M interfaces) contain large synaptosomes in groupings of two or more, along with nuclear contaminants. Many of these large synaptosomes contain several synaptic ribbons, numerous synaptic vesicles, and have retained their dyad and triad postsynaptic elements. Two fractions (1.4M and the 1.4M/1.6M interface) contain large, ribbon-containing single and double synaptosomes, without nuclear contamination. The double synaptosomes most likely represent synaptic terminals from principle and accessory members of double cone photoreceptors whereas the single synaptosomes could represent terminals from cones or rod photoreceptors. Using a modification of the method of Cohen et al (1977), lysed synaptosomes are put on a second sucrose gradient (0.85M, 1.0M, 1.5M and 2.0M) at 90,000g for 30 min. SPM and PSD are obtained at the 1.0M/1.5M and 1.5M/2.0M interfaces, respectively. PAGE of 10 ug of the SDS solubilized PSD fraction yields four faint Coomassie blue stained bands which may be doublets. The molecular weights are approximately 48K, 65K, 70K and 74K Daltons. PAGE of 10-20 ug of SDS solubilized SS fractions yields 10 prominent Coomassie blue stained bands at 32K, 39K, 40K, 46K, 48.5K, 52K, 64K, 65K, 70K, and 74K Daltons. Preliminary EM of the PSD fractions shows densely stained oval PSD's in the en face plane of section and some contamination by synaptic junction complexes. In conclusion, a protocol has been established based on cell isolation techniques used elsewhere that appears to be suitable for characterizing the macromolecular components of photoreceptor synapses within subcellular fractions of the retina. Supported by USPHS EY 02708.

102.14 CALCIUM-PROMOTED FLUORESCENT RESONANT ENERGY TRANSFER IN AGGREGATING CHROMAFFIN GRANULE MEMBRANES. S. J. Morris, T. C. Südhof and D. H. Haynes (Spon: H.B. Pollard). Neurotoxicology Section, NINCDS, NIH, Bethesda, MD 20205; Dept. of Neurochemistry, Max Planck Institute for Biophysical Chemistry, Göttingen, FRG and Dept. of Pharmacology, U. of Miami Medical School, Miami, FL 33101

Isolated adrenal medullary chromaffin granules will aggregate and fuse upon the addition of Ca^{2+}. These reactions may serve as a model for exocytosis in vivo. We have previously reported on the kinetics of granule aggregation using light scattering techniques. Reactions consonant with or subsequent to granule-granule contact can be followed using resonant energy transfer between suitably placed donor and acceptor fluorophores.

Proteins of intact granules are labelled with sulfhydryl-specific maleimide and mercury acetate agents, then lysed and washed extensively to remove soluble or loosely bound proteins. The quenching of donor fluorescence is followed as an unambiguous signal of probe interaction. When both donor and acceptor are placed on proteins of the same membrane, Ca^{2+} increases donor quenching with a K_m of ~ 200 μM. This Ca^{2+}-concentration is too low to promote significant aggregation. The effect is also independent of membrane concentration and must result from intra-membrane protein clustering or patching. This constitutes the first demonstration of the fluid mosaic nature of a subcellular organelle. If the donor and acceptor labels are placed on separate membranes, mixing in the absence of Ca^{2+} produces a slow decline in donor fluorescence. This effect can be greatly reduced by extensively washing the membranes and is ascribed to exchange of membrane proteins either through the medium or by granule-granule collisions. If the two sets are mixed and Ca^{2+} added immediately or if they are incubated overnight to allow for complete protein exchange, addition of Ca^{2+} produces protein concentration-dependent energy transfer with a K_m of ~ 2 mM and rates 5-10 times slower than Ca^{2+}-promoted aggregation (K_m ~ 4 mM) measured in parallel. We attribute this fluorescence change to slow rearrangements of membrane components which follow aggregation.

We have postulated that granule-granule aggregation is mediated by proteins which protrude several Å from the membrane p-surface (Haynes, Kolber and Morris, J. Theoret. Biol 81:713 (1979)). No component with rates similar to aggregation is seen by this labelling method. Ca^{2+} aggregates labelled or unlabelled granules at the same rate. If such proteins are responsible for granule-granule recognition, they either contain no free sulfhydryl groups or the labelled sites are far enough apart when the proteins interact for no significant Förster energy transfer to occur.

103.1 CHOLINEACETYLTRANSFERASE: PURIFICATION AND IMMUNOFLUORESCENT LOCALIZATION IN RETINA OF FOUR VERTEBRATE SPECIES. F. Eckenstein*, M. Schwab* and H. Thoenen. Max-Planck-Institute for Psychiatry, Dept. of Neurochemistry, D-8033 Martinsried, FRG.

The acetylcholine (ACh) synthesizing enzyme choline acetyltransferase (ChAT), which represents the only undisputable marker for cholinergic neurons, has been purified from pig brain by ammonium sulphate fractionation, ion exchange and hydrophobic chromatography, followed by elution of the enzyme from blue dextran Sepharose by 100 µm coenzyme A. After concentration on hydroxylapatite ChAT was purified by high pressure liquid chromatography to a final specific activity of 150 µmoles ACh·min^{-1}·mg^{-1} protein and to a purity of over 90% as judged by SDS-gel electrophoresis. Antisera to this preparation raised in mice were specifically directed against ChAT as demonstrated in double immunodiffusion tests.

By indirect immunofluorescence cholinergic neurons were demonstrated in the retina of the rat, chick, goldfish and frog (Xenopus). In all four species amacrine cells located mostly at the inner margin of the bipolar cell layer and less often in the ganglion cell layer were stained. Their fibers projected into the inner plexiform layer where their terminals form a double band. In the frog retina, a second type of labeled cells - probably ganglion cells - was found in the ganglion cell layer.

103.2 HORSERADISH PEROXIDASE LABELING OF INDIVIDUAL LATERAL HYPOTHALAMIC NEURONS. M. J. Wayner, F. C. Barone and S. L. Scharoun. Brain Research Lab., Syracuse Univ., 601 University Ave., Syracuse, NY 13210.

Electrical recordings were made from lateral hypothalamic (LH) neurons in urethane anesthetized rats. Single barrel electrodes or the center barrel of seven barrel electrodes, filled with 1% horseradish peroxidase (HRP, Sigma Type VI) in 2 M NaCl, were used to record neuronal activity. Following establishment of a stable baseline in a single neuron, the effects of mesencephalic central gray and reticular formation single pulse electrical stimulation and/or the microiontophoretic ejection of dopamine or norepinephrine were determined. HRP was then ejected from the recording electrode utilizing 500 nA anodal current for 30 sec. Only if the discharge frequency was not affected by the HRP was the tissue retained for further histological examination. Animals were perfused with wash and fix solutions and stored overnight at 4°C. Hypothalamic tissue was sliced at 40 µm and sections were processed with DAB for the brown reaction and counterstained with neutral red. Sequential sections were examined and positively labeled neurons were identified and photographed. In most cases, individual neurons in the LH were densely labeled and had a Golgi-like appearance. Dendritic and axonal processes could be easily identified and followed for several hundred micrometers. Since small ejection sites were located adjacent to the cell bodies and no other neurons could be identified nearby in the Nissl stained tissue, labeled neurons were apparently those cells from which electrical recordings had been made. Labeled neurons were located within and adjacent to the medial forebrain bundle (MFB). In general, the dendritic processes of these neurons spread out perpendicularly to the path of the MFB fibers and are similar to the "path" neurons identified previously (Millhouse, Brain Res. 15: 341-363, 1969). The location of these neurons within the MFB allows for a great amount of synaptic convergence from within the hypothalamus and other MFB associated structures. (Supported by NIH Grant NINCDS USPHS No. 13543.)

103.3 ORGANIZATION OF NEOCORTICAL PROJECTION NEURONS IN RAT NUCLEUS LOCUS COERULEUS. B.D. Waterhouse, C.-S. Lin, R.A. Burne, W.K. Smith and D.J. Woodward. Dept. of Cell Biology, The Univ. of Texas Health Science Center, Dallas, Texas 75235.

The present study was conducted to examine the spatial organization of locus coeruleus (LC) neurons that project to rat cerebral cortex. Twenty-three Long-Evans hooded rats (180-275 gm) received unilateral pressure injections (0.6-1.5 µl) of HRP (Miles Lab., conc. in Tris buffer) in either frontal (n=3), somatosensory (n=7) or visual (n=7) cortex in the rostro-caudal plane or lateral to somatosensory (n=3) or visual (n=3) cortex in the medio-lateral plane to localize LC neurons projecting to specific cortical areas. Coronal and sagittal sections (40-100 µ) through the LC were examined after incubation with TMB and H_2O_2 and counterstaining with neutral red. The locations of retrogradely labeled cells were recorded relative to a three dimensional biological coordinate system maintained by a computer linked to a light microscope.

LC neurons labeled from cerebrocortical injections of HRP were primarily located in the ipsilateral nucleus and to a lesser extent (5-10% of total labeled cells) in the contralateral nucleus. Routinely, cortical projection neurons were concentrated in the caudal half of LC. Within this region, the most rostrally labeled cells were observed after frontal cortex injections. In the dorso-ventral dimension, neurons labeled from neocortical injections were scattered in the dorsal division of the caudal LC, with a tendency for individual groups of cells projecting to visual, somatosensory or frontal areas of cortex to be aligned in a dorsal to ventral pattern. Only the frontal cortex received a projection from the ventral division of the caudal LC. Neurons projecting to lateral regions of the neocortex tended to be localized toward the medial aspect of the ipsilateral LC, in contrast to a more lateral tendency for cells projecting to medial portions of the cortex. The zone of labeling resulting from injections confined to neocortical areas overlapped with but was not coextensive with that observed following injections into 1) neocortex and caudate, 2) neocortex and hippocampus and 3) parafloccullus of the cerebellum.

In summary, the data from the brains analyzed has delimited a portion of the ipsilateral LC nucleus which projects to rat cerebral cortex. Moreover, the results suggest that within this zone of LC there exists a rough topographic organization reflecting the rostro-caudal and medio-lateral distribution of noradrenergic axons within the cortex. In view of this evidence, the hypothesis may now be considered that selective noradrenergic interaction with various CNS regions may occur as a result of activity in topographically organized subsets of LC neurons. (Supported by NIDA DA-02338 and Biological Humanics Foundation)

103.4 MORPHOLOGY AND ULTRASTRUCTURE OF AN ACETYLCHOLINE AND NEUROPEPTIDE CONTAINING NEURON IN A MOLLUSK. B.Masinovsky*, P.E.Lloyd* and A.O.D.Willows. (SPON: K.Graubard). Dept. of Zoology and Friday Harbor Labs. Univ. of Washington, Seattle WA 98195.

A large white appearing neuron (B11) is located on dorsal surface of each of the paired buccal ganglia in the gastropod, Tritonia diomedia. This neuron innervate the animal's gut and has been shown to contain acetylcholine and neuropeptide, SCP$_A$ (Lloyd, Neurosci. Abstr., 5, 252; Lloyd et al, Neurosci. Abstr., this volume). Because of these interesting properties, we have analyzed the morphology of B11 in detail.

We visualized the peripheral axonal morphology of B11 by intracellular injection of Lucifer yellow into the cell body. By combining this procedure with an electrophoretic gradient, we were able to fill even small axonal branches at distances of over 1 cm from the cell body. Each B11 is an unipolar neuron which sends its axon into the ipsilateral gastro-esophageal ganglion where it divides into one major and two minor branches. The minor branches exit gastro-esophageal ganglion in small nerves and immediately ramify over the muscle surface apparently innervating the most proximal part of the esophagus. The major axonal trunk travels along the gastro-esophageal nerve and occasionally sends out small branches which also ramify into the gut. We have not been able to fill the axon to their final termination but they may contribute to the neural plexus on the surface of the stomach. This morphology is consistent with known physiological properties of B11.

Intracellular injection of HRP was used to visual the neurites of B11 in the neuropil of the buccal ganglia. Each B11 produces a relatively sparse network of neurites, and although a few processes extend towards the buccal commissure, none cross over to the contralateral ganglion.

The cell body of B11 contains large quantities of acetylcholine and a neuropeptide. We were therefore interested in the vesicular content of the cytoplasm. Ultrastructural examination of the cytoplasm of B11 soma, using 2.5% glutaraldehyde in 0.2 M s-collidine buffer (pH 7.35; 970 mOsmols) as a primary fixative, indicated that B11 contained a large number of vesicles, many associated with Golgi complexes. These vesicles were electron luscent and varied from round to ellipsoid. The average diameter in the longest plane was 141±29 nm (mean ± S.D.; N=80). These vesicles are largerthan those associated with cholinergic neurons and are not electron dense as are many peptide containing granules. (Supported by NSF BNS 7906280).

103.5 ULTRASTRUCTURE OF SPECIALIZED NEUROMUSCULAR JUNCTIONS IN A BLOOD VESSEL OF APLYSIA.

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Electrophysiological evidence for multiple neurotransmitter involvement in the excitation and inhibition of Aplysia vascular smooth muscle prompted an ultrastructural study of neuromuscular junctions (NMJ) in the largest vessel, the anterior aorta (AA). The wall of the AA is composed of three layers: an inner, single-cell thick myoendothelial lining of the lumen with adherent amebocytes; a 2-5 cell thick layer of circular muscle (~5 μm dia); and an outer, several cell thick layer of longitudinal muscle (~8 μm dia) and small nerves embedded in a matrix of collagen and connective tissue. Circular muscle fibers are tightly grouped with extensive membrane interdigitation and are rarely innervated. Longitudinal muscle cells occur as well-separated single fibers with rare inter-fiber contact and are extensively innervated. Contact between circular and longitudinal muscle was seen and possible regions of specialization identified.

Three distinctly different types of NMJ's were observed. Type I: rare, relatively flat terminal with long, straight contact with sarcolemma; spherical clear vesicles (~70 nm dia) with no obvious clustering; pre-synaptic densities and electron dense cleft material present but little post-synaptic specialization. Type II: common, large volume terminal with junctional area often several μm long and enwrapping sarcolemma; small dense core vesicles (~85 nm dia) and more numerous small clear vesicles (~55 nm dia) and clustering of clear but not dense core vesicles; pre- and post-synaptic membrane specializations were minor. Type III: very common, small volume bulbous terminals with small but well-defined synaptic regions; numerous large vesicles (~160 nm dia) of variable electron density and fewer numbers of small clear vesicles (~65 nm dia) clustered primarily at synaptic area; pre- and post-synaptic membranes thickened, pre-synaptic triangular-shaped densities, cleft contains little material.

On the basis of earlier electrophysiological work on the AA (Sawada et al., Brain Res., 207:486-490, 1981) and the anatomical and neurochemical evidence from this study, speculation on the neurotransmitter content (Type I=ACh; II=5-HT; III=glycine) and function of each terminal type is made and probable parent neurons in the parietovisceral ganglion identified. The pattern of innervation observed and frequency of muscle-muscle contacts are discussed in relation to the overall contractile performance of the aorta.

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104.1 APOMORPHINE-INDUCED HYPOTHERMIA IN MICE TREATED PRENATALLY WITH PHENOBARBITAL. R. Kuprys, P. Hoffman and B. Tabakoff. Dept. of Physiol. Biophys., Univ. of Illinois Med. Ctr. and VA Westside Med. Ctr., Chicago, IL 60612.

Our previous work indicates that central dopamine (DA) receptor sensitivity is altered in adult mice exposed prenatally to phenobarbital (PB). In the present study, the effects of the DA receptor agonist apomorphine (APO) upon body temperature was further examined in these mice.

Heterogeneous strain mice were fed a milled diet containing 3 mg PB/g diet or control diet and water ad libitum on days 9-18 of pregnancy. Male and female offspring of PB and control (C) dams were subsequently tested as adults for their hypothermic response to APO HCl (0.3-3 mg/kg, ip). Rectal body temperature was measured up to 120 min. after drug injection, using a telethermometer probe.

Body weights and baseline body temperatures of both male and female PB mice did not differ from their respective C values. Male PB mice were less sensitive than male C mice to the hypothermic effects of APO following treatment with 0.4 or 0.5 but not 1 mg/kg APO. In contrast, female PB mice were more sensitive than female C mice to the hypothermic effects of APO following treatment with 0.3 or 0.5 but not 1 or 3 mg/kg APO. The differences observed between PB and C mice could not be attributed to changes in metabolism of APO. Following ip injection of 3 mg/kg APO, wholebrain µg/gm levels of APO (obtained spectrofluorimetrically) in C and PB male mice were 0.95±0.29 (n=5) and 0.94±0.18 (n=5), respectively, at 15 min. post-injection, and 0.53±0.21 (n=5) and 0.46±0.06 (n=5), respectively, at 30 min. post-injection. For C and PB female mice, wholebrain APO levels were 0.50±0.39 (n=10) and 0.61±0.45 (n=10), respectively, at 15 min. post-injection. In addition, the differences in APO-induced hypothermia could not be attributed to alterations in environment-dependent thermoregulation capacity, or to changes in α-noradrenergic regulation of body temperature following acute administration of clonidine HCl.

The results suggest that prenatal PB exposure produces long-term changes in the sensitivity of DA receptors which regulate body temperature and that the alteration produced is expressed differently in the male and female adult offspring.

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104.2 EFFECTS OF ETHANOL ON CENTRAL DOPAMINERGIC FUNCTIONS IN THREE INBRED STRAINS OF MICE. B. Tabakoff and K. Kiianmaa*. West Side VA Medical Center, Chicago, IL 60612 and Department of Physiology and Biophysics, University of Illinois Medical Center, Chicago, IL 60680.

The effects of an acute dose of ethanol on the synthesis and release of dopamine (DA) were studied in the striatum of Balb/c, C57Bl/6 and DBA mice. The rate of DA synthesis and release was monitored by measuring the accumulation of DOPA and DOPAC levels, respectively, after inhibition of the aromatic amino acid decarboxylase with NSD-1024. The DA precursor and metabolite were quantitated using high performance liquid chromatography with electrochemical detection. Ethanol, in doses ranging from 0.8 - 3.5 g/kg i.p., stimulated the rate of DA synthesis in Balb and DBA mice in a dose-dependent manner. The highest dose of ethanol (3.5 g/kg) increased the levels of DOPA in DBA and Balb mice by 200-300%, relative to the saline-injected controls. However, the lower doses of ethanol (0.8 - 2.0 g/kg) did not significantly affect the rate of DA synthesis in C57Bl mice, while administration of 2.5 - 3.5 g/kg of ethanol stimulated synthesis only 90-130%. Ethanol was found to have a biphasic effect on DA release. Thus, ethanol decreased the DOPAC levels in the striata of Balb and DBA mice by 25-40% in doses of 0.8 - 1.35 g/kg, and increased them 20-70% when higher doses of 2.5 - 3.5 g/kg were injected. The C57Bl mice showed only a small suppression of DA release at the lower dose range of ethanol, whereas the higher doses induced an increase of 75-135% in DOPAC levels. These results show that the central DA systems of Balb and DBA mice are, in general, more sensitive to the effects of ethanol than those of C57Bl mice.

The behavioral effects of a hypnotic and non-hypnotic dose of ethanol in these strains of mice are being studied. The results of these experiments will be discussed in terms of whether the ethanol-induced changes in the central dopaminergic functions found here can explain differences in the stimulatory and depressant effects of ethanol between these strains of mice.

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104.3 ALTERATIONS IN BEHAVIOR AND BRAIN CHOLINERGIC FUNCTION AFTER CHRONIC ALCOHOL AND DIHYDROERGOTOXINE MESYLATE (HYDERGINE) TREATMENT OF AGING MICE. K. Persson* and T. Samorajski. Texas Research Institute of Mental Sciences, Houston, TX 77030.

It is now well established that cholinergic deficits in the brain may be responsible for some motor and cognitive impairments that may occur in normal aging and age-related neurodegenerative disorders. We sought to determine whether the ergot alkaloid dihydroergotoxine mesylate (Hydergine, a potent α-adrenergic blocking agent with significant glycolytic activity in the brain) might prevent brain deterioration associated with aging and/or prolonged ingestion of alcohol. Two hundred aging male mice of the C57BL/6J strain were used. The animals were distributed according to weight into four equal groups of 50 mice each. One group received 1mg/kg of Hydergine orally, 3 times per week. A second group (controls) received corresponding volumes of distilled water. A third group was maintained on a 10% v/v ethanol-water solution. The fourth group received both Hydergine (1mg/kg orally, 3 times per week) and 10% ethanol in their drinking water. To preclude residual treatment effects, behavioral and neurochemical tests were conducted two weeks after termination of treatment. We found age-related decreases in choline acetyltransferase (ChAT) and cholinergic muscarinic binding in the cortex and striatum of mouse brain. Alcohol treatment increased ChAT activity only in the striatum. Receptor binding, however, was not effected by alcohol ingestion. The administration of Hydergine increased barbiturate narcoses, indicating an adverse effect on the liver and/or brain. Hydergine treatment had no effect on cholinergic muscarinic receptor binding or ChAT activity in the corpus striatum, cortex or cerebellum of the mouse brain. Combined administration of alcohol and Hydergine had a "sparing effect" on alcohol induced impairments in ChAT activity. Neuronal redundance, plasticity and recovery offer some prospects for intervention by drugs (and possibly hormones, and neurotransmitter precursor substances) in brain disorders caused by chronic alcohol ingestion.

104.4 RESPONSE TO PENTOBARBITAL OF PIGMENTED VS. ALBINO LONG-EVANS RATS. I. S. Westenberg and J. M. Bolam* (SPON: K. R. Swiatek). Instit. for Study of Developmental Disabil., Chicago, IL 60608.

Compared to pigmented strains of rats, albino strains are more susceptible to pentobarbital. In such between-strains comparisons the single-gene variable of albinism is confounded with other genetic variables (strain). To eliminate this problem we made a within-strain comparison of partially inbred pigmented vs. albino rats. Subjects were pairs of male rats, an albino and a black-hooded littermate from each of three breeding lines of Long-Evans ancestry [Sim:(LE), coefficient of inbreeding 0.67 to 0.73]. In each pair the rats differed in one gene at the albino (c) locus but were otherwise genetically very similar. They weighed about 500g and were 16 to 17.5 months old at the beginning of the study. Each rat was housed with its littermate with food and water ad libitum. Each rat was injected i.p. weekly with sodium pentobarbital; its dosage began at 10 mg/kg and increased 10 mg/kg/wk until the rat died. To measure the onset of drug effects we recorded the post-injection times for the rat to fall off a walkway (droptime) and lose righting reflexes (LRR-time). To measure the duration of drug effects we recorded the time of the return of righting reflexes (RRR) and calculated the time from LRR to RRR (sleeptime).

In the few cases of significant pigmented-albino differences in the onset measures, the pigmented rats showed the more rapid onset of the drug's effects. In neither onset measure did an albino appear consistently more susceptible than its pigmented counterpart. Sleeptimes generally increased steadily with increasing dosage. In two pairs the pigmented rats consistently slept longer than their respective albino littermates, and the pigmented-albino difference grew steadily as dosage increased ($p<0.01$, rank correlation coefficient). In the third pair results for the albino and its pigmented littermate were more similar. In no case did an albino consistently sleep longer than its pigmented littermate as dosage increased. Thus, in the duration measure, as in the onset measures, an albino never appeared consistently more susceptible than its pigmented counterpart. Hence, the rapid onset and long duration of pentobarbital effects in some albino strains are related to genetic variables other than just the albino mutation.

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104.5 THE EFFECTS OF ETHANOL AND TEMPERATURE ON CRAB NEUROMUSCULAR PHYSIOLOGY. P.J.Stephens and R.E.Lazarus* Dept. of Biology, Villanova Univ. Villanova, Pa. 19085.

The stretcher muscle in the crab limb is innervated by branches from three motor axons; the excitor (E), the specific inhibitor (SI) and the common inhibitor (CI). Preparations were made from autotomized limbs of the shore crab Pachygrapsus so that the three motor axons could be electrically stimulated either individually or in combination. At normal temperatures E axon shocks produced single excitatory junctional potentials (ejp's) in stretcher muscle fibers. Increasing the temperature caused a decline in ejp amplitude until, above a critical temperature threshold, a single E axon shock provoked the generation of spikes in the peripheral E axon branches and concomitant ejp's in the stretcher muscle fibers (Stephens and Atwood, Soc. Neurosci. 6, 1980). Further temperature increases caused an increase in the number of ejp's and E axon spikes following an E axon shock, whereas cooling reversibly abolished the repetitive response.

Bathing preparations in a crab saline containing low levels of ethanol (<5%) decreased the temperature threshold at which repetitive axon spikes and ejp's were recorded. Latency measurements and extracellular recordings from two locations along the E axon confirmed that the spikes produced after the initial orthodromic action potential were generated peripherally.

Synchronous stimulation of the E and SI axons abolished the peripherally generated E axon response. Furthermore, when trains of spikes and ejp's were produced by E axon shocks, stimulation of the SI axon shortly after the E axon shock abolished only a portion of the peripherally generated E response. These results suggest that SI axon activity can modulate the peripheral generation of spikes in the E axon branches. CI axon activity was not observed to modulate the peripheral E axon response.

We conclude that ethanol decreases the threshold temperature for the peripheral generation of E axon activity. This result, together with the observation that the threshold temperature is dependent upon acclimation (Stephens and Atwood, J.Comp.Physiol. In press 1981), suggests that the peripheral generation of E axon spikes is closely related to membrane fluidity.

104.6 EFFECT OF PENTOBARBITAL IN A GERBIL MODEL OF STROKE. Charles J. Hannan, Jr. and Robert A. Patterson*. Clinical Investigation Department, Eisenhower Army Medical Center, Ft Gordon, GA 30905

A previously described modification of the gerbil stroke model (Neurosci Abstr 6:826, 1980) developed in this laboratory allows the production of regional ischemia leading to infarction and death in about 80% of prepared gerbils within 3 days. A further 10% of animals survive with histological evidence of infarction. The procedure involves the total occlusion of the left common carotid artery, restriction of the right common carotid artery (to about 1.4mm with a tantalum ligating clip), and the i.p. injection of 2.5 gm/kg glucose in ketamine anesthetized gerbils. To evaluate the sensitivity of the model to therapeutic measures, the effect of pentobarbital on edema formation 6 hours post occlusion was measured. Edema was shown to be demonstrable by 6 hours in a group of 13 gerbils not given pentobarbital. Brains were removed, divided into quadrants, and a wet weight followed by a dry weight was obtained. The calculated percent water was significantly greater in the left hemisphere, both anterior and posterior quadrant, when compared to the right hemisphere by students t-test. When pentobarbital was used as the anesthetic (50 mg/kg) rather than ketamine, there was no difference in percent water between left and right hemispheres:

	MEAN % WATER	
QUADRANT	LEFT HEMI	RIGHT HEMI
NO TREATMENT:		
Anterior	82.2±0.90*	81.7±0.64
Posterior	80.9±1.06**	79.8±0.66
PENTOBARBITAL:		
Anterior	81.6±0.64	81.6±0.61
Posterior	80.6±0.96	80.3±0.73

*:p>.05; **:p>.01

The regional ischemia produced by this model is considered a more appropriate model for evaluation of experimental therapies than total global ischemia, and the high incidence of infarction is an advantage over the simple single common carotid occlusion model most widely used.

104.7 RESPONSE OF LOCUS COERULEUS NEURONS TO DIRECT APPLICATION OF ETHANOL. Jean C. Strahlendorf and Howard K. Strahlendorf. Departments of Physiology and Medical and Surgical Neurology, Texas Tech University Health Sciences Center, Lubbock, Texas, 79430.

Ethanol (ETOH) is one of society's oldest and most widely used drugs yet the neuropharmacological actions of alcohol at the cellular level are poorly understood. The pharmacologic profile of ETOH bears many similarities to that of the benzodiazepines and opiates, e.g., anti-anxiety activity. Some recent reports indicate ETOH may influence GABA-ergic neurotransmission in the spinal cord and cerebral cortex. The noradrenergic neurons of the locus coeruleus (LC) possess GABA receptors and are strongly inhibited by iontophoretically applied GABA. Furthermore, the LC may be involved in the expression of anxiety, fear and stress reactions. We are currently examining the effect of ETOH applied directly to LC neurons by microiontophoresis (electro-osmosis) and micropressure ejection.

Chloral hydrate anesthetized male rats were used in all experiments. Conventional 5 or 7 barrel micropipettes containing 2M NaCl saturated with fast green in the center barrel were employed to record single LC units. Drug barrels contained 0.3M ETOH, 1M ETOH, 0.2M GABA, 0.1M glycine (all in distilled water) and 0.01M clonidine HCL in 165mM NaCl. Four molar NaCl was used in the balance barrel. LC neurons were identified by characteristic electrophysiologic criteria at the time of recording and verified histologically at the end of an experiment. Preliminary results indicate ETOH markedly slows LC firing in the absence of any detectable membrane effects when applied directly to the neuron. Percent inhibition of firing rate ranged from 6% to 24% over a "dose" range of 100nA-sec to 300nA-sec (n=10 cells). ETOH was applied for periods of up to 40 sec with 60 sec off times between ejections. With this protocol there was no evidence of cumulative (carry-over) effects or of rebound excitation. One cell appeared to become tolerant to repeated applications of ETOH at high ejection currents. These data demonstrate ETOH can directly suppress LC unit activity and suggest that the LC may be an important substrate for some pharmacologic actions of ETOH.

104.8 BEHAVIORAL AND BIOCHEMICAL CHANGES IN RATS EXPOSED TO CHRONIC ETHANOL TREATMENT. S. Liljequist*, J. Engel and K. Yoshida*.

Previous studies from our laboratory have indicated that chronic ethanol treatment (ethanol administered in various concentrations as the sole drinking fluid for up to 270 days)produced significant alterations in the behavior and in the activity of central catecholamine neurons in rats. One such change was characterized by the observation that local application of dopamine into the nucleus accumbens caused a marked locomotor stimulation in chronic ethanol rats in doses which per se had no effect on the locomotor activity of age-matched control rats thus indicating that chronic ethanol treatment increased the functional responsiveness of central dopaminergic mechanisms at or beyond postsynaptic dopamine receptors.

In order to further characterize the observed alteration of central dopamine receptor sensitivity, we have examined the effects of various doses of the specific dopamine receptor antagonist haloperidol on the catecholamine synthesis (measured as the accumulation of DOPA after inhibition of aromatic amino acid decarboxylase by NSD-1015) in various brain regions. It was observed that haloperidol significantly less enhanced the accumulation of DOPA in dopamine-rich brain structures of chronic ethanol rats as compared to the biochemical changes seen in age-matched control rats, thus lending further evidence to the hypothesis that chronic ethanol treatment may increase the sensitivity at or beyond central postsynaptic dopamine receptors.

The interaction between central dopaminergic and cholinergic neurons in the brain is well established. To investigate whether the present ethanol regimen affect central cholinergic mechanisms we have studied the binding of ^{3}H-nicotine to membranes of the hippocampus, thalamus, and hypothalamus of chronic ethanol rats. Chronic ethanol treatment for 150 days significantly decreased the binding of ^{3}H-nicotine to membranes of the hippocampus while increased binding of ^{3}H-nicotine was observed in the thalamus and in the hypothalamus.

Taken together, the results from the present study seem to further support the view that various central neurotransmitter mechanisms are affected by chronic ethanol treatment.

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104.9 Behavioral Effects of Apomorphine and Ethanol in Lines of Mice Which Differ in Neurosensitivity to Alcohol. Michael E. Abbott* and Bruce C. Dudek. Dept. of Psychology, SUNY-Albany, Albany, NY 11222.

The selective breeding program of McClearn and Kakihana (Behav. Genet.3:409-410, 1973) produced lines of mice which differ markedly in sensitivity to the hypnotic effect of ethanol (ETOH). This reflects a differential neurosensitivity to ETOH. Furthermore, SS mice(Short sleep) are highly activated by subhypnotic doses while LS(Long sleep) show depressed locomotion and severe uncoordination. ETOH reduces dopamine (DA) turnover more in the LS mice than in SS mice. Additionally several agents which affect DA function have differential behavioral and biochemical effects in these mice, implicating DA systems as a basis for the differential response to ETOH. The present studies examined the effects of the DA agonist apomorphine(APO) in these mice and replicated earlier work showing that APO could block the activitional effects of ETOH on locomotor activity. The first experiment examined the APO dose response curve. Mice were treated with one of seven doses of APO (.15-2.5 mg/kg i.p.). Ten minutes later they were placed in a photocell apparatus and locomotor activity was recorded for 15 min. Both lines showed similar depression to .6075 mg/kg, slight activation to 2.5 mg/kg, and depression to 5 mg/kg. The second experiment assessed APO-induced stereotypy with similar doses. Dose dependent stereotypy did not differ between the two lines. Thus from the first two experiments it appears that the agonist actions of APO are similar in these lines. However, the third experiment shows that the ability of APO to modify the dose response curve of ETOH is different in these two lines. The mice were treated with APO at 0, .15, .6075 or 2.5 mg/kg. Ten minutes later they received ETOH at 0, 1.5, 2.0, or 2.5 g/kg, and locomotor activity was recorded for 15 min. At the lower doses of APO (.15 and .6075) the activational effects of 1.5 g/kg ETOH were blocked in the LS mice and slightly diminished in SS mice. The 2.5 mg/kg dose of APO blocked the depressant effects of 2.5 g/kg in the LS mice and reduced the activational effect in the SS mice. Thus both presumed presynaptic and postsynaptic effects of APO on the ETOH responses were genotype dependent, providing added evidence that DA systems may control the differential response of these mice to ethanol. Althougn the genetic control of the ETOH response is complex (polygenic), it appears that at least one major neurotransmitter system(s) may have been strongly affected by the selective breeding. These mice thus serve as a valuable tool for investigations of DA involvement in ETOH actions.

104.10 EVIDENCE FOR ALTERATION OF THE BRAIN MEMBRANE CALCIUM BINDING IN ETHANOL-DEPENDENT RATS PROBED BY TERBIUM (TB^{3+}) FLUORESCENCE. Harish C. Pant*, Charles E. Swenberg*, Edward Majchrowicz*, and Forrest F. Weight. (SPON: J. S. Hong). Laboratory of Preclinical Studies, National Institute on Alcohol Abuse and Alcoholism, Rockville, MD 20852

Calcium (Ca^{2+}) interaction with biological membranes in the presence of ethanol has attracted considerable attention since many cellular processes are controlled by Ca^{2+}. However, reported data on Ca^{2+}-membrane interaction after chronic treatment with ethanol or after in vitro addition of ethanol to isolated synaptosomal plasma membranes (SPM) are conflicting. In the present study, we utilized Tb^{3+} fluorescence to probe membrane Ca^{2+} binding in the cerebellum and cerebral cortex of ethanol dependent and withdrawing rats. Ethanol was administered to male Sprague-Dawley rats (250-350 g) using the intubation technique of Majchrowicz (Psychopharmacolgia 43: 245, 1975). Four groups of rats were studied: (1) water treated controls; (2) acutely treated rats (4 hr after po dose of ethanol, 6 g/kg); (3) dependent-intoxicated (prodromal detoxication phase), blood ethanol concentration usually 300-500 mg/dl; (4) ethanol withdrawal syndrome group (4-6 hr after the onset of overt signs of CNS hyperexcitability). SPM were prepared by the method of Whittaker (Ann. N.Y. Acad. Sci. 137:982, 1976). Fluorescence measurements were performed with an Aminco-Bowman Spectrophotofluorometer (excitation wave length 285 nm, emission wave length, 545 nm). The addition of Ca^{2+} decreased the Tb^{3+} fluorescence in all synaptosomal preparations. A double reciprocal analysis of the Tb^{3+} fluorescence as a function of Tb^{3+} concentration indicates a decrease in the Ca^{2+} dissociation constant relative to control for Ca^{2+} binding in dependent-intoxicated animals and its partial restoration during withdrawal. No change in the Ca^{2+} dissociation constant was observed in the acutely intoxicated rats. The data suggest that membrane Ca^{2+} binding is modified in the cerebellum and cerebral cortex of ethanol-dependent rats.

104.11 CHANGES IN GLUCOSE UTILIZATION IN THE BRAIN DURING THE WITHDRAWAL SYNDROME IN ETHANOL-DEPENDENT RATS. Gerald A. Campbell*, Michael J. Eckardt, Edward Majchrowicz*, Cheryl A. Marietta* and Forrest F. Weight. Laboratory of Preclinical Studies, National Institute on Alcohol Abuse and Alcoholism, Rockville, Md. 20852

EEG and behavioral studies have indicated that sudden reduction in blood ethanol concentration (BEC) due to abstinence in humans and experimental animals which are physically dependent on ethanol can result in the development of CNS hyperexcitability and a withdrawal syndrome characterized by hyperactivity, hyper-reflexia, tremors, spasticity and convulsions. We have used 2-deoxy-D-[^{14} C]-glucose (2-DG) as a metabolic tracer to investigate whether there are local differences in glucose uptake in the CNS of ethanol-dependent rats exhibiting the withdrawal syndrome. Male Sprague-Dawley rats (200-300g) were made physically dependent upon ethanol using the intubation technique of Majchrowicz (Psychopharmacologia, 43:245, 1975). The last dose of ethanol in the early evening of the fourth day of ethanol administration marked the beginning of the withdrawal period. The onset of the behavioral signs of the withdrawal syndrome typically occurred about 12 hours later. Following the onset of the withdrawal syndrome, the animals were observed for 4-6 consecutive hours and rated for the severity of withdrawal. Animals were selected to be studied using the 2-DG technique when the manifestations of the withdrawal syndrome appeared maximal; at that time, the BEC had fallen to negligible levels. Glucose utilization was determined by means of the autoradiographic 2-DG method of Sokoloff (J. Neurochem., 28:897, 1977). Examination of the autoradiographs of brain sections from withdrawing animals revealed alternating light and dark columnar bands in frontal-sensorimotor and parietal cortex and in cerebellar vermis. Such columnar bands were not observed in controls. Cerebellar flocculus and paraflocculus showed patchy increases in density, whereas these structures were relatively uniform in controls. In addition, in controls, the globus pallidus, the zona incerta, the preoptic area of the hypothalamus, and several specific thalamic nuclei had little contrast compared with adjacent structures; however, in withdrawing animals, the density of these areas was considerably greater than that of adjacent structures.

104.12 The Actions of Ethanol on Basal and DA-Stimulated Adenylate Cyclase: Alteration of the Energy of Activation. S.N. Deyo*, M.V. Wagner*, S.J. Henriksen, W.J. Shoemaker, L.M. Randolph* and F.E. Bloom. A.V. Davis Center for Behavioral Neurobiology, LaJolla, CA 92037.

Ethanol (E) may produce its central pharmacological actions in part by disrupting the organization of neuronal membranes. Indeed, E increases the mobility of a membrane viscosity probe. This effect of E is dose dependent and may be interpreted as an indication that E decreases regional membrane viscosity. Proteins embedded in the membrane are thought to be dependent on the lipid environment for the maintenance of their structure and function. Therefore, it is possible that low and intoxicating doses of E might induce their pharmacological effects by altering the activities and interactions of membrane-bound proteins. We have studied E's affect on the kinetics of a membrane-bound enzyme, as reflected by the energy of activation (Ea) for the reaction that is catalyzed by the enzyme, to examine E's action(s) on membrane-embedded proteins. The Ea is determined from an Arrhenius plot which relates the rate of a reaction to the reaction temperature. In many cases the Ea's of reactions that are catalyzed by membrane-bound enzymes have been found to be directly related to membrane microviscosity. Therefore, changes in membrane viscosity induced by E may be expected to alter the rate of a membrane protein catalyzed reaction by changing the Ea. We have performed in vitro experiments that examine the effect of low concentrations of E (20-100 mM) on the Ea for basal and dopamine (DA)-stimulated adenylate cyclase from the rat striatum. In these experiments adenylate cyclase activity was measured in crude, striatal homogenates. E was added at the start of the incubation period, and the samples were incubated for 0, 3, 6, 9 or 12 minutes. From these data an equation relating cAMP production to time was generated. The velocities of enzyme activity and of DA activation of enzyme activity were determined from the first and second derivatives, respectively, of the equation. E (40 mM) decreased the Ea for the stimulation of adenylate cyclase activity by DA (Ea-control = 17.8 kcal/mole;Ea-Etoh = 5.3 kcal/mole), but it did not change the Ea for non-stimulated (basal) adenylate cyclase activity (Ea-control = 8.9 kcal/mole;Ea-Etoh = 9.8 kcal/mole). These results suggest that low concentrations of E facilitate the activation by DA of adenylate cyclase activity by enhancing the lateral mobility of the DA receptor. This is consistent with the idea that E produces some of its pharmacological effects by decreasing the viscosity of membranes and modifying the interactions between membrane proteins. (supported by AA 03504 and AA 07273)

104.13 THE EFFECT OF CHRONIC ALCOHOL CONSUMPTION ON NEUROTRANSMITTER SYSTEMS IN ALCOHOL-PREFERRING RATS. J.M. Murphy, W.J. McBride, D.J. Ciancone,*L. Lumeng* and T.-K. Li*, Depts. Psych. Med. & Biochem., Indiana Univ. Sch. of Med. & VA Medical Center, Indianapolis, IN 46223.

Selectively bred alcohol-preferring (P) rats voluntarily drink large quantities of alcohol that approach their daily metabolic capacity. In three experiments, the effects of chronic alcohol consumption on brain transmitter systems were assessed by comparing P-line animals maintained on water (P-W) with P rats allowed free choice between water and a 10% ethanol solution (P-EtOH) for 6-8 weeks.

In the first experiment, five P-W and eight P-EtOH rats were killed by the near-freezing technique; the brains were dissected into cerebral cortex, striatum, hippocampus, thalamus, hypothalamus, midbrain, pons-medulla, and cerebellum. The brain regions were then assayed for the content of GABA, glutamate, aspartate, and tryptophan. No significant differences occurred in any brain region between the P-W and P-EtOH groups for any of the amino acids measured. The results suggest that chronic EtOH consumption does not alter steady state CNS levels of the amino acid neurotransmitters and does not compromise dietary availability of tryptophan. The second experiment compared 10 P-W and 10 P-EtOH rats for CNS levels of norepinephrine (NE), dopamine (DA) and serotonin (5-HT), 3,4-dihydroxyphenylacetic acid (DOPAC) and 5-hydroxyindoleacetic acid (5-HIAA) in the cerebellum, hippocampus and hypothalamus. EtOH consumption had no effect on NE levels in the three regions and did not alter DA or DOPAC levels which were detected in sufficient amounts only in the hypothalamus. The levels of 5-HT and 5-HIAA were 5-20% lower for P-EtOH rats, but only 5-HIAA in the cerebellum (0.33 $\pm$ 0.02 nmoles/g tissue) differed significantly from the P-W group (0.42 $\pm$ 0.03 nmoles/g). The third experiment compared receptor binding to synaptic plasma membranes (SPM) from the telencephalon (TEL) and dienmesencephalon (D-M) of P-W and P-EtOH rats. The binding of [^{3}H]-5-HT, -dihydroalprenalol (DHA), and -spiroperidol were decreased 15-25% in the P-EtOH group relative to the P-W group while [^{3}H]-GABA binding was not affected by EtOH consumption. Binding of [^{3}H]-5-HT and -DHA to SPM from D-M was not significantly changed by EtOH consumption.

Taken together, the findings indicate that a major consequence of chronic ethanol consumption at the receptor level occurs in the TEL. Some evidence was also obtained to suggest that chronic EtOH consumption decreased 5-HT synthesis, release and/or turnover, as indicated by lower 5-HIAA in the cerebellum. (Supported in part by AA 003243, MH 00203, and Assoc. Adv. Mental Health Res. & Ed., Inc.).

104.14 MODULATION OF BENZODIAZEPINE AND GABA RECEPTORS BY BARBITURATES. R. J. Fanelli, M. J. Iadarola, W. A. Wilson* and J. O. McNamara (SPON: R. R. Miller). Departments of Medicine (Neurology) and Pharmacology, Duke University; and Epilepsy Center, VA Medical Center; Durham, NC 27705

We have examined the ability of a series of barbs to enhance 1) GABAergic recurrent inhibition and 2) GABA stimulated and basal BDZ binding.

Transverse 400μ slices of hippocampus were maintained in vitro and drugs were added to the bath. Extracellular field potentials were recorded from the pyramidal layer of CA1. The time course of recurrent inhibition was mapped by varying the latency between stimulation of the alveus and stratum radiatum. The amplitude of the orthodromic population spike was reduced if it fell into the inhibitory period of the recurrently evoked IPSP. Pentobarb (PB, 100μM) enhanced the efficacy and greatly prolonged the duration of recurrent inhibition. Modest effects were observed at 10μM and intermediate effects obtained at 50μM PB. Secobarb (SB) also caused a dose dependent enhancement and prolongation of recurrent inhibition. In contrast phenobarb (PhB) or diphenylbarbituric acid (DPB; 50-500μM) appeared to produce only a minimal enhancement of inhibition in our system; DPB being less effective than PhB. However doses of PhB or DPB in this same range were capable of altering abnormal discharges induced by bicuculline methiodide (50μM) indicating that the drugs were active in the tissue.

The effect of SB, PB, PhB, and DPB on BDZ ([^{3}H]-flunitrazepam) binding in the presence and absence of chloride ion (Cl^-, 100mM) and/or maximal concentrations of GABA (10μM) was studied in extensively washed freeze-thawed membranes from rat forebrain. BDZ binding could be stimulated by Cl^- alone (24% enhancement), GABA alone (49% enhancement), and in a dose-dependent fashion by either SB or PB (maximal effect 76% and 29% respectively). Stimulation of BDZ binding by SB and PB could be significantly enhanced by inclusion of GABA in the assay. In contrast, PhB and DPB alone and in the presence of GABA failed to augment BDZ binding. Also, Cl^- stimulated BDZ binding was markedly enhanced by SB and P B but not by PhB and DPB. These data demonstrate a striking correlation between the efficacy of barbs to enhance GABA-coupled BDZ binding as well as a GABA-mediated synaptic response and suggests that the molecular action may be related to the synaptic action. The slice experiments also suggest that, at least in this preparation, enhancement of GABAergic inhibition may not be essential for the anticonvulsant activity of the barbs.

104.15 NEURONAL PHOSPHOLIPID METABOLISM IN C57/6 MICE MADE PHYSICALLY DEPENDENT TO ETHANOL. Thomas L. Smith and Mary Gerhart*. Dept. Pharmacol., University of Arizona and Veterans Administration Medical Center, Tucson, AZ 85723.

Alteration of the physical state of neuronal membranes is believed to play a central role in the expression of functional tolerance and physical dependence to ethanol. Recent evidence suggests that these changes may occur in the lipid bilayer.

To test this hypothesis, mice were subjected to a liquid diet containing 7% (v/v) ethanol for 8 days. Mice were judged to be physically dependent by monitoring tremor and convulsions during withdrawal. At the time of sacrifice blood ethanol levels were > 250 mg%. Lipid analyses were performed on forebrain subcellular fractions (crude, myelin, light and heavy synaptosomes) isolated by discontinuous sucrose - density gradients. Lipids were extracted with chloroform - methanol (2:1) and in some cases were further separated by TLC. Neither total phospholipid content per mg protein nor the degree of phospholipid fatty acid saturation was altered in any of the above fractions. However, small increases in total cholesterol were observed in the crude synaptosomal fraction after ethanol treatment. This is in agreement with previous observations with DBA mice (Biochem. Biophys. Acta, 513:358, 1978).

In a series of in vitro experiments, neither basal nor muscarinic stimulation of ^{32}P incorporation into various phospholipids of light synaptosomes was affected by ethanol treatment. These results do not support the hypothesis that changes in phospholipid metabolism/levels or saturation of their fatty acids play a critical role in the development of physical dependence to ethanol. (Supported by Veterans Administration High Priority Alcohol Research Grant).

104.16 CALMODULIN ACTIVATED CA UPTAKE DURING ETHANOL TOLERANCE D.H.Ross,Div.Mol.Pharm.,Univ. Texas Health Sci.Center San Antonio,Texas 78284

Calcium ions are required for neuronal membrane stability and neurotransmitter release. The influx of calcium during depolarization and subsequent levels of free cytosolic calcium are buffered by a Ca ATPase and ATP dependent uptake of calcium by the membrane. This buffering mechanism is regulated by calmodulin(CM). Since ethanol has been shown to interfere with release of neurotransmitters and calcium movements across the membrane it was of interest to study the CM activation of Ca uptake during short and long term ethanol exposure. Male mice were exposed to a seven day ethanol-Sustacal(7%) drinking regimen. Behavior was measured by bar holding following a 1.5gm/kg dose of ethanol. Behavioral tolerance was 65% by day 4 and 100% by day 7. Synaptic membranes were prepared and ATP dependent Ca uptake measured by millipore filtration. Uptake was inhibited 35% on day 1, 22% on day 4, and 7% on day 7. Membranes were treated with EGTA to strip off CM and the uptake studies were repeated. CM addition to EGTA treated membranes increased uptake by 35% and 50% on day 4 and 7. These results demonstrate that Ca uptake is inhibited following one day of ethanol. This inhibition disappears by day 7 in parallel with the development of behavioral tolerance. CM addition to membranes produces maximal stimulation on day 7.These results suggest that during ethanol drinking, more calmodulin binds to the membrane such that by day 7 the membranes have increased CM content compared to pair fed controls. The increase in bound CM parallels the development of tolerance and may account for part of the adaptation to ethanol seen in nerve cells.
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104.17 FACILITATORY EFFECTS OF ETHANOL ON NEUROMUSCULAR TRANSMISSION IN INTERCOSTAL MUSCLE FROM PATIENTS WITH MYASTHENIA GRAVIS.

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Ethanol potentiates synaptic transmission at normal vertebrate neuromuscular junctions by both pre- and post-synaptic actions. It has been suggested that ethanol might improve the efficacy of neuromuscular transmission in diseases such as myasthenia gravis (MG) (Bradley, R.J., et. al., Nature 284:60, 1980) where an apparent curare-like block caused by antibody binding to the post-synaptic membrane impairs receptor function. We have investigated the facilitatory effects of ethanol on neuromuscular transmission in intercostal muscle from patients with MG.

Conventional, intracellular recording techniques were used to measure miniature end-plate potentials (MEPP) in in vitro experiments. MEPP amplitudes from myasthenic muscles were small, ranging from 0.1mV to 1.0mV. Ethanol concentrations of 0.01, 0.05, 0.1% (found in humans after alcohol consumption), 0.5 and 1.0% were applied to different muscle bundles and MEPP amplitudes and waveform characteristics were analyzed. At 0.05, 0.1, 0.5 and 1.0% ethanol, MEPP amplitudes were larger than in the control solution and the duration of the MEPP was increased. No increase in MEPP amplitude was seen with 0.01% ethanol but MEPP duration was slightly prolonged. The concentrations of ethanol used in this study appear to enhance the post-synaptic response to the neurotransmitter and thus antagonize the disease-induced neuromuscular blockade of MG.

105.1 ACTIONS OF IDENTIFIED ACETYLCHOLINE/PEPTIDE CONTAINING NEURONS IN REGULATION OF MOLLUSKAN FEEDING BEHAVIOR. A.O.D. Willows, P.E. Lloyd*, and B. Masinovsky*. Dept. of Zoology and Friday Harbor Laboratories, University of Washington, Seattle, WA 98195.

Feeding behavior in the gastropod mollusk, Tritonia diomedia is controlled principally by neurons of the buccal ganglia, some of which are large (100-200 um. dia.) and individually reidentifiable. Certain of these neurons control the initiation, and execution of a cyclic pattern of motor output which ressembles the pattern observed during feeding behavior (Willows, A.O.D., J. Neurophysiol. 44:849). One pair of buccal ganglion neurons, the large dorsal white cells (LDWC, since re-named B11) contain a neuroactive peptide which has been isolated, and purified. We have found recently that this same neuron pair also contains the neurotransmitter, acetylcholine(Lloyd, P.E., et. al. Soc. Neurosci. Abst. this volume). Thus it is of increasing interest to know the site and mechanisms of action of these neurons.

The B11's are electrically active during spontaneously occurring or electrically driven episodes of the cyclic motor output in buccal ganglion neurons. Recordings of co-ordinated bursting activity in B11 and other neurons reveal low frequency bursts of impulses (3-7 Hz.) in the B11's phase coupled to the output of the motor pattern generator. Increases in the frequency and number of endogenously occurring impulses in B11 are accompanied by similar increases in motor neurons and in the amplitudes of the motor output measured with a phototransducer. Brief stimulation of both B11's simultaneously elicits first a polysynaptic excitatatory synaptic volley, and then one or more complete cycles of motor output. In spontaneously active preparations, stimulation of B11's results in prolonged enhancement of the bursting output. The excitation of the motor pattern generating system by B11 is apparently not mediated by electrical junctions because simultaneous strong hyperpolarization of B11 bilaterally has no detectable influence on the frequency of bursting output from spontaneously active motor neurons.

Earlier, we showed that the peptide from B11 directly applied to the buccal ganglia elicits or (in spontaneously active preparations) enhances the cyclic motor output (Lloyd P.E. and Willows, A.O.D., Soc. Neurosci. Abst. 240.9, 1980). As yet we have not determined what, if any, role acetylcholine plays in this control mechanism. However, B11 and its central and peripheral targets offer an unusual opportunity to determine whether there is an interaction between this neural peptide and the acetylcholine originating in the same neuron.

105.2 DIFFERENTIAL ACTION OF CHOLINERGIC AGENTS ON THE BODY WALL MUSCULATURE OF A SABELLID WORM. L.Córdova*and C. Escalona de Motta.Lab.Neurobiology UPR Sch. Med.San Juan,PR 00901.

The somatic muscles of annelid worms exhibit multicomponent contractile responses to ACh and related agonists(Alvarez et al,Comp.Biochem.Physiol. 29:931,1969). This could imply the existence of cells with different pharmacological properties in apparently homogeneous muscles. A pharmacological analysis of the responses displayed by strips of dorsal longitudinal muscles of S. magnifica was undertaken to explore this possibility. Strips 2-3cm long were isolated from fresh specimens and placed in a sea water chamber at 26°C to record isometric tension. Addition of 10^{-6} to 10^{-4} w/v ACh evoked a 'fast'contraction (time to peak:44s^{+} 4s, SE,n=38) that returns slowly to resting tension with an average half time of 158s^{+} 15s,SE,n=31. In 10^{-6}w/v d-tubocurarine(dtC) contraction time is slightly increased but in the presence of $2x10^{-6}$w/v α bungarotoxin (αBuTX) no change occurs.$2x10^{-7}$to $5x10^{-5}$w/v nicotine elicits a biphasic response: an early relaxation followed, at concentrations above 10^{-5}, by a slow contraction that reaches a maximum in 10 to 25 min. dtC blocks the contraction phase without affecting the relaxation while αBuTX does not affect either phase. No early relaxation component was revealed in the response to ACh by 10^{-6}w/v atropine. Methacholine, a classical muscarinic agent, produces biphasic responses similar to nicotine. Neither of these agents alone is able to evoke responses with the same time course as those initiated by ACh in this preparation.

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105.3 PEPTIDE MODULATION OF THE ISOLATED APLYSIA HEART. Sandra Wernham and Ken Lukowiak*. Dept. of Medical Physiology, Faculty of Medicine, University of Calgary, Calgary, Alberta, Canada T2N 1N4.

In the past 20 years investigations performed on the molluscan cardiovascular system and its central control have yielded some valuable information in relation to cardioactivity not associated with the usual neurotransmitters serotonin(5HT), acetylcholine(Ach) and catecholamines. Although most of this cardioactivity has been characterised as resulting from endogenous peptide extracts, with few exceptions further identification of these peptides and their mode of action remain to be determined.

In the marine mollusk, Aplysia californica, however, the presence in the anterior ganglia of an arginine vasotocin (AVT)-like neuropeptide has been positively identified (Moore et al, Brain Res., 206:213-218, 1981). We have shown that AVT has both long-term positive chronotropic and inotropic effects on the isolated Aplysia heart at concentrations as low as 10^{-14} M. In comparison, perfusion of 10^{-12} M AVT is sufficient to increase basal heart rate to the same level achieved by a 10^{-9} M 5HT perfusion of the same heart tissue. The inotropic effects produced by AVT are both cummulative and of greater amplitude than those produced by 5HT. Further, the effects produced by AVT are not immediate - they have a consistent latency of 10$\pm$1 mins; and both the chronotropic and inotropic effects persist for hours following washout compared to minutes as found for 5HT.

Preliminary evidence also suggests that dopamine, met-encephalon and angiotensin-II may have varying degrees of long-term effects as well on heart behavior when applied directly to the isolated Aplysia heart at similar concentrations as those found for AVT. The effects of these and other neuropeptides, therefore, will also be discussed in comparison to similar, shorter-term effects produced by 5HT and Ach, known molluscan cardioregulators (Liebeswar et al, J. Neurophysiol., 38:767-779, 1975).

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105.4 ENDOGENOUS HISTAMINE IN THE LOBSTER STOMATOGASTRIC NERVOUS SYSTEM. Brenda J. Claiborne. Department of Biology, Univ. of Calif., San Diego, La Jolla, CA 92093

Physiological and pharmacological studies suggest that histamine may function as a neurotransmitter in the lobster stomatogastric ganglion (Claiborne, B., Neurosci. Abstr., 6:626, 1980). The thirty neurons in the stomatogastric ganglion control the rhythmic movements of the lobster stomach and receive inputs from neurons located in other ganglia of the stomatogastric nervous system. I report here the presence of endogenous histamine in the ganglia and nerves of the stomatogastric nervous system of the spiny lobster, Panulirus interruptus.

Histamine was assayed according to the procedure of Weinreich, Weiner and McCaman (Brain Res., 84:341, 1975). Extracts of individual nerves or ganglia were incubated at 37°C for 30 minutes with ^{3}H-S-adenyl-L-methionine and histamine methyltransferase in phosphate buffer. The reaction was stopped by the addition of NaOH, the ^{3}H-methylhistamine was extracted into chloroform, and, following two washes with NaOH, aliquots of the organic phase were counted in a liquid scintillation counter. The results indicate that histamine is distributed throughout the system, but not uniformly: the stomatogastric ganglion contains 115 pmoles of histamine per mgram of tissue protein, the commissural ganglia contain 60 pm/mg, and the esophageal ganglion contains 12 pm/mg. The nerves connecting these ganglia contain between 3 and 11 pm/mg, and the motor nerve exiting the stomatogastric ganglion contains 5 pm/mg. These histamine levels are similar to those found in ganglia and nerves of the mollusc Aplysia, for which there is extensive evidence that histamine functions as a neurotransmitter.

Preliminary data from assays of individual cell bodies and the neuropil region of the stomatogastric ganglion indicate that the histamine present in the stomatogastric ganglion is located in the neuropil region and is not present in neuronal cell bodies. Further, results from nerve ligation experiments suggest that histamine is transported into the stomatogastric ganglion from neuronal somata located in other ganglia. Current work is therefore focused on locating histamine-containing neurons in the commissural, esophageal or supraesophageal ganglia, all of which are known to send inputs to the stomatogastric ganglion.

105.5 SYNTHETIC OCTOPAMINE AGONISTS: STRUCTURE-ACTIVITY RELATIONSHIPS OF THE FORMAMIDINE PESTICIDES. James A. Nathanson. Depts. of Neurology and Pharmacology, Harvard Medical School, Mass. General Hospital, Boston, Massachusetts 02114.

Octopamine appears to be an important neurotransmitter and neuromodulator in invertebrates. Electrophysiological studies suggest that more than a single receptor for octopamine may exist, and biochemical experiments indicate that at least some octopamine receptors may activate adenylate cyclase. A significant handicap in the investigation of octopaminergic function has been a lack of potent octopamine analogs. However, a recent report (Nathanson and Hunnicutt, Mol. Pharmacology, in press) indicates that the formamidine pesticide, demethylchlordimeform, is a potent activator of octopamine-sensitive adenylate cyclase in the firefly light organ (a tissue rich in octopamine-sensitive adenylate cyclase). We now find that several other formamidine analogs are also potent octopaminergic compounds.

Octopamine-sensitive adenylate cyclase activity was measured in washed particulate preparations of firefly light organs. The assay system contained 80mM Tris-maleate, pH 7.4; 10mM theophylline; 8mM $MgCl_2$; 0.1mM GTP; 0.5mM EGTA; and 2mM ATP, plus or minus drug. The formamidines tested were chlordimeform (CDM); N-demethylchlordimeform (DCDM); N,N-didemethylchlordimeform (DDCDM); BTS 27271 (DCDM with methyl substituted for chloro); and two symmetrical triazapentadienes, BTS 27419 and BTS 23376, with structures derived from BTS 27271 and DCDM, respectively.

Compared with octopamine, all compounds with the exception of CDM (K_a = 30 micromolar; Vmax = 9% of octopamine) were more potent than octopamine. DCDM (K_a = 2.6 micromolar) and its symmetrical analogue, BTS 23376, were both about 6 to 10 times more potent than octopamine. The dechloro methylated derivatives, BTS 27271 (K_a = 8 micromolar) and BTS 27419 (K_a = 10 micromolar, were about 4 times less potent than DCDM. DDCDM was considerably more potent than CDM but less potent than DCDM, indicating that, as with octopamine, synephrine and N,N-dimethyloctopamine, the N-monomethyl analogue is the most potent. All of the formamidines except CDM showed a Vmax of between 70 and 90% that of octopamine. In other experiments, DCDM and CDM were found to have little effect on either dopamine- or isoproterenol-activated adenylate cyclase. The results indicate that the formamidines are very potent octopamine analogs which appear to have a degree of specificity for certain octopamine receptors.

105.6 DOPAMINE ACTION ON THE CRUSTACEAN CARDIAC GANGLION. M. W. Miller*, R. E. Sullivan, J. A. Benson* and A. Berlind (Spon: D. Russell) Bekesy Laboratory of Neurobiology, University of Hawaii, Honolulu, HI 96822

Dopamine (DA) has been shown to be present in the neurosecretory pericardial organs of decapod crustacea. We have examined the mechanisms by which this catecholamine exerts its effects upon the semi-isolated cardiac ganglion, an autonomously rhythmic 9-cell neural network which controls the heartbeat. Dopamine ($5X10^{-8}$ M - $5X10^{-6}$ M) has strong excitatory effects on the cardiac ganglion of the crab Portunus sanguinolentus. It increases the burst rate, prolongs burst duration, and increases the number of impulses per burst. Moreover, DA appears to have an 'organizing' effect on ganglia in which bursting is irregular.

Experiments designed to determine the locus of DA action indicate that its principle effects are on the small "pacemaker" cells. In experiments utilizing a two-pool chamber, DA effects were limited to applications to the posterior small cell region. When DA is applied to the whole ganglion, its enhancement of small cell intraburst spike frequency is especially pronounced. Also, in ligatured ganglia in which small cell activity could be recorded via electrotonic coupling with a posterior large cell, DA was noted to exert strong excitatory effects on small cells while having minimal effects on anterior large cells.

One means by which dopamine could have such excitatory action is via an effect upon the driver potential (DP), a slow (200-300 msec), Ca^{++}-dependent (TTX-resistant), voltage- and time dependent potential which underlies burst formation in the cardiac ganglion (Tazaki & Cooke, 1979). In the presence of TTX ($3X10^{-7}$ M), DA produces small (1-5 mV) repetitive potential oscillations in large cell recordings in Portunus. The occurrence of these oscillations is limited to posterior application in two-pool experiments and their time-course and form suggest that they are electrotonically conducted small cell DP. Although DA has minimal effects on the form of the DP of large cells, it does slightly decrease the threshold for evoked large cell DP and cause a small decrease in the apparent large cell input resistance.

These experiments suggest 1) that the excitatory actions of dopamine on the crustacean cardiac ganglion are primarily produced by effects upon the small cells and 2) that these effects are due to enhancement of DPs and/or lowering of threshold for DP generation in these cells.

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105.7 METACEREBRAL GIANT CELL AND SEROTONIN EFFECTS ON SALIVARY ACINAR CELLS IN THE TERRESTRIAL MOLLUSK, LIMAX MAXIMUS. Jonathan Copeland. Department of Zoology, University of Wisconsin-Milwaukee, Milwaukee, Wisconsin 53201.

A re-identifiable serotonergic neuron, one in each cerebral ganglion, has been found in a number of different species of gastropod mollusks. This neuron, called the metacerebral giant cell (MGC) in Limax maximus, produces conventional and modulatory effects at buccal ganglion synapses, modulatory effects at buccal mass neuromuscular junctions and muscle, and an overall facilitory effect on the neural networks that underlie feeding. I have found that the MGC and serotonin also have a modulatory (excitatory) effect in Limax at an unusual type of neuroeffector, secretory epithelium.

Limax possesses paired salivary glands (SGs), each innervated by a salivary nerve (SN) which arises from the ipsilateral buccal ganglion (BG), paired BGs, and paired cerebral ganglia. Previous work has shown that each SN contains several thousand axons. One axon is that of the MGC, and two axons are from the paired bilateral salivary neurons (BSNs). One BSN soma is found in each BG, and each BSN has an axon in the ipsilateral and contralateral SN. BSN is a secretomotor neuron and it innervates less than 10% of the secretory salivary acinar cells (SACs). BSN-evoked EJPs can be recorded from these SACs, and these occasionally give rise to action potentials. BSN is an autoactive burster neuron with slow, regular bursts. Intracellular stimulation of MGC delays or accelerates BSN burst frequency.

Cobalt nitrate fills reveal that the axon of the MGC travels the length of the ipsilateral SN and enters the SG. Intracellular stimulation of MGC (10 spikes/2sec.) produces changes in BSN-evoked EJPs recorded in SACs. Both EJP amplitude and EJP number increase within seconds of MGC stimulation and remain elevated for up to 14 seconds. SAC potential hyperpolarizes slightly (2 - 5 mv) and input resistance increases (< 43%) within 2 seconds of MGC stimulation, and these effects last for 30 seconds. Superfusion of an isolated SG with a saline containing 10^{-9} M serotonin produces a small (4 - 6 mv) depolarization of the SAC resting potential, an increase in input resistance, and an increase in excitability. After treatment with serotonin for 5 minutes, SACs, previously silent in response to current injection, now produce action potentials when stimulated. SACs respond to superfusion of an elevated (10^{-3} M) serotonin-containing saline and intracellular current injection (1 nanoampere) with a tonic train of action potentials.

Collectively, these results are consistent with the hypothesis that the MGC and serotonin modulate the excitability of a secretory epithelium.

105.8 OCTOPAMINE AND DOPAMINE EVOKE VOLTAGE-DEPENDENT INWARD CURRENTS IN APLYSIA. T. Pellmar. Lab. Preclin. Stud. NIAAA, Rockville, MD 20852.

In LB and RB neurons of the abdominal ganglion of Aplysia californica, serotonin elicits a slow inward current which is present only at potentials more depolarized than -40mV and has a maximum amplitude near 0mV. To determine if this voltage-dependent response was unique to serotonin, several transmitters were tested in these two cell clusters. Dopamine (in LB cells) and octopamine (in both RB and LB cells), but not histamine, acetylcholine, gamma aminobutyric acid or glutamate, elicit a similar current. Like the serotonin-evoked response, the slow inward currents resulting from iontophoretic application of octopamine and dopamine reach a maximum amplitude in 10 to 20 seconds and last at least one minute. The currents are insensitive to changes in extracellular potassium concentration, persist after replacement of sodium with bis tris propane, are not reduced by a decrease in chloride concentration and are blocked by 2 mM cadmium. Because of the similarities in voltage-dependence, time course and ionic sensitivities, the responses to octopamine, dopamine and serotonin are concluded to have the same ionic mechanism. Associated with the voltage-dependent response to serotonin, appears to be a broadening of the action potentials recorded in the presence of 50 mM tetraethylammonium. Like serotonin, octopamine is capable of increasing spike duration in LB cells.

Because intracellularly injected cyclic AMP elicits an identical voltage-dependent current in LB and RB cells (Pellmar, Cell. and Molec. Neurobiol., 1981), it is tempting to suggest that the currents evoked by serotonin, dopamine and octopamine are all mediated by the cyclic nucleotide. Yet pharmacological data for the response to serotonin tend to dispute this hypothesis. Agents which alter cyclic AMP metabolism do not affect the serotonin-induced current in the predicted way. Although it seems unlikely that current evoked by serotonin is mediated through cyclic AMP, it is possible that the responses to octopamine and/or dopamine are activated through the second messenger. This would result in an interesting situation where two transmitters would elicit nearly identical responses through very different intracellular mechanisms.

105.9 (^{3}H)SPIROPERIDOL BINDING IN CILIATED TISSUES OF THE MARINE BIVALVE MYTILUS CALIFORNIANUS. J. R. SMITH* (SPON: C. SCHASTEEN). Marine Sci. Center, Oregon St. Univ., Sch. of Pharm., Corvallis, OR 97331.

Pharmacological, biochemical, and histochemical evidence suggests that the endogenous substance dopamine (DA) acts as a neurotransmitter in marine mussels in a cilioregulatory capacity (Malanga, C.J., Comp. Biochem. Physiol., 51C:25-34, 1975). In an effort to further clarify the role of DA as a neurotransmitter in this species we have carried out a series of experiments designed to identify and characterize putative DA receptor sites in ciliated tissues. The DA antagonist (^{3}H)spiroperidol (SPDL) (New England Nuclear; specific activity=25.7 Ci/mmole) was used as the radioligand in all experiments. Tissue suspensions were prepared in a manner similar to that previously reported by Burt et al., (Mo. Pharmac., 12:800-812, 1976). Incubation buffer consisted of 50mM Tris, 0.1% ascorbic acid, and 10μM pargyline (pH=7.4 at 4°C). Samples were incubated for 60 min. in an ice bath after which they were filtered through a glass fiber filter and given 3 (5ml) washes under vacuum pressure. The protein concentration in each tube ranged from .1 to .2 mg and the (^{3}H)SPDL concentration was .2nM. All samples were run in triplicate and data from 2-6 separate experiments were pooled for estimates of K_D, B_{max}, and IC_{50}. Total (^{3}H)SPDL binding never exceeded 10% of the total (^{3}H)SPDL added. All competition experiments utilized 6-8 concentrations of competing agents. Specific binding of (^{3}H)SPDL was defined as the difference between the amount of (^{3}H)SPDL bound in the absence of unlabeled SPDL and that bound in the presence of excess SPDL (10^{-6}M). (^{3}H)SPDL binding to gill tissue homogenates was found to be saturable with an apparent K_D of 0.6nM and a B_{max} of 92pmoles/gm protein. Competition experiments using d and l-butaclamol show that the pharmacologically active d-form had an IC_{50} of 1.5nM while the l-form showed no competition up to a 10^{-6}M concentration. DA (up to 10^{-4}M) also failed to compete for (^{3}H)SPDL binding sites. Serotonin also showed no competition up to a 10^{-6}M concentration. The addition of 440mM NaCl (mussel body fluid concentration) to the incubation media shifted the K_D for (^{3}H)SPDL to 0.1nM while the addition of 9.5mM $CaCl_2$ resulted in a rise in K_D to 80nM. We conclude that gill tissue of Mytilus c. has high affinity binding sites which appear to be highly specific for dopamine antagonists. This offers further support for the theory that DA acts as a neurotransmitter in ciliated tissue of bivalves and indicates that gill tissue may be a valuable source of a relatively pure DA-receptor type. This study was funded by PHS grant ES01926.

105.10 PROTEIN BIOSYNTHESIS IN THE X-ORGAN-SINUS GLAND COMPLEX OF DECAPOD CRUSTACEANS. E. Stuenkel* (Spon: C. Zomzely-Neurath). Bekesy Laboratory of Neurobiology, University of Hawaii, Honolulu, HI 96822.

The x-organ-sinus gland (XOSG) peptide neurosecretory system located in the eyestalk of decapod crustaceans is morphologically and functionally subdivided into a cell soma biosynthetic region, an axonal transport and processing portion and a discrete nerve terminal storage and release area. The XOSG from the land crab Cardisoma carnifex was utilized for in vitro radiolabeling studies on crustacean neuropeptide biosynthesis. In vitro studies designed to determine the time dependence of tritiated leucine (20 µM) uptake into the x-organ indicate ^{3}H-leucine accumulates and reaches a plateau in approximately 3 hours. Incorporation of ^{3}H-leucine into protein is directly proportional to incubation time for up to 7 hours. Additionally, radiolabeled proteins produced in the x-organ are axonally transported to the sinus gland as determined in preparations with intact versus ligated XOSG nerves.

In all experiments ^{3}H-leucine incorporation into protein was determined by analyzing counts remaining at the origin after high voltage paper electrophoresis (50-60 V/cm) at pH 1.9 for 1-2 hours. The protein nature of origin counts was confirmed by acid hydrolysis and subsequent electrophoresis. ^{3}H-leucine incorporation into origin counts was reduced 90% by puromycin, an inhibitor of eukaryotic protein synthesis. Moreover, origin counts eluted in the void volume of Sephadex G-25 and Biogel P-2, suggesting a molecular weight greater than 2500. A 5 hr pulse 24 hr chase paradigm (at 20°C) was found to optimize conditions for examining this high MW protein fraction. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) of non-radiolabeled XOSG revealed at least two distinct protein bands of <10,000 MW which are specific to the XOSG neurosecretory system. One of the bands (MW ≃ 6000) has previously been identified as hyperglycemic hormone by other workers and represents 10% of the total sinus gland protein. Electrophoretic examination of extracts of ^{3}H-leucine incubated XOSGs using SDS-PAGE revealed at least three distinct peaks of radiolabel incorporated into protein. One of these peaks corresponds with a band of approximately 6,000 MW which exhibits hyperglycemic hormone activity. This appears to be the first demonstration of in vitro biosynthesis of an identified peptide neurohormone in the arthropods. Supported by NIH grant NS15453 to I. M. Cooke.

106.1 DIFFERENTIATION OF RAT BRAIN GABA RECEPTOR SITES. M. Browner*, J. W. Ferkany*, and S.J. Enna (SPON: S.J. Strada). Depts. of Pharmacology and Neurobiology, Univ. Texas Med. Sch., Houston, Texas 77025.

Using in vitro ligand binding assays, studies were undertaken in an attempt to identify pharmacologically and functionally distinct receptor binding sites for γ-aminobutyric acid (GABA) in rat brain membranes. The results indicated that there were regional differences in the potency of bicuculline, a GABA receptor antagonist, to inhibit specifically bound ^{3}H-GABA. Bicuculline was most active in midbrain and cerebral cortex, while being significantly weaker in the corpus striatum and cerebellum. In the presence of 50mM ammonium thiocynate (SCN), the potency of bicuculline to inhibit specific ^{3}H-GABA binding was increased 10-100-fold in the brain regions studied. The possible biological relevance of this finding was indicated by the fact that 50mM SCN also increased the potency of bicuculline to inhibit GABA-activated benzodiazepine (BZ) receptor binding, a biochemical measure of GABA receptor function. In contrast, SCN did not affect the potency of GABA to activate BZ binding. Analysis of GABA receptor site saturation data indicated that SCN treatment selectively abolished the high affinity binding site for this amino acid, without altering the low affinity component. These findings suggest the existence of GABA receptor sites having a differential sensitivity to bicuculline and they provide direct evidence to support the hypothesis that only low affinity GABA binding sites are linked to the BZ receptor. Thus, SCN may be useful in identifying and defining pharmacologically and functionally different GABA receptor sites. (Supported in part by USPHS grants NS-13803, NS-00335 and MH-07688).

106.2 SOLUBILIZATION OF THE PICROTOXININ RECEPTOR: EVIDENCE THAT PICROTOXININ AND DIAZEPAM DO NOT BIND TO THE SAME PROTEIN. W.C. Davis* and M.K. Ticku. Dept. Pharmacology, University of Texas Health Science Center, San Antonio, TX 78284

The binding sites for [^{3}H]-α-dihydropicrotoxinin (DHP), which is a radioligand for the picrotoxin-sensitive site at the benzodiazepine-GABA receptor-ionophore complex, were solubilized from rat brain with 1% Lubrol. Lubrol-solubilized fraction bound [^{3}H]-DHP to two sites (K_{D1}=0.038 μM; K_{D2}=1.85 μM), in contrast to only one site observed for membrane receptors (Ticku et al, Mol. Pharmac. 14:391, 1978). DHP binding to the crude Lubrol-solubilized fraction was inhibited by depressant and convulsant drugs with potencies similar to those required to inhibit DHP binding to membrane receptors. The signal-to-noise ratio of DHP binding was significantly improved from 0.14 in membranes to 0.35 in the Lubrol fraction. Besides [^{3}H]-DHP binding, specific binding of [^{3}H]-diazepam and [^{3}H]-muscimol was present in the crude Lubrol fraction. Furthermore, GABA agonists (muscimol), pentobarbital and ethanol produced a dose-dependent enhancement of [^{3}H]-diazepam binding in the Lubrol fraction. Pentobarbital inhibited the binding of [^{3}H]-DHP in the Lubrol fraction. These results indicate that under the conditions used for this study, Lubrol solubilizes the benzodiazepine-GABA receptor-ionophore complex. Gel filtration of the Lubrol-solubilized fraction revealed that [^{3}H]-DHP and [^{3}H]-diazepam bind to two distinct fractions, with apparent molecular weights of 185,000 and 61,000 daltons, respectively. [^{3}H]-DHP bound to the 185,000 dalton fractions with two binding constants and [^{3}H]-flunitrazepam bound to the 61,000 dalton fraction to a single site. [^{3}H]-Muscimol and [^{3}H]-GABA binding (∿90%) co-migrated with the 185,000 dalton fraction, and approximately 10% was associated with the 61,000 dalton fraction. Muscimol, pentobarbital and ethanol, while enhancing [^{3}H]-diazepam binding to membrane and crude Lubrol-solubilized fractions, failed to enhance [^{3}H]-diazepam binding to the 61,000 dalton fraction. Pentobarbital produced a dose-related inhibition of [^{3}H]-DHP binding to the 185,000 dalton fraction with an IC_{50} value of 60 ± 12 μM. Binding of [^{3}H]-DHP to the 185,000 dalton fraction was inhibited by several depressant and convulsant drugs (e.g. barbiturates) which affect GABAergic transmission. These results provide evidence that picrotoxinin and diazepam bind to two distinct proteins. Furthermore, these results support our earlier contention (Ticku and Olsen, Life Sci. 22:1643, 1978; Ticku, Br. Res. Bull. (Suppl. 2) 5:919, 1980) that the picrotoxinin site may be a receptor site for depressant and convulsant barbiturates.

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106.3 IN VITRO BINDING STUDIES WITH [^{35}S] CYSTEINE TO RAT SYNAPTIC MEMBRANES. C. H. Misra, R.C. Smith and J.C. Schoolar. Section of Biological Psychiatry, Texas Research Institute of Mental Sciences and Department of Pharmacology, Baylor College of Medicine, Houston, TX 77030.

Cysteine was shown to have neurotoxic property (Olney, J.W. et al, Brain Res. 5: 309, 1972) like other exicitatory amino acids (Curtis, D.R. and Watkins, J.C., J. Physiol. 166, 1, 1963), glutamic and asparatic acids. However, recently it has been shown that cysteine has a unique high affinity uptake system in a synaptosomal preparation of rat cerebral cortex (Misra, C.H., J. Neurosciences Res. 5, 507, 1980; Wang and Seigal, J. Neurochem 33, 1303, 1979) which is considered one of the characteristic properties for amino acid neurotransmitters (Logan, W.J. Snyder, S.H., Brain Res. 42, 413, 1972). Now we have found that [^{35}S] cysteine can bind to the synaptic membrane preparation from rat cerebral cortex, in a very elegant fashion. Binding of [^{35}S] cysteine (0.254 Ci/mmole, Amersham Searle) to synaptic vesicles fraction from rat cerebral cortex was studied by incubating 0.1 μm [^{35}S] cysteine for 5 min. at 0°C and then finally terminating by filtration through GF/B glass fiber filters. Non-specific binding was estimated with 20 mM non-radioactive cysteine. The binding was greater in buffer (50 mM Tris-HCl, pH 7.0 containing KCl, $MgCl_2$ and $CaCl_2$) containing sucrose instead of Na^+ in the same molarity. Binding was found satusable between 0.4 to 1.5μm concentration of [^{35}S] cysteine; and a K_n, 1.0μm and B_{max} 250 P-mole/ mg of protein were estimated in rat cerebral cortex membrane. About 90% of bound [^{35}S] cysteine was detected unmetabolized by TLC, using a solvent butanol-acetic acid water (120: 30:50 v/v) after reacting with N-ethyl-maleimide. As with other amino acid ligands, the binding of cysteine reaches equilibrium very quickly. DL-homocysteine, and L-glutamic acid were weak inhibitors while N-ethyl-L-cysteine and butaperazine were most potent inhibitors of [^{35}S] cysteine binding.

106.4 ^{3}H-NIPECOTIC ACID (Nip) SPECIFICALLY IDENTIFIES GABA UPTAKE SITES IN MEMBRANES PREPARED FROM CEREBRAL TISSUE. K.G. Lloyd and F. Vargas, LERS, 31 Ave. P.V. Couturier, F. 92220 Bagneux, France.

Nip is reported to be a relatively specific inhibitor of GABA uptake in rat brain slices (Krogsgaard-Larsen and Johnston, J. Neurochem., 25, 797, 1975). As this compound is available commercially at a relatively high specific activity (Amersham, 41 Ci/mMole) we have attempted to use ^{3}H-Nip as a radioligand for the GABA neuronal uptake site. In brief, fresh or frozen rat brains were homogenized (Polytron, 1:20, w/v) in Tris-citrate buffer (pH 7.4, 50 mM, 4°C), centrifuged at 50,000 x g x 10 min and the pellet washed thrice in buffer. The final pellet was resuspended in buffer containing 100 mM NaCl and an aliquot was exposed to predetermined concentrations of ^{3}H-Nip for 12 min. The binding was terminated by centrifugation (20 min x 50.000 x g) followed by a rinse of the pellet with buffer. "Blanks" to account for non-specific binding consisted of all components plus 5 mM GABA. The protein was dissolved with Soluene and the radioactivity determined in Demilume. Saturation curves (0.1 - 100 μM ^{3}H-Nip) showed a single Kd of 12 μM for fresh, and 5 μM for frozen tissue, with a Bmax of 100 pmol/mg. prot. for fresh and 75 pmol/mg. prot. for frozen tissue. ^{3}H-Nip (5 μM) was displaced from frozen tissue by (IC50's in parentheses) : Nip (9 μM) ; GABA (4 μM); Chlorpromazine (160 μM) ; diaminobutyric acid (DABA, 150 μM) ; muscimol (1600 μM) but not by beta-alanine, THIP or isoguvacine (all with IC50's greater than 5 mM). Boiled membranes did not exhibit any specific binding for ^{3}H-Nip. These results parallel similar studies on : (1) ^{3}H-Nip uptake into purified synaptosomes from rat brain : Km = 4.2 μM, Vmax = 304 pmol/mg. prot./min ; IC50's = Nip, 5,5 μM ; GABA 4 μM ; chlorpromazine, 30 μM ; DABA, 85 μM ; beta-alanine, 1300 μM; isoguvacine, and THIP inactive at 2 mM ; (2) ^{3}H-GABA uptake into purified rat brain synaptosomes : Km = 3.4 μM, Vmax = 570 pmol/mg. prot./min ; IC50's = Nip, 4.5 μM ; GABA, 5 μM; chlorpromazine, 30 μM ; DABA, 75 μM, beta-alanine, 1100 μM; isoguvacine and THIP were inactive at 1 mM. Both ^{3}H-Nip and ^{3}H-GABA uptake were temperature and sodium dependent, were sensitive to osmotic shock and to ouabain. Thus, these studies indicate that ^{3}H-Nip specifically identifies the GABA uptake site in either fresh or frozen membranes or in cerebral synaptosomal preparations.

106.5 CHARACTERISTICS OF Na^+ AND Cl^--DEPENDENT BINDING OF ^{3}H-GABA (γ-AMINOBUTYRIC ACID) TO GABA TRANSPORT SITES. Diana N. Krause, Esther Wong* and Eugene Roberts. Division of Neurosciences, City of Hope Research Institute, Duarte, CA 91010.

Na^+-dependent GABA binding sites are thought to reflect the recognition sites for GABA transport systems. We have been characterizing these sites in mouse brain microsomal membrane fractions that were well-washed, osmotically-shocked, and stored frozen in liquid nitrogen with no loss of binding capacity. ^{3}H-GABA binding, which was assayed at 0°C by filtration, was found to have a definite requirement for Cl^- as well as Na^+. Hill plot analyses indicated that at least two molecules of Na^+ and one of Cl^- are necessary at each site. Divalent cations inhibited Na^+, Cl^--dependent ^{3}H-GABA binding with uranyl being the most potent cation tested (IC_{50} = 0.6 mM) followed in decreasing potency by Ca^{2+}, Sr^{2+}, Mn^{2+} and Mg^{2+}. Preincubation of the membranes with Na^+ (but not Cl^-) also decreased binding. Ouabain had no effect, but the Na^+ ionophores gramicidin, monensin and nigericin all inhibited association of ^{3}H-GABA with the membrane fraction. Tributyltin, which can act as a Cl^- ionophore, also inhibited binding, suggesting that Na^+ and Cl^- gradients may be involved in the process under study. However, no effects were observed in attempts to reverse the gradients by subsequently incubating the ^{3}H-GABA-associated membranes in lowered Na^+ and Cl^- concentrations. Bound ^{3}H-GABA also did not appear to be sensitive to hypo-osmotic conditions. The nature of the Na^+, Cl^--dependent "binding," including such possibilities as ^{3}H-GABA accumulating into osmotically-insensitive particles or partitioning into the membrane itself, is being investigated. Supported by USPHS grant NS-12116 and Nelson Research and Development Company.

106.6 AN INTRACELLULAR ANALYSIS OF AN UPTAKE SYSTEM FOR GABA AND ITS ANALOGUES AT THE CAT DORSAL ROOT GANGLION (DRG). Joel P. Gallagher, Jun Nakamura* and Patricia Shinnick-Gallagher, Dept. of Pharmacology and Toxicology, Univ. Texas Med. Br., Galveston, TX 77550.

We are using the cat DRG as a model system to study the pharmacology of a mammalian GABA receptor with electrophysiological techniques. We have previously demonstrated that GABA produces a Cl^--dependent depolarization of these neurons. Although a GABA synapse is not present, there have been conflicting reports as to whether or not an active uptake system for GABA is present in these neurons. To test this possibility we applied several GABA analogues by bath superfusion and/or iontophoresis to isolated DRG and recorded the resultant membrane changes. We determined complete concentration (10^{-6} to 10^{-2}M) response curves for muscimol (M), GABA, and 3 aminoproprane sulfonic acid (3-APS) under voltage clamp conditions. Muscimol was the most potent at all concentrations, however at concentrations greater than 10^{-4}M, GABA was more potent than 3-APS while at concentrations less than 10^{-4}, 3-APS was more potent than GABA. Using a low Na^+-Krebs solution or the GABA uptake inhibitor, nipecotic-acid, produced a shift in the GABA concentration curve to the left and upwards throughout its concentration range. It appears that at concentrations less than 10^{-4}M, GABA was taken up by glial cells in the DRG whereas 3-APS which is not a substrate for glial uptake was effective. Other GABA agonists whose action could be facilitated during uptake inhibition included isoguvacine and SL75102. Thus, we have concluded that there is an active uptake process for GABA in the cat DRG and this process must be considered when evaluating drugs at the DRG. Supported by NIH Grant NS 13727.

106.7 REGIONAL GABA BINDING DIFFERENCES BETWEEN AGGRESSIVE AND NON AGGRESSIVE HAMSTERS. A. Perumal, M. Potegal, A. Barkai, G. Cannova* and A. Blau*. N.Y. State Psychiatric Institute, New York, NY 10032.

Previous pharmacological and neurochemical studies suggest that the aggressive behaviors of mice and rats may correlate with endogenous GABA activity (Mandel, et al, 1979). However, the specificity of this relationship is not entirely clear. We pursued this question in a study of GABA mechanisms in ovariectomized female hamsters selected for either high or low aggressiveness. Neuroanatomical specificity was established through comparisons of regional rather than whole brain values. Neurochemical specificity was evaluated by (1) assaying more than one neurotransmitter and (2) evaluating neurotransmitter receptor binding rather than neurotransmitter concentrations. Behavioral specificity was determined by also measuring behaviors other than aggression.

Experiment 1: Six singly-housed, ovariectomized adult female hamsters which attacked a small methotrimeprazine treated target hamster (Potegal, et al., Psych. Rec., 30:191, 1980) 4 or more times in 10 min were the aggressive group; 6 subjects failing to attack formed the non-aggressive group. After decapitation, the following 4 regions were pooled within groups: cortex, "mid-region" (limbic, striatal and diencephalic structures), cerebellum and pons/medulla. GABA binding was evaluated by using ^{3}H-GABA as ligand; non-specific binding was carried out in presence of 2mM cold GABA. Noradrenergic binding was assayed with ^{3}H-DHA and specific binding was calculated after subtracting the non-specific binding obtained in presence of an excess of alprenolol. The "mid region" GABA binding in the aggressive animals was 32% higher than that of the non aggressive animals on two replications. Differences in GABA binding in other regions and differences in DHA binding in all regions were 14% or less.

Experiment 2: Ten hamsters were screened twice for aggression. Between screenings they were tested for activity, reactivity to handling, sexual behavior after hormonal priming, hoarding and nest building. The differential "mid region" GABA binding for aggressive vs non aggressive subjects was replicated in a statistically significant fashion; there were no significant differences between the other regions. There were also no significant differences in behaviors other than aggression between the two groups.

The higher level of GABA binding in aggressive animals suggests lower endogenous GABA levels consistent with the previous evidence for an aggression-inhibitory effect of GABA.

106.8 PURIFICATION AND PARTIAL CHARACTERIZATION OF A GLUTAMATE BINDING PROTEIN FROM BOVINE BRAIN MEMBRANES. E. Michaelis, L. Chittenden*, B. Johnson*, J. Fennimore* and M. Adamson* Dept. of Human Development, Neurobiology Section, Univ. of Kansas, Lawrence, KS. 66045.

A small molecular weight glycoprotein (M_r~13,000) which binds L-glutamic acid with a high degree of stereoselectivity and specificity has been purified in the past from rat brain synaptic plasma membranes (Michaelis, BBRC 65, 1004, 1975). Binding of L-[^{3}H] glutamic acid to this protein has been shown to be competitively inhibited by the neuroexcitatory amino acids L-aspartate, L-cysteine sulfinic acid, and D,L-homocysteic acid, and by the antagonist of glutamate physiologic activity, L-glutamate diethyl ester. On the other hand γ-aminobutyric acid, glycine, and some non-neuroactive amino acids did not displace L-[^{3}H] glutamate bound to this protein. This protein purified from rat brain synaptic membranes was considered to be the recognition site of the physiologic glutamate receptors. We have recently been able to purify larger quantities of a glutamate binding protein from bovine brain cerebral and cerebellar cortex which has many of the characteristics described for the rat brain binding protein.

The purification scheme involves the preparation of a crude mitochondrial fraction which is subsequently treated with 0.5% (w/v) Na-cholate according to the procedure described in Michaelis et al., FEBS Lett. 118, 55, 1980. The membrane pellet obtained after 39,000 x g for 60 min centrifugation contains all of the glutamate binding activity. These membranes are solubilized in an alkaline Triton X-100 medium (Michaelis et al., BBA 367, 338, 1974) and the glutamate binding proteins are isolated by batch affinity separation on glass fiber with co-reticulated L-glutamate as described previously (Michaelis, BBRC 65, 1004, 1975). The final eluate of the glutamate binding protein after treatment with 6% glycerol and concentration against polyvinyl pyrrolidone contains only one protein as determined by SDS-PAGE. On SDS gels this protein migrates to a position identical to that of the purified glutamate binding protein from rat brain. Its affinity for the L-[^{3}H] glutamic acid varies within the range of 0.4 - 1.1 µM for different batches. L-Aspartate and L-cysteine sulfinic acid are the most potent competitive antagonists. In terms of its binding activity, of its molecular size, of its amino acid composition, and of its isoelectric point, this bovine brain glutamate binding protein is very similar to that purified from rat brain synaptic membranes. (This research was supported by grant # DAAG 29-79-C-0156 from the U.S. Army Research Office and by grant AA 04732 from NIAAA).

106.9 UPTAKE AND RELEASE OF PUTATIVE AMINO ACID NEUROTRANSMITTERS BY CHICK EMBRYO RETINA NEURONS AND NONNEURONAL CELLS IN PURIFIED CULTURES. Arnold Hyndman[++] and Ruben Adler[+]. [+]Dept. of Biology, University of California, San Diego, La Jolla CA 92093 and [++]Dept. of Biology, Livingston College, Rutgers University, New Brunswick NJ 08903

Selective combinations of media and substrata allow the preparation of different monolayer cultures from chick embryo neural retina cell suspensions, namely: i) glia free, purified neuronal cultures; ii) complex cultures, containing a confluent monolayer of nonneuronal flat cells of apparent glial origin underlying a network of multicellular clumps; and, iii) purified nonneuronal monolayers, derived from complex cultures by mechanical removal of the clumps. They are used here to investigate the development of uptake and release mechanisms for several putative amino acid neurotransmitters with autoradiographic and biochemical techniques.

At 3 days in vitro (d.i.v.), 65% of the neurons in purified neuronal cultures become labeled when incubated with 5 x 10^{-8}M [^{3}H]GABA. As expected from a high affinity uptake mechanism, neuronal labeling is totally abolished at 0-4°C or in a Na^+-free medium. Uptake is also completely blocked by diaminobutyric acid (DABA), but not by β-alanine. High (56 mM) K^+ does not cause any significant increase in GABA release. Important developmental changes occur between 3 and 6 d.i.v., namely: i) GABA high affinity uptake is only present in 50% of the neurons; ii) in 80% of these neurons the uptake is insensitive to DABA, and iii) high K^+ causes a 2-3 fold increase in GABA release, which can be partially blocked by 10 mM Co^{++}, suggesting the development of synaptic terminals in the cultures. Nonneuronal cells present in complex cultures show Na^+-dependent GABA uptake only during the first 10 d.i.v.; this uptake is insensitive to β-alanine but is reduced 85% by DABA. Neurons as well as nonneuronal cells can also be labeled by incubation in micromolar concentrations of radioactive glutamate, aspartate, glycine or taurine. The properties of these uptake mechanisms are currently under investigation.

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106.10 COMPARISON OF THE γ-AMINOBUTYRIC ACID UPTAKE SITE DERIVED FROM RAT MIDBRAIN SYNAPTOSOMES OR MOUSE GLIAL ASTROCYTES GROWN IN CULTURE. B. R. Lester, M. Burman*, G. I. Moonsammy*, and L. M. Yunger. Smith Kline & French Labs., Dept. of Biol. Sci., Box 7929, 1500 Spring Garden St., Philadelphia, PA 19101

γ-aminobutyric acid (GABA) uptake sites are present on neurons and glia. It has been conjectured that diaminobutyric acid (DABA) and cis-3-aminocyclohexanecarboxylic acid (cis-3-ACHC) exhibit a putative preference for neuronal GABA uptake and β-alanine, β-proline and 4-hydroxynipecotic acid display a selectivity for glial GABA uptake. We have examined the pharmacologic preference of these various ligands for their ability to compete with [^{3}H] GABA using both cultured glial astrocytes from neonatal Swiss mice and synaptosomal preparations from the diencephalon-midbrain of rats. The accumulated data indicates that these compounds display a relatively constant ratio of IC_{50}'s (glial astrocytes:synaptosomes) of from 4.0 to 9.0 with little or no selectivity observed between the preparations employed. This lack of specificity could be due to glial contamination of the synaptosomal preparations or neuronal overgrowth of astrocytic cultures which would result in heterogenous patterns of GABA uptake as measured by saturation kinetics as well as mixed patterns of inhibition using the putative selective GABA analogs. These possibilities were examined by characterization of uptake saturation kinetics using [^{3}H] GABA and [^{3}H] cis-3-ACHC and by inhibition studies utilizing the putative selective GABA analogs to challenge labelled GABA uptake. It was observed that cis-3-ACHC challenge of [^{3}H] GABA uptake exhibited a competitive inhibition pattern indicating a single site in both cultured glial astrocytes (K_i=41 μM) and synaptosomal preparations (K_i=43 μM) by an apparently single site homogenous mechanism. The putative glial selective analog, 4-hydroxynipecotic acid, exhibited a high affinity for the GABA uptake site in cultured astrocytes, as predicted (IC_{50}=43.9 μM); however, it also appeared to inhibit synaptosomal uptake with an even higher affinity (IC_{50}=4.6 μM). These data indicate a lack of differential specificity towards glial versus neuronal GABA uptake by these GABA analogs at least in so far as cultured astrocytes and synaptosomes represent populations of glial and neuronal cells respectively.

106.11 TAURINE BINDING IN RAT RETINAL MEMBRANES. J.B. Lombardini. Department of Pharmacology and Therapeutics, Texas Tech University Health Sciences Center, Lubbock, Texas 79430.

While taurine is highly concentrated in the retina especially in the photoreceptor cells (Kennedy et al., J. Neurochem. 29, 157, 1977) the role of taurine in retinal function is not well defined. It has been postulated that taurine may act as an inhibitory neurotransmitter and as a regulator of intracellular calcium levels in the retina (Pasantes-Morales et al., Brain Res. 172, 131, 1979). In taurine binding studies utilizing chick retinal membranes, López-Colomé and Pasantes-Morales (J. Neurochem. 34, 1047, 1980) have demonstrated two sodium dependent components with different affinity constants (K_{D_1}=0.68 μM; K_{D_2}=9.32 μM).

In the present study the binding of taurine to retinal membranes obtained from the rat was studied. Retinal membranes were prepared from adult Wistar rats by homogenizing in 30 volumes of distilled water in a glass homogenizer. The homogenate was centrifuged for 20 min at 15,000 x g. The pellet was washed once in distilled water and once in Krebs-Tris buffer (pH 7.4; 118 mM NaCl; 1.2 mM KH_2PO_4; 4.7 mM KCl; 2.5 mM $CaCl_2$; 1.17 mM $MgSO_4$; 26 mM Tris-HCl) and recentrifuged after each wash as above. After the final wash the pellet was resuspended in Krebs-Tris buffer. Scatchard analysis of the taurine binding profile demonstrated the presence of two distinct components of taurine binding with a K_{D_1} for the high affinity binding site of 20 μM and a K_{D_2} for the low affinity binding site of approximately 900 μM.

Optimal binding of taurine to retinal membranes required sodium ions. In the absence of sodium ions approximately 60% of the total binding was observed. Removal of potassium ions from the incubation medium decreased taurine binding to 20% of total. No binding was observed in the combined absence of sodium and potassium ions nor in the absence of calcium ions.

Structural analogues of taurine, β-alanine and guanidinoethanesulfonate, at a final concentration of 1 mM inhibited taurine binding by 70% (taurine concentration = 30 μM). 2-Aminoethanephosphonic acid (isosteric replacement of the sulfonic acid moiety with a phosphonic acid moiety) decreased taurine binding by 20%. Aminomethanesulfonic acid, the one carbon analogue of taurine, was ineffective in displacing taurine as was isethionic acid (the hydroxy analogue).

106.12 SOLUBILIZATION OF A NON-NEURONAL BENZODIAZEPINE BINDING SITE. J. W. Thomas and J. F. Tallman. Section on Biochemistry and Pharmacology, Biological Psychiatry Branch, NIMH, Bethesda, MD 20205

While initial attempts using nonionic detergents were unsuccessful, the benzodiazepine binding site in mammalian kidney has been solubilized using a bile salt detergent. A single extraction step releases approximately 15% of the membrane-bound binding sites into the high-speed supernatant. The affinity of the binding site is decreased about 5 fold by solubilization (apparent K_D for membrane-bound site, 10 nM; apparent K_D for soluble site, 51 nM), and a series of drugs inhibited [^{3}H]flunitrazepam binding to the soluble binding site with the same relative potencies observed for the membrane-bound binding site. In particular, RO5-4864, a specific inhibitor at peripheral and glial benzodiazepine binding sites (D.W. Gallager et al., J. Neurosci. 1: 218-225, 1981), when included at a concentration of 100 nM, decreases the binding of 5 nM [^{3}H]flunitrazepam by 68.5%. In contrast, drugs which specifically inhibit binding to the neuronal site are considerably less potent at the soluble kidney binding site; clonazepam at a concentration of 1 μM decreases binding of 5 nM [^{3}H]flunitrazepam only 7.0% and CL 218,872 at a concentration of 10 μM decreases binding only 15.2%. While further analysis of the soluble non-neuronal binding site is required, these results suggest that the pharmacological differences in the neuronal and non-neuronal benzodiazepine binding sites are due to the inherent properties of binding sites since an intact membrane is not required. Properties of the neuronal and non-neuronal sites will be compared.

106.13 SELECTIVE RETROGRADE LABELLING OF CENTRAL AND SPINAL GANGLION NEURONS AFTER INJECTIONS OF D-ASPARTATE AND GABA IN THE SPINAL CORD AND CUNEATE NUCLEUS OF RATS. A. Rustioni and M. Cuénod. Brain Research Institute, University of Zürich (Switzerland) and Dept. of Anatomy Univ. N. Carolina, Chapel Hill, NC 27514 (USA).

Recent studies have shown that after injection of ^{3}H-D-Asp in CNS structures the perikarya of some but not all pathways projecting to the injected structure are labelled. Since the labelled neurons are believed to use glutamate (glu) and/or aspartate (asp) as neurotransmitter(s) and since D-Asp is known to compete for the L-glu and L-asp high affinity sites, it is postulated that a transmitter-related affinity might be at the basis of the selective retrograde transport. In the present experiments the selectivity of the retrograde labelling of neurons has been tested after injection of ^{3}H-D-Asp in the cuneate n. and in the cervical cord of rats. Twenty-four hours after injections in the cuneate n., autoradiographic labelling is present over the pyramidal tract, internal capsule and over layer V pyramids of the sensorimotor cortex. No perikarya of the other central sources of afferents to the cuneate n. appear labelled. After ^{3}H-GABA injections in the same nucleus no labelling attributable to retrograde translocation can be detected in spinal segments, brain stem or cortex. These observations are consistent with existing evidence indicating that the cortico-cuneate path uses glu and/or asp as neurotransmitter. ^{3}H-D-Asp injections in the cervical cord label some perikarya of the substantia gelatinosa and larger neurons in laminae IV to VI for three to five segments above and below the injection. No brain stem neuron is labelled though silver grains overlie pyramidal tract fibers on the side contralateral to the injection. Retrograde labelling of corticospinal axons for at least some distance may suggest a special affinity of their terminals for glu and/or asp. This also is consistent with previous indications of a transmitter role for glu or asp in the corticospinal tract.

Silver grains accumulation overlies about one third of cervical ganglia neurons whose perikarya is 100 um^2 or more 24 hours after injection of ^{3}H-D-Asp, but not of ^{3}H-GABA, in the cuneate nucleus. After spinal injection of the same ^{3}H-amino-acids, labelled neurons of cervical ganglia have mainly large perikarya and represent approximately 5% (after D-Asp injection) and 12% (after GABA injection) of the neuronal population of the ganglion(a) related to the injected segment(s). While these observations are consistent with the idea that a fraction of large primary afferents use glu and/or asp as neurotransmitters the mechanism underlying selective labelling of spinal ganglion neurons by ^{3}H-GABA remains to be elucidated.

106.14 PIPECOLIC ACID: A NEUROMODULATOR OF MOUSE CNS? E. Giacobini, H. Nishio*, J. Ortiz* and M.d.C. Gutierrez, Lab. of Neuropsychopharmacology, Univ. of Conn., Storrs, CT 06268

The following results obtained in our laboratory suggest that the imino acid pipecolic acid (PA) may be selectively localized in neuronal populations of the mouse brain where it could act as a physiological modulator:

1. The identity of PA in the brain has been unambiguously established by mass spectrometric identification. Its endogenous levels in the adult mouse brain were found to be 18 + 4 nmoles/g (Schmidt-Glenewinkel et al., Neurochem.Res. 2:619, 1977).
2. PA is synthesized both in vitro and in vivo from lysine in brain, liver, kidney, heart and the large intestine of the adult mouse and chick. In addition, the embryonic brain in both species is able to synthesize PA from lysine in vitro (Nomura et al., Dev. Neurosci. 1:239, 1978).
3. PA is taken up in synaptosomes of mouse brain by a high affinity, temperature and Na+ dependent, ouabain sensitive mechanism (Nomura et al., Neurochem. Res. 5:1163, 1980).
4. PA is released by a Ca++ dependent mechanism from brain slices (Nomura et al., J. Neurochem. 33:803, 1979).
5. PA, when injected in tracer amounts intracarodically, is taken up in the brain with a BUI (Brain Uptake Index) of 3.9 which is within the low range of aminoacid transport. This uptake is saturable and its kinetics suggest the presence of two transport systems. The corresponding BUI for lysine, the PA's precursor, is 8.5 (Nishio and Giacobini, Neurochem.Res. 6, 1981).
6. Following pulse-injection of PA, i.p. or i.v., three features are most salient: its rapid accumulation in brain; its rapid secretion in urine, and the long-lasting steady levels of radioactivity maintained in brain. A significant decline of the percentage recovered as PA in brain and kidney following i.p. injection suggests cerebral metabolic activity. PA accumulates following pretreatment with probenecid.
7. Following intraventricular injections, high levels of PA are maintained in brain. The labeled compound is accumulated in four brain regions (neocortex, hippocampus, diencephalon and striatum) and slowly metabolized.
8. Accumulation of ^{3}H-PA following i.p. inject. is greater in new born than in adult mice.
9. A high affinity binding for ^{3}H-PA in whole brain homogenates, as well as in crude mitochondrial (P_2) preparations, has been detected in preliminary experiments. This suggests the presence of a specific binding site for PA in mouse brain.

(Supported by PHS grants NS 11430 and 14086 to E.G.).

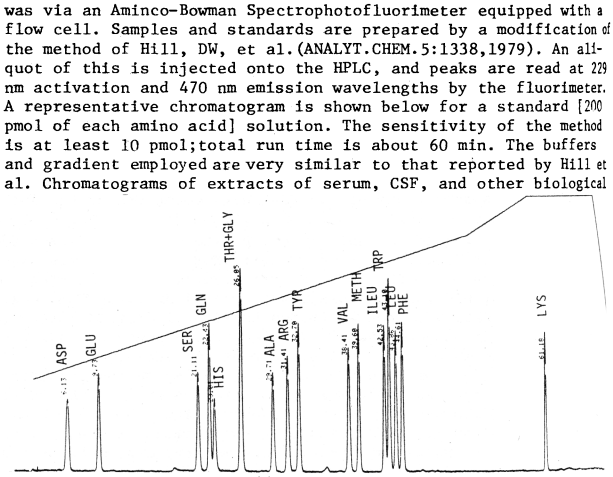
106.15 SPONTANEOUS AND DEPOLARIZATION-INDUCED TURNOVER OF GABA IN RAT CORTICAL SLICES: EFFECT OF A GABA-T INHIBITOR. J.C. Szerb, Dept. Physiol. & Biophys., Dalhousie U., Halifax, N.S. B3H 4H7.

In a previous study (Szerb et al., J. Neurochem., in press) it was shown that when both the synthesis and metabolism of GABA was inhibited with amino-oxyacetic acid only 10% of endogenous GABA present in slices was in a compartment from which elevated K^+ induced release. To see the turnover of GABA both in the releasable and non-releasable compartments, slices were first incubated with [^{3}H]GABA then were superfused with Krebs containing 3 mMK^+ either in the absence of an inhibitor or starting after 15 min with 5 μM gabaculine (GCU), a specific GABA-T inhibitor. Endogenous GABA released or remaining in the slices was measured with HPLC which also separated [^{3}H]GABA from its labelled metabolites. In the absence of GCU the endogenous GABA content of the slices remained constant while the labelled GABA content declined to about 1/3 at the end of 60 min superfusion as compared to that found after 15 min. With GCU present endogenous GABA content increased by 65% over 45 min and [^{3}H]GABA content remained constant. In the absence of GCU metabolites were responsible for 90% of radioactivity released spontaneously and the release of metabolites was reduced by 80% by GCU without increasing the efflux of [^{3}H]GABA. No spontaneous release of endogenous GABA was detected. Depolarization with 50 mMK^+ caused a large efflux of endogenous GABA which was only 25% higher in the presence of GCU than in its absence. However 50 mMK^+ released 70% more [^{3}H]GABA in the presence than in the absence of GCU. The specific activity of GABA released during 25 min exposure to 50 mMK^+, declined by 75% both in the absence or presence of GCU, indicating a depolarization induced synthesis of releasable GABA. Results suggest a considerable rate of spontaneous turnover of all GABA compartments and a depolarization induced enhanced synthesis of GABA in the releasable compartments. Gabaculine, by inhibiting the metabolism of GABA, increased the total GABA content to a lesser extent than the size of the releasable compartment.

(Supported by the MRC of Canada and the Banting Research Foundation.)

106.16 A SIMPLE, RAPID HPLC/FLUORESCENCE METHOD FOR MEASURING PICOMOLE QUANTITITES OF AMINO ACIDS IN PHYSIOLOGICAL SOLUTIONS. Madelyn Hirsch Fernstrom and John D. Fernstrom. M.I.T., Cambridge, MA 02139.

Recently, several reports have appeared concerning the measurement of amino acids using reversed-phase, liquid chromatography (HPLC) coupled with fluorescence detection. Amino acid samples are derivatized with ortho-phthalaldehyde (OPT) and then run over the HPLC. We have explored the utility of this method for routine analysis of amino acids in physiological solutions, and find the reliability and sensitivity to exceed that of conventional amino acid analyzers. The system we employ was obtained from Waters Associates (2 6000A pumps, a 720 Controller, & a 730 data analyzer; a 10 mm x 30 cm μBondapak C-18 reverse-phase column); post-column detection was via an Aminco-Bowman Spectrophotofluorimeter equipped with a flow cell. Samples and standards are prepared by a modification of the method of Hill, DW, et al. (ANALYT.CHEM. 5:1338, 1979). An aliquot of this is injected onto the HPLC, and peaks are read at 229 nm activation and 470 nm emission wavelengths by the fluorimeter. A representative chromatogram is shown below for a standard [200 pmol of each amino acid] solution. The sensitivity of the method is at least 10 pmol; total run time is about 60 min. The buffers and gradient employed are very similar to that reported by Hill et al. Chromatograms of extracts of serum, CSF, and other biological



fluids show the same excellent separation. For serum, we compared values from runs on the HPLC to those on a Beckman 119C Amino Acid analyzer for 15 amino acids, and found the data to be indistingusihable. These data, and those to be present d, show this method of analysis to be superior in many regards to that accomplished on a standard amino acid analyzer.

(Supported in part by a grant from the NIMH.)

106.17 DETERMINATION OF γ-AMINOBUTYRIC ACID IN WHOLE RAT BRAIN BY ELECTROREDUCTION FOLLOWING LIQUID CHROMATOGRAPHIC SEPARATION.
W. Lowry Caudill* and R. Mark Wightman. Department of Chemistry, Indiana University, Bloomington, IN 47405.

γ-Aminobutyric acid (GABA) has been recognized for many years as a neurotransmitter. However, measurements of its neuronal function have been hampered by the lack of a sensitive analytical method for its determination. In the past several years, amperometric detection with carbon electrodes following liquid chromatographic separation has become a widely accepted analytical method because of its high sensitivity, selectivity and low cost. We have developed a relatively simple microderivatization scheme which renders GABA electroactive. In basic solution, the derivatizing agent, 2,4,6-trinitrobenzenesulfonic acid, reacts rapidly with GABA to give the desired derivative in 100% yield. Following the quenching of the reaction at low pH and the introduction of trinitrophenyl-δ-aminovaleric acid as an internal standard, the mixture is extracted with toluene. The toluene fraction is extracted with an aqueous basic solution. This basic solution is then separated using C-18 reversed phase liquid chromatography and the derivatives are detected by reduction at -0.8 V vs. SCE with a pressure annealed pyrolytic graphite electrode. The technique has been applied to the analysis of whole rat brain samples. The method provides a routine and accurate analysis of the derivatized amine at picomole levels.

107.1 VASOPRESSIN LEVELS IN REAGGREGATE CULTURES OF THE DEVELOPING HYPOTHALAMUS AND PITUITARY. M.F.D. Notter*, C.D. Sladek, and D.M. Gash. Depts. of Anatomy, Center for Brain Research, and Neurology, Univ. of Rochester School of Medicine, Rochester, NY 14642.

Vasopressin released into culture media as well as vasopressin content of dispersed cell cultures taken from developing hypothalamus and pituitary tissue were measured by radioimmunoassay (RIA). The hypothalami and pituitaries, dissected from fetal 19 day postcoitus (dpc) and neonatal one-day-old Long Evans rats, were dissociated by a typsinization procedure. Dispersed cells were seeded into siliconized multiwelled tissue culture dishes containing Eagles minimum essential medium (medium supplemented with 0.6% glucose, .1% glutamine, 10% fetal calf serum, 100 μg/ml penicillin and 2.5 μg/ml fungizone) and rotated at 70 rpm in a humid CO_2 incubator. The cells reaggregated into spheroids, roughly 150 μm in diameter, within four hours. When media or homogenized cells from cultures containing only hypothalamus cells were examined for vasopressin by RIA after 4 days, vasopressin levels ranging up to 388 pg/10^6 cells were measured in the tissue and 2418 pg/ml in the media. Vasopressin was also present in cultures after eight to ten days, but the quantities measured were variable and tended to be lower than those measured in the four day cultures. When newborn hypothalamus was co-cultured with dispersed posterior pituitary cells, the amount of vasopressin in the reaggregates increased up to 6900 pg/10^6 cells in the tissue and approximately 3500 pg in the media. This same effect was seen after nine days of culture although less dramatically. The increase in vasopressin content of co-cultivated hypothalamus and posterior pituitary was not seen when neonatal anterior pituitary was substituted for posterior tissue nor when 19 dpc hypothalamus was co-cultured with 19 dpc pituitary tissue.

These data indicate that vasopressin containing neurons survive cell culture for at least nine days in vitro and that at a critical time in development the posterior pituitary may either promote survival of the neurons or stimulate secretion by them.
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107.2 IMMUNOHISTOCHEMICAL ANALYSIS OF DISPERSED, HYPOTHALAMIC TRANSPLANTS. J. Schöler*, J.R. Sladek, Jr., M.F.D. Notter* and D.M. Gash (SPON: W.E. Armstrong). Depts. of Anatomy and Microbiology and Center for Brain Research, University of Rochester School of Medicine and Dentistry, Rochester, NY 14642.

It has recently been shown that fetal hypothalamic tissue can survive transplantation into the third ventricle of a host animal. The present study was undertaken to determine the ability of dispersed hypothalamic neurons to survive transplantation and structurally integrate into the host brain. Hypothalamic tissue from 19 day, post-coital, Long Evans rats was dispersed with trypsin. Cells were counted with a hemocytometer and viability determined by .1% erythrocin dye exclusion. 500,000 viable hypothalamic cells were stereotaxically placed into the third ventricle of adult Brattleboro rats, homozygous for the diabetes insipidus trait. The hosts were allowed to survive for 21 to 60 days. Prior to tissue processing, the animals received 1mU of vasopressin (VP) in tannate oil per day for two days. The animals were then anesthetized and perfused through the ascending aorta with 4% paraformaldehyde in 0.01M PO_4 buffer. The brains were sectioned on a vibrating microtome and processed for light microscopic immunohistochemistry. Alternate 30μm free-floating sections were stained for neurophysin (provided by Dr. A.G. Robinson), oxytocin and VP (supplied by Dr. G. Nilaver). The respective antigenic sites were visualized with the peroxidase-anti-peroxidase technique.

Analysis of those animals in which the transplant could be located revealed that in each case the dispersed cells had reaggregated to form a large, viable clump of cells located either in the floor of the ventricle or juxtaposed to the ventricular walls. Sections stained for neurophysin revealed two populations of cells present within the reaggregated transplant. One cell population consisted of small neurophysin-positive neurons possibly parvicellular in nature; the other consisted of larger, magnocellular-type neurons. In each case, the neurons appeared normal and had processes extending throughout a large portion of the transplant. Studies are currently under way to determine what percentage of the neurophysin-positive neurons contain either VP or oxytocin. The present study indicates, however, that the third ventricle and its CSF provides a favorable milieu for transplanted hypothalamic neurons and demonstrates their ability to colonize within certain areas of the central nervous system.
Supported by USPHS Grants NS 15109 (DMG), NS 15816 (JRS) and AG 00847 (JRS).

107.3 LIGHT MICROSCOPIC IMMUNOCYTOCHEMICAL EVIDENCE SUGGESTING COUPLING OF HYPOTHALAMIC PEPTIDERGIC NEURONS. M.V. Sofroniew. Dept. of Anatomy, Ludwig-Maximilians University, Pettenkoferstr. 11, 8000 Munich 2, FRG.

Evidence in support of electrotonic transmission and coupling of neurons has now been observed in various areas of the mammalian central nervous system. Recently this evidence has been extended to magnocellular neurosecretory neurons (MNN's) of the rat hypothalamus by the observation of dye coupling and gap junctions occurring between such neurons (Andrews et al., Science 211, 1187). During the course of various light microscopic immunohistochemical studies on hypothalamic peptidergic neurons, in particular a morphological analysis of MNN's based on Golgi-like immunoperoxidase staining for vasopressin, oxytocin or neurophysin, we noted a number of neural configurations suggestive of the coupling of neurons. Although it is clear that light microscopic data alone cannot be taken as direct verification of coupling, these findings may provide information on the various 3-dimensional forms by which coupling may occur; information which may be difficult to obtain from ultrastructural studies relying largely on 2-dimensional analysis. Four types of contacts between neurons were observed which were considered possible substrates for coupling. The first three constituted dendro-dendritic, dendro-somatic and soma-somatic contacts occurring between MNN's via short interconnecting processes. These interconnecting processes generally arose as juxtaposed protuberations on both neurons which connected into single short extensions providing an uninterrupted (at the light microscopic level) transition between the neurons. In some cases several such connections appeared to be made between a single pair of neurons. Most frequent were dendro-dendritic, followed by dendro-somatic, followed by soma-somatic contacts of this nature. The fourth type of contact observed was soma-soma apposition between the perikarya of neurons. Since most perikarya did not appear to directly contact other perikarya, direct close apposition was a conspicuous well definable configuration, Apposition of this nature was identified between a number of pairs of MNN's, as well as between some chains of MNN's in certain magnocellular accessory groups. Soma-soma apposition was noted not only between neurons containing the same peptide, but also between neurons which contained two different peptides, i.e. somatostatin or neurophysin, stained different colors using two-color immunoperoxidase staining. Even though the light microscopic findings presented here cannot be taken as the direct demonstration of coupling of neurons, the findings may be useful in visualizing the various 3-dimensional forms by which coupling may occur, and as such warrant further study.

107.4 RECURRENT INHIBITION AND PHASIC BURSTING IN RAT PARAVENTRICULAR NEURONS: A CHALLENGE FROM THE HYPOTHALAMIC IN VITRO SLICE. Glenn I. Hatton. Neuroscience Program and Psychology Department, Michigan State University, East Lansing, MI, 48824.

It is not known by what mechanism the so-called "phasic bursting" patterns are generated in certain hypothalamic magnocellular neuropeptidergic cells (MNCs). Recurrent inhibition has been invoked to explain phasic bursting. Although there is evidence for recurrent inhibition in MNCs, it has never been reported for a phasic bursting cell. Thus, it was of interest to investigate the possibility that bursting patterns of activity might be displayed by MNCs independently of such inhibitory feedback. This was accomplished using the in vitro hypothalamic slice preparation in which all chemical synaptic transmission could be blocked. In order to be certain that chemical synaptic transmission was blocked, a monosynaptic pathway in the hippocampal slice was first monitored. Both hippocampal and hypothalamic slices were prepared (Brain Res. Bull., 1980, 5, 391 & 405) and placed side by side in an in vitro recording chamber. The field potential from Schaffer collateral stimulation was recorded in the CA1 region of the hippocampus. Blocking of this synaptic response was monitored while the medium bathing the slices was changed from one containing 2.4 mM Ca^{2+} and 1.3 mM Mg^{2+} to one with .05 mM Ca^{2+} and 18.7 mM Mg^{2+}. In some experiments this was reversed by changing back to a standard solution. When synaptic transmission was completely blocked in the hippocampal slice, recordings of spontaneous activity of MNCs in the paraventricular nucleus (PVN) were made in adjacent hypothalamic slices. Also, in some experiments the phasic bursting neuron was antidromically activated by extracellular electrical stimulation in order to monitor its presence or activity during the sometimes prolonged silent periods.

To date, 20 cells displaying phasic bursting activity have been recorded from 12 preparations in which synaptic transmission had been blocked. The activity patterns recorded from these cells are similar to those recorded in vivo, often consisting of bursts of 100-200 spikes and lasting 7-25 seconds. Nearly all the cells displaying this activity were located in the lateral subnucleus of the PVN. These results suggest that phasic bursting activity in MNCs is not generated by recurrent synaptic inhibition.

This research was supported by NIH grant NS 09140.

107.5 THE DISTRIBUTION AND CELLS OF ORIGIN OF SOME AFFERENT PROJECTIONS TO THE PARAVENTRICULAR AND SUPRAOPTIC NUCLEI IN THE RAT. P. E. SAWCHENKO* and L. W. SWANSON, The Salk Institute, La Jolla, California 92037.

Axonal transport and immunohistochemical techniques were used to clarify the organization of projections to the paraventricular (PVH) and supraoptic (SO) nuclei. Six rats received a 30 nl injection of the retrogradely-transported fluorescent tracer, true blue, centered in the PVH. The material was used to plot the distribution of retrogradely-labeled neurons, and to demonstrate simultaneously true blue and an antigen (Brain Res. 210:31, 1981), using antisera directed against dopamine-β-hydroxylase (DBH), serotonin, or ACTH (1-39). Then, the distribution of major inputs to the PVH and SO was charted, using the autoradiographic method. 20-40 nl of ^{3}H-amino acids was injected into each of the cell groups that were identified in the retrograde transport studies as projecting to the PVH. The parvocellular (pc) division of the PVH receives inputs from many sites in the brainstem, including DBH-positive neurons in the A1, A2 and A6 (locus coeruleus) catecholamine groups. Projections from the A1 and A2 groups end primarily in dorsal and medial parts of the pc division, while that arising from the locus coeruleus is restricted to the periventricular part. A light projection to most parts of the pc division arises from serotonin-stained cells in and near the dorsal and median raphe. The parabrachial nucleus, the bed nucleus of the stria terminalis and the subfornical organ all project substantially to most pc areas. Relatively light projections, primarily to the periventricular and medial pc zones, arise from nearby hypothalamic regions, including the lateral, medial, median and periventricular preoptic areas, the suprachiasmatic nucleus, the ventromedial nucleus and the posterior hypothalamic area. A dense projection to the periventricular, dorsal and medial pc areas arises from ACTH (1-39)-positive cells centered in the arcuate nucleus. Relatively few inputs to the magnocellular (mc) division of the PVH, and to the SO, were identified. A dense projection arising from DBH-positive neurons in the A1 catecholamine cell group is preferentially distributed to those mc areas of the PVH and SO in which vasopressin-containing cells are concentrated. The oxytocinergic parts of the PVH and SO receive inputs from midbrain serotonin-positive cells, and from ACTH (1-39)-stained neurons in and near the arcuate nucleus. Thusfar, only the subfornical organ appears to innervate both cell types, although this input was more dense over the vasopressinergic portions of the PVH and SO. These results indicate that limbic, hypothalamic and brainstem cell groups project to the PVH, where each appears to innervate discrete parts of the parvocellular division. Specificity was also clear in the inputs to the magnocellular parts of the PVH and SO, since each appears to terminate preferentially in regions where either oxytocin-or vasopressin-containing cells are concentrated. (We thank K.B. Helle, H. Steinbush, and S.A. Joseph for geneous supplies of antisera.)

107.6

Withdrawn

107.7 ADRENALECTOMY INDUCES SPROUTING IN PROJECTIONS FROM THE PARAVENTRICULAR NUCLEUS TO THE MEDIAN EMINENCE OF THE RAT. A.J. Silverman, C. Moodhe*, and E.A. Zimmerman. Depts. of Anatomy and Neurology, Columbia Univ., P&S, New York, NY 10032.

In normal rat median eminence (ME), few immunoreactive vasopressin or neurophysin (NP) fibers are found in zona externa (ZE) by immunocytochemistry when compared to rhesus monkeys. It was previously reported that unilateral lesions (U_x) of the paraventricular nucleus (PVN) in monkey hypothalamus caused an ipsilateral loss of these ZE fibers, and in rats that bilateral PVN lesions (B_x) abolished the progressive visible reactivity in these fibers after bilateral adrenalectomy (ADX). The results of the next experiment prompted the studies reported: U_x of PVN followed by ADX for 2 weeks in rats did not result in significant ipsilateral ZE fiber loss suggesting two possibilities: either (a) the PVN-ME vasopressin-NP system in rat is not unilateral as in monkey, or (b) ADX and/or U_x causes sprouting to the opposite ZE. In the next group of animals, anterograde HRP-tracing from PVN was used to investigate this projection in intact animals. HRP was iontophoresed into PVN and visualized with the TMB procedure of Mesalum. Only pvl or pvm subnuclei (Armstrong et al., Neuroscience 5:1931, 1981) resulted in labeled terminals in ZE. Innervation of ZE was mostly unilateral with a small number of fibers just crossing midline. U_x of PVN 1-4 weeks preceding HRP into the intact PVN gave similar results. One animal surviving ADX, U_x and with correct placement of HRP showed bilateral innervation of ZE, suggesting sprouting. To further test this possibility, and to determine the time course of sprouting, rats were ADX for 2 weeks followed by U_x of PVN. Animals were killed 3-21 days post U_x and the brains processed for immunoperoxidase technique using antiserum to rat neurophysin. At 3 days post U_x NP fibers were numerous on the side of the intact PVN and few remained on the side of the U_x. At 6 and 21 days NP input to ZE was bilateral, although the side of U_x was less heavily innervated. Bilaterality was more prominent in rostral ME and infundibular stalk. These results demonstrate that the PVN-ME NP projection is normally unilateral in rat as in the rhesus monkey. These experiments suggest that unilateral denervation of the ZE is not sufficient to cause sprouting of contralateral NP-VP fibers. The additional stimulus of ADX, known to activate the VP neurons in PVN, is also involved in the sprouting process. In addition, therefore, to the known stimulatory effects of adrenal insufficiency on the synthesis of VP in this system, these experiments suggest an additional and separate effect on the ability of these cells to initiate sprouting. Supported by NIH grant AM20337; AJS is a recipient of an Irma T. Hirschl Career Scientist Award.

107.8 SUPRAOPTIC NEUROSECRETORY NEURONS: MODIFICATIONS OF EXCITABILITY BY ELECTRICAL STIMULATION OF LIMBIC AND SUBFORNICAL ORGAN AFFERENTS. L.P. Renaud, E. Arnauld*, M. Cirino*, B.S. Layton, S.Sgro* and Y.S. Siatitsas*. Division of Neurology, Montreal General Hospital and McGill University, Montreal, Canada.

The frequency of action potentials generated by magnocellular neurons of the neurohypophyseal system is an important determinant of the amount of hormone released at the level of the posterior pituitary. Investigation of synaptic pathways that alter the excitability of these neurosecretory neurons should assist the understanding of neural mechanisms that govern the release of neurohypophyseal hormones. We therefore examined the excitability of supraoptic neurohypophyseal (SON) neurons during electrical stimulation in several forebrain regions that provide afferent fibres to the supraoptic nucleus in the rat i.e. the cortico-medial or basal amygdala (AMYG), lateral septum (LS), hippocampus and subfornical organ (SFO).

Extracellular action potentials were recorded from antidromically identified SON neurons in urethane or pentobarbital anesthetized male Sprague-Dawley rats. Isolated stimulators delivered single current pulses (0.05msec, 0.1-0.6mA) at 1Hz or 10Hz to implanted bipolar electrodes. Excitability patterns based on frequency and poststimulus histograms were analyzed with a PDP 11/40 computer. Special attention was directed to 'phasic' SON neurons since these are deemed to be vasopressin secreting cells (cf Poulain et al, Proc. Roy. Soc. Lond.B. 196:367-384, 1977). Single or repetitive stimulation in AMYG and LS evoked a transient depression of excitability among more than 2/3 of active SON neurons. Among phasic SON neurons, bursts of repetitive stimuli applied during periods of activity shortened the duration of phasic discharges. In contrast, SFO stimulation evoked an increase in excitability for more than 50% of SON neurons. With phasic SON cells, repetitive SFO stimuli could trigger or prolong a phasic discharge. Tests conducted on adjacent SON cells indicated variability of responsiveness to the same stimulus.

These data suggest that limbic afferents to SON neurons in the rat serve to decrease their excitability and hence the release of neurohypophyseal hormones, while SFO afferents appear to exert a predominantly excitatory effect on SON cells and therefore to enhance the release of neurohypophyseal hormones. These actions on phasic or putative vasopressin-secreting SON neurons may indicate at least a part of the cellular basis for hormonal (vasopressin release) and behavioural (drinking) functions attributed to limbic brain regions and to the subfornical organ.

(Supported by the Canadian MRC)

107.9 THE INFLUENCE OF ANGIOTENSIN II ON IDENTIFIED MAGNOCELLULAR NEURONS OF THE SUPRAOPTIC NUCLEUS. L. D. Mitchell & A.K.Johnson
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The supraoptic-hypophysial tract is a primary system for the synthesis and release of antidiuretic hormone (ADH). Angiotensin II (AII) has been shown to release ADH both when injected into the cerebral ventricles (IVT) or delivered intravenously (IV). The present studies were conducted to examine the effects of AII delivered by either route on the unit activity of SON magnocellular neurons.

Fourteen male albino rats were prepared with intracranial cannulae which insured delivery of drugs to the lateral ventricles, and with polyethylene catheters into the right jugular vein and femoral artery for systemic injections and arterial pressure recordings.

A transbuccal approach permitted visualization of the pituitary stalk, the cerebral arteries and the optic tracts. This allowed positioning of bipolar stimulating electrodes directly into the posterior pituitary and glass microelectrodes into the SON, without violating the ventricular-SON partition. Magnocellular neurons were identified by antidromic excitation and collision inhibition of action potentials.

Either 30ng of AII or 5ug of Saralasin (P113) in 10ul of 0.9% saline were delivered through the IVT cannulae.

In 7 identified cells, IVT AII produced increased firing in 6, and decreased firing in the seventh with latencies of between 4 and 40 sec. In 4 neurons, no change was observed. In 2 cases where P113 was given after AII, excitation was inhibited; in 4 cases where IVT P113 was given alone, firing was reduced to below basal levels with a latency of 2 to 10 sec.

In 6 neurons, pressor doses of AII (IV) produced an initial burst (2-20 sec) of firing followed by inhibition (2-4 min) while blood pressure was elevated.

Based on latency, the results indicate that IVT AII acts on a periventricular site to influence SON magnocellular neurons. Because P113 alone reduced the firing rate of identified cells, it is likely that in the anesthetized rat (urethane) there is a tonic influence of AII on the SON magnocellular neurons. Further more, although systemic AII may briefly excite SON neurosecretory cells, the sustained effect is inhibition which may be mediated via baroreceptor mechanisms.

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107.10 MODULATION OF PARAVENTRICULAR UNIT ACTIVITY AND THE MILK EJECTION REFLEX BY POSTERIOR HYPOTHALAMIC AND PERIPHERAL MAMMARY NERVE STIMULATION. E. W. Haller* and J. B. Gearns* (SPON: E. K. Stauffer). Dept. of Physiol., Sch. of Med., Univ. of Minnesota, Duluth, MN 55812.

In view of the unit discharge pattern of oxytocinergic neurosecretory neurons when correlated with oxytocin secretion (and milk ejection), the question arises why oxytocin is not secreted from the neurohypophysis as a constant, elevated output under conditions of constant mammary receptor stimulation. The present experiments examine the possibility that the afferent input of the milk ejection reflex is modulated at the level of the posterior hypothalamus to account for the characteristic firing pattern of oxytocinergic neurons (J. B. Wakerley & D. W. Lincoln. J. Endocrinol. 57: 477, 1973) in relation to episodic oxytocin secretion. A stainless steel microelectrode (ME) (1-2 tip) and a concentric steel stimulating electrode (HSE) (0.3 mm tip, 0.3 mm exposed barrel) were placed stereotaxically on ipsilateral sides of the paraventricular nucleus (PVN) and posterior hypothalamus, respectively, of 5-10 day post-partum rats under urethane (1.3 g/kg b.w.) anesthesia. Contralateral mammary nerve stimulation was accomplished with bipolar Ag/AgCl electrodes (MSE); the nerve was looped over the electrode and kept moist by submerging in mineral oil. Trains of electrical pulses were applied to HSE (1-2 v, 10 Hz, 30 s, 0.5 s on/off) or MSE (5-20 v, 10 Hz, 30 s, 0.5 s on/off) either separately or simultaneously. Unit spike discharge rates and patterns recorded by ME were analyzed by computer program and correlated with mammary duct pressure and cortical EEG monitored during the experiment. Units responding to MSE stimulation were usually associated with a rise in milk duct pressure (96%). HSE stimulation per se inhibited PVN units or failed to elicit a unit response (79%). When MSE and HSE stimuli were applied simultaneously, PVN units displayed attenuated firing rates (61%) and milk duct pressure failed to rise (83%). Alternatively, unit firing patterns of some PVN units were altered without effect on their mean firing rates but reducing milk duct pressure nevertheless. Previous studies have indicated that the afferent path of the milk ejection reflex divides in two portions in the brain stem, but that both innervate PVN. Present experiments suggest that afferents coursing through posterior hypothalamus in close relation to mammillo-thalamic tract may serve a modulating inhibitory function in the milk ejection reflex. Supported by NIH HD13906.

107.11 METABOLIC RESPONSES TO SUCKLING IN POSTPARTUM LACTATING RATS. Theresa O. Allen, Judith M. Stern* and Norman T. Adler, Depts. of Psychology, Univ. Penn., Philadelphia, Pa. and Rutgers Univ., Piscataway, N.J.

In response to suckling by pups, lactating females are known to release pulses of oxytocin from the posterior pituitary and prolactin from the anterior pituitary. In order to investigate the central mediators of these systems, we used the (14C) deoxyglucose (2DG) method of metabolic mapping. All females were deprived of their pups and of food for 6 hours on the 5th or 6th day after parturition and assigned to one of three groups. Four suckled females were reunited with their pups and allowed to nurse during the incorporation period (45 min.) of 2DG. Three nonsuckled females were not reunited with pups during this period. Four postsuckled females were injected with 2DG after a bout of suckling had terminated. Females were killed, brains and pituitary glands were removed and processed for x-ray autoradiography. Sections were later stained with cresyl violet (brain) or aldehyde fuchsin(pituitary).

The data were analysed with a computerized image-processing system (UPenn-Drexel Autoradiography Project) which allowed precise alignment of the autoradiograph and the histology. The following anatomical areas were analysed: posterior pituitary, anterior pituitary, subdivisions of supraoptic and paraventricular nuclei, optic tract, hippocampal commissure, and corpus callosum. To make comparisons between animals, the data are expressed as a ratio of the concentration of 14C in a structure to that in white matter. The posterior pituitary is more active in both suckled ($P \leq 0.05$) and postsuckled ($P \leq 0.05$) females than in nonsuckled rats. This increase may reflect oxytocin release and restoration of ionic gradients (Mata,M. et al., J. Neurochem.1980 34, 213). The anterior pituitary is more active in suckled than in either nonsuckled($P \leq 0.005$) or postsuckled females ($P \leq 0.05$). This increase may be a consequence of depletion/transformation/repletion of prolactin during a bout of suckling. The subdivisions of the supraoptic(Sokol, H. et al., Endocrinol. 1976, 98, 1176) and paraventricular nuclei(Armstrong, W. et al.,Neurosci. 1980, 5, 1931) did not differ between suckled and nonsuckled females. In postsuckled females the dorsal and ventral parts of the supraoptic nuclei and the more posterior parts of the paraventricular nuclei were more active than in nonsuckled females. These increases may reflect synthesis of oxytocin and vasopressin in the cell bodies following a bout of suckling.

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107.12 DOPAMINE SYNTHESIS IN THE POSTERIOR PITUITARY MAY BE REGULATED BY PLASMA CONCENTRATIONS OF SODIUM. R.H. Alper and K.E. Moore, Department of Pharmacology/Toxicology, Michigan State University East Lansing, MI 48824.

The in vivo rate of dopamine (DA) synthesis in selected regions of the rat brain was estimated by measuring the rate of accumulation of DOPA 30 min after the administration of a decarboxylase inhibitor (NSD 1015, 100 mg/kg, i.p.). Water deprivation for up to 5 days had no effect on the rate of DA synthesis in the striatum or median eminence, which contain terminals of nigrostriatal and tuberoinfundibular neurons, respectively. On the other hand, water deprivation caused a gradual increase in the rate of DA synthesis in the posterior pituitary, which contains the terminals of tuberohypophyseal neurons; the increase attained statistical significance ($p<0.01$) by 72 hr. Increases in plasma sodium concentrations and hematocrit preceeded the change in DOPA accumulation; they were significantly elevated by 24 hr and increased progressively with time as water deprivation was continued.

In rats deprived of water for 3 days the increased DOPA accumulation in the posterior pituitary and the increased plasma sodium concentration returned to control values within 3 hr after allowing the animals access to water; the hematocrit did not return to control until 24-48 hr later. When rats deprived of water for 3 days were given access to 2% NaCl the hematocrit returned to control values within 48 hr while the rate of DA synthesis in the posterior pituitary and the sodium concentration in the plasma remained elevated.

An injection of hypertonic saline (15% of NaCl, 5 ml/kg) caused a marked but brief (less than 2 hr) increase in the plasma sodium concentration but did not alter the hematocrit. In these animals the rate of DOPA accumulation in the posterior pituitary was increased 24 hr later. Thus, the injection of hypertonic saline causes a brief pulse of increased plasma sodium which apparently triggers a delayed increase in DA synthesis in the terminals of tuberohypophyseal neurons.

The results suggest that in rats subjected to dehydration, rehydration or hypertonic saline injections there is a relationship between changes in DOPA accumulation in the posterior pituitary and the sodium concentration in the plasma, but not with changes in the hematocrit. Thus, the activity of tuberohypophyseal DA neurons appears to be regulated, in part, by activation of sodium but not of volume receptors. (Supported by USPHS Grant NS 09174 and 15911).

107.13 BRAIN STEM PROJECTIONS TO NEURONS OF THE CAUDAL NEUROSECRETORY SYSTEM: HRP TRACER STUDIES. J.P. O'Brien* & R.M. Kriebel* (SPON: J. Wells). Department of Anatomy & Neurobiology, The University of Vermont, College of Medicine, Burlington, Vermont 05405.

The caudal neurosecretory system of P. sphenops(molly) is an isolated population of neurosecretory cells located in the caudal most aspect of the spinal cord. From our earlier studies it was suggested that the caudal system provided a suitable structure in which to study the synaptic control of neurosecretory processes. Morphological and electrophysiological studies have shown that caudal neurosecretory system neurons of fishes receive synaptic input from descending spinal projections. Previously, we have shown several axon terminal types forming axosomatic and axodendritic synapses with the neurosecretory cells in the caudal system. In this study HRP tracer techniques were used to determine the source of the descending spinal cord projections. By determining the source of the synaptic input we may begin to determine possible neurochemical pathways that are involved in synaptic control of these neurosecretory cells and to elucidate how this unique piscine caudal neuroendocrine system functions in concert with cranial neuroendocrine systems. Animals were anesthetized with MS222 and the spinal cord exposed through small lateral incisions. HRP-polyacrylamide implants (15% HRP) were inserted into the spinal cord. Implants were placed at various spinal cord levels in twenty fish, each fish receiving only one implant. Animals survived for 60 hrs; tissue fixed in 2.5% glutaraldehyde, 2% paraformaldehyde in phosphate buffer, transversely and parasagittally sectioned at 50µ, and processed by the TMB technique of Mesulam. All sections were counterstained with a Nissl stain. Using light and darkfield microscopy axons containing HRP were traced cranially in the spinal cord. There appeared to be no specific orientation within the spinal cord of these descending axons, but most were located in ventral funiculi. In the brain stem two groups of neurons were observed containing HRP granules. At mid-medulla levels cells were seen bilaterally in a ventrolateral location. In other species, medullary cells at this location were identified as catecholaminergic. At approximately the junction of the diencephalon and midbrain, just below the subependyma, a second group of cells was found containing HRP granules. These cells were also bilaterally observed and were close to the midline. Our preliminary ultrastructural studies suggest that these cells are peptidergic. Studies are in progress to examine the proposal that caudal neurosecretory cells receive both peptidergic and catecholaminergic descending synaptic input. (Supported by PHS 5429-19-4).

107.14 MATURATION OF NEURONAL PROJECTIONS AND ULTRASTRUCTURE OF THE GOLDFISH PREOPTIC NUCLEUS. W.A. Gregory and C.D. Tweedle. Dept. of Anatomy, Michigan State University, East Lansing, MI. 48824

Recent evidence has shown the continuation of neurogenesis in certain CNS regions and substantial increases in gross brain size throughout the lives of fishes. The preoptic nucleus was studied to observe the development of a neurosecretory system. This nucleus begins rostroventrally with small subependymally located neurons. A spatially graded increase in cell size has led to ill-defined terminology for subdivisions (e.g. parvocellular, magnocellular). Several workers report that cells of all sizes in all regions can contain the same hormones. Using the retrograde transport of HRP we have reported that neurons in the rostralmost as well as more caudal portions of the nucleus project to the pituitary in small (2-8g) fish. Some cells in these regions, as well as those further caudad cells were shown to project to the spinal cord. Findings for fish up to 75g are similar, with correspondingly placed cells larger in larger fish. Also, the most caudal neurons which were only shown to project to the cord in small fish project to the pituitary as well in larger fish (whether via collaterals or separate cells is undetermined). The positional and maturational transition from small to large neurosecretory cells was examined ultrastructurally in the preoptic region of various sized fishes by sectioning each throughout its rostrocaudal extent. Some fish had previously received HRP IP injections or HRP applied to the transected spinal cord to label populations of preoptic neurons. Evidence to date supports a gradual transition in ultrastructure with variation in either cell position or body weight. Morphological variation may be related to the cells' degree of hormone synthesis and release and the requirement for total hormone release as influenced by body size. The most rostral cells were densely packed, with extensive neuron-neuron appositions. Spherical nuclei were surrounded by a thin rim of cytoplasm with a rudimentary complement of organelles (small profiles of rough ER and Golgi (G), and no dense core vesicles (DCVs)). Slightly caudad cells had small stacks of rough ER and an occasional DCV. Neuronal form gradually transformed, yielding a cell rich in cytoplasmic organelles typical of neurosecretory cells (extensive G, large stacks of rough ER, and many DCVs and lysosomes). Some "magnocellular" cells had diameters < 10µm in small (2g) fish. Extensive neuronal appositions were still seen caudally, but neuropil was more evident between neurons. Richer cytoplasm was seen in corresponding larger cells of larger fish. The more caudal and largest cells of large fish had extremely invaginated nuclei, while other preoptic neurons of these or smaller fish had spherical nuclei.

(Supported by BRSG Funds).

107.15 LIGHT AND EM LOCALIZATION OF HRP-INJECTED, PHYSIOLOGICALLY IDENTIFIED MGC NEUROENDOCRINE CELLS IN GOLDFISH PREOPTIC NUCLEUS. T.A. Reaves, Jr., R. Cumming*and J.N. Hayward. H. Houston Merritt Electron Microscopy Lab., Dept. Neurology and Neurobiology Program, Univ. North Carolina, Chapel Hill, N.C. 27514.

By intracellular recording, cell marking with fluorescent dyes combined with immunocytochemistry, we described three chemical magnocellular (MgC) neuroendocrine cell types in the goldfish preoptic nucleus (NPO; JCN 193:777 '80). To further characterize the structure and connections of these physiologically identified NPO neurons, we report here on intracellular horseradish peroxidase (HRP) injection for light and electron microscopic analysis.

We recorded intracellularly from adult goldfish (Carassius auratus) pituitary-antidromically identified NPO neurons using 3% HRP-filled glass micropipettes. We injected HRP into identified cells with 5-10 nA cationic current for 30-180 sec, then vibratome sectioned the fixed brain at 50-100 uM. We processed the hypothalami with either DAB or TMB for light or EM study.

By light microscopy the HRP-filled MgC resembled Golgi impregnated neurons with a greater complexity of processes than found previously with fluorescent dye-marking. Densely labeled axons and dendrites projected to several sites: 1) to other NPO neurons; 2) to small blood vessels and capillaries; 3) to the third ventricular ependyma; 4) to fiber tracts in the preopticohypophysial tract, in the lateral forebrain bundle, in the medial forebrain bundle and others. We occasionally observed a lightly labeled large perikarya lying adjacent to a heavily labeled MgC, suggesting cell-to-cell connections.

Electron microscopic examination of these MgC showed a dense HRP-reaction product in perikarya and processes. Heavily labeled perikarya reveal elaborate networks of endoplasmic reticulum, somal appendages or spines, axosomatic contacts from unlabeled neurons and close somal apposition of unlabeled profiles or rarely a large adjacent lightly labeled perikarya. Most HRP-labeled perikarya and processes are separated from brain capillary endothelium by a thin glial sheath. HRP-labeled dendrites show heavily labeled spines or appendages and have axodendritic contacts from unlabeled neurons. HRP-labeled axons contact unlabeled perikarya, axons and dendrites.

These results demonstrate that MgC neuroendocrine cells in goldfish NPO have complex connections to other neurons, to brain capillaries, to ventricular ependyma and to the pituitary. We conclude that these data support non-endocrine as well as endocrine functions for MgC neurons. (Supported by NIH Grant NS-13411 and a Neurobiology Fellowship to R.C.)

107.16 NEURAL INNERVATION AND CONTROL OF THE TESTES: A ROLE FOR THE PARAVENTRICULAR NUCLEUS? D.M. Nance. Dept. of Anatomy, Fac. Med., Dalhousie University, Halifax, Nova Scotia, B3H 4H7.

Hemiorchidectomy of prepubertal male rats produces a selective increase in serum FSH with little or no change in serum LH levels. Previously, we found that unilateral hypothalamic knife cuts prevent this rise in serum FSH following hemiorchidectomy on the ipsilateral, but not the contralateral side (Neurosci. Abst. 189.8, 1980). This endocrine difference between ipsi- and contralateral hemi-castrated rats with identical brain surgeries can only be accounted for by postulating that FSH release is, in part, under direct neural control.

Using the fluorescent tracer Bisbenzimide (Bb), cell bodies providing afferent nerves to the testes were identified in dorsal root ganglions (DRG) T_{12} - L_4. All labelled cells were ipsilateral and the L_2 DRG generally showed the largest number of labelled neurons. Efferent nerves showed a similar spinal distribution and cell bodies were localized in lamina IX in the anterior horn of the spinal cord. Bilateral thoracolumbar cord injections of Bb produced extensive retrograde labelling of neurons in the paraventricular nucleus (PVN) of the hypothalamus. Labelled neurons were also localized in a lateral and posterior direction with respect to the PVN. Unilateral retrochiasmatic knife cuts (1.5 mm radius) located anterolateral to the PVN dramatically reduced or eliminated the labelling of PVN neurons on the ipsilateral side following bilateral Bb injections into the thoracolumbar cord. However, labelled neurons were still present lateral to the knife cuts. Thus, PVN neurons innervating the thoracolumbar cord project primarily in a lateral and somewhat posterior direction. These results provide direct evidence on afferent and efferent innervation of the testes and further suggest that the PVN may be involved in the endocrine response to hemi-castration. Research supported by MRC Grant # MA 6956.

107.17 VASOPRESSIN: A REQUIREMENT FOR ENDOTOXIN-INDUCED FEVER IN THE BRATTLEBORO RAT. P.C. Eagan*, W.L. Veale and K.E. Cooper. Department of Medical Physiology, Faculty of Medicine, University of Calgary, Calgary, Alberta, Canada T2N 1N4.

The Brattleboro (DI) rat, a genetic mutant of the Long Evans strain, lacks the ability to synthesize the neuropeptide arginine vasopressin (AVP). The DI rat does not develop fever in response to bacterial endotoxin. These experiments were done to examine the possibility that the absence of AVP is the cause of this lack of a febrile response.

Male, DI rats (300 - 350 gm), which are homozygous recessive for the genetic mutant, were used in these experiments. Stainless steel guide cannulae were implanted stereotaxically into the brains of each animal and were directed towards the lateral cerebral ventricles. The post-operative recovery period was seven days. Colonic temperature was measured continuously using a thermistor probe inserted 9 cm into the rectum and taped to the base of the tail. Colonic temperatures were recorded for two hours prior to each injection and for three hours following. On Day 1, each animal received an injection into a lateral cerebral ventricle of 1 µg of the bacterial pyrogen derived from Salmonella abortus equi (SAEP). The volume injected centrally was 10.0 µl and the vehicle used was pyrogen-free sterile saline. No significant change in body temperature was observed during the three hours following injection, the time during which the fever would be expected to be at its peak. The following day (Day 2), each animal received a subcutaneous injection of 1.0 Units of AVP (Pitressin) in 0.5 ml of sterile oil suspension. Thirty minutes later, 1 µg of SAEP was injected intraventricularly. During the three hours following SAEP administration, no significant change in body temperature had occurred. This procedure was repeated on Days 3 and 4. On Day 3, the increase in colonic temperature was 0.51 ± 0.21°C, and on Day 4, the rats developed a fever of 1.15 ± 0.20°C during the three hours following SAEP. On Days 5, 6, and 7, the animals received 1 µg of SAEP, but the AVP injection was replaced by 0.5 ml of sterile oil subcutaneously. The febrile response on Day 5 was reduced (0.59 ± 0.12°C) and no significant change in body temperature occurred on Days 6 and 7.

The appearance and absence of the febrile response in the DI rat corresponds to the gradual rise and fall in circulating levels of AVP produced by the subcutaneous injections of the peptide on Days 2, 3, and 4. These results suggest that AVP must be present in the body to produce endotoxin fever in the DI rat.

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107.18 CENTRAL SEROTONERGIC CONTROL OF SODIUM EXCRETION. J.M. Stein, R.W. Lind, and A.K. Johnson. Department of Psychology and the Cardiovascular Center, University of Iowa, Iowa City, IA 52242.

Our understanding of the CNS neurochemical control of body sodium balance is still developing. Experiments involving central cholinergic and catecholaminergic stimulation have implicated these neurotransmitters in the control of sodium excretion ($U_{Na}V$) and vasopressin release. The present studies indicate a serotonergic (5-HT) component in the central control of sodium excretion.

We have previously reported (Stein, Lind, & Johnson, Neurosci. Abst. 6:228, 1980) that peripherally applied p-chloroamphetamine (PCA) (2.0-10.0 mg/kg) produces a natriuresis in the rat. Since this natriuresis was also found following intraventricular (IVT) infusions of 200-600 µg of PCA, some component of this effect is likely to be centrally mediated. The following experiments were performed to investigate this phenomenon further.

In Experiment 1, rats were implanted with intraventricular cannulae, femoral arterial and venous catheters, and urinary bladder catheters. A hydrating solution (10mM NaCl, 4mM $NaHCO_3$, 130mM Glucose) was continuously infused (0.2 ml/min) into the femoral vein during the experiment. Following a 2-3 hr equilibration period, the unanaesthetized rats were administered PCA (400 µg) intraventricularly with or without pretreatment with the serotonin reuptake blocker, fluoxetine (10 mg/kg, ip). The increased $U_{Na}V$ seen following PCA was totally abolished by fluoxetine. The antidiuretic and acute hypertensive actions of IVT PCA were unaffected or enhanced by fluoxetine pretreatment. Since fluoxetine treated rats could still show a natriuresis following IVT angiotensin II, a direct effect of fluoxetine on the kidney was unlikely. PCA has been shown to release 5-HT from serotonergic nerve terminals and this action is inhibited by 5-HT reuptake blockers (Ross & Kelder, Acta Physiol. Scand. 99:27-36, 1977). It appears that increases in synaptic 5-HT concentrations are necessary for the PCA induced natriuresis to be seen.

In Experiment II, rats were similarly implanted but administered 5-HT (0,1,5,20 or 100 µg) IVT. Dose dependent natriuretic responses were seen. Small (0-20 mmHg) increases in blood pressure and antidiuretic responses were observed at the highest doses tested.

Taken together, these findings suggest that stimulation of central 5-HT receptors increase sodium excretion independent of blood pressure elevation and antidiuretic responses.

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107.19 PSYCHOPHARMACOLOGICAL ACTION OF BETA 2 STIMULANTS AGENTS AND VASOPRESSIN. B. Delbarre, G. Delbarre, O. Christin *. Lab. Chir. Exp., Fac. de Méd., 37032 Tours Cedex, France.

Pharmacological (FRANCES H.J. and al., J. Pharm., Paris, 1978 9,1-25 - B. DELBARRE and al., Mol. Biol. Pharmacol. of Cyclic Nucleot., 1978), and clinical studies (WIDLOCHER and al., Lancet 1977, 767) suggest that beta adrenergic agents have antidepressant properties. The precise mechanism of their antidepressant action remains to be elucitated. It is known that beta sympathomimetic amine : isoprenaline stimulates the renin angiotensin system and also vasopressin (VP) release (W. KNEPEL ans al., J. Card. Pharm., 1980, 2, 815).
GOLD P.W. and al., Lancet, 1978, 1233, makes this peptide (VP) a suitable candidate for involvement in complex behavioural syndromes. But no pharmacological data are available on this subject.
To investigate this action, we have used the antagonism to reserpine induced hypothermia in mice. The drugs were administered 4 hours after reserpine. Intraventricularly (IVL), lysine VP (SANDOZ) 0,01 to 0,1 U/mouse and arginine VP (SIGMA) 0,01 to 0,1 U prevent hypothermia induced by reserpine. Intraperitoneal (IP) 5 to 50 U/kg of lysine and arginine VP are ineffective. Angiotensin II (SIGMA) IVL 0,01 to 2 µg/kg/mouse and 5 to 20 µg/kg IP is ineffective. Drugs known to release VP from hypophysis, histamine (1 to 10 µg/kg/mouse),dimaprit (1 to 10 µg/kg/mouse), Db C.AMP (20 to 100 µg/kg/mouse), met.enkephalin (1 to 20 µg/kg/mouse) have no action.
These results suggest that beta 2 stimulant agent act on VP located in brain. Indeed, recently, S.M. GLICK (Lif. Sci., 1980, 27, 1103) found VP in amygdala, septum, thalamus and striatum of rat brain.
F. HIRATA and al. (Proc. Nat. Acad. Sci., 1979, 76, 6, 2640) reported that stimulation of the beta adrenergic receptor increases phospholipid methylation. To test this hypothesis, we have used a prostaglandin synthetase inhibitor : lysine acetylsalicylate. This drug IVL (50 µg/kg/mouse) does not prevent reserpine induced hypothermia, but increases the action of salbutamol (1 mg/kg i.p.) if administered 15 min. before.
It may be suggested that beta 2 stimulant agent exerts its effect in brain through a adenylcyclase system in connection with metabolism of phospholipids and arachidonic acid.

107.20 MECHANISM OF ANTIDIURETIC EFFECT OF BETA ADRENERGIC STIMULATION. G. Delbarre, B. Delbarre. Lab. Chir. Exp., Fac. de Méd., 37032 Tours Cedex, France.

It is considerable amount of experimental data which suggest that the adrenergic system is involved in the control of water metabolism. The effect of vasopressin (VP) appears to be mediated by adenylcyclase, an enzyme which in several tissues has been shown to be activated by beta adrenergic stimulation. Further support to this hypothesis is derived from studies in the rat, and cat which have demonstrated that beta adrenergic stimulation with intravenous isoproterenol (ISO) is associated with an antidiuretic response. The mechanism responsible for ISO antidiuresis is not clear. Data suggest that the antidiuretic effect of ISO is due to a direct action at the renal tubule rather than to the release of ADH. J. LEVI and al. (Am. J. Phys. 1971, 221-6, 1728) have demonstrated that ISO causes an antidiuresis by using the Brattleboro strain of rats which have no circulating or stored VP. ISO exerts its effect through the stimulation of beta adrenergic receptor in the renal tubule since the beta adrenergic antagonist propranolol abolished this effect. Recently, F. HIRATA and al. (Proc. Nat. Acad. Sci., 1979 76, 6, 2640) found that stimulation of the beta adrenergic receptor increases phospholipid methylation.
To investigate the action of a beta 2 stimulant agent, we have used salbutamol.
To investigate the enhancement of phospholipid methylation by stimulation of beta 2 receptor, we have used indomethacin, a prostaglandin synthetase inhibitor. In the normal rats, indomethacin (5 mg/kg per os), salbutamol (5 mg/kg i.p.) have no action on the diuresis. In contrast, in association the decrease of diuresis is significant. In the Brattleboro, indomethacin (5 mg/kg per os), salbutamol (0,1 mg/kg i.p.) decrease diuresis and in association have a more pronounced effect.
These results suggests that there is an interaction between stimulation of the beta adrenergic receptor and phospholipid methylation. Indeed, blockade of prostaglandin synthesis by indomethacin increases action of beta 2 stimulant : salbutamol. Moreover, the different action in Brattleboro and normal rats may be explained by the fact that Brattleboro have a modification of prostaglandin synthesis (WALKER L.A. and al., Am. J. Physiol., 1978, 4, 3, F. 180.

108.1 THE EFFECT OF α-MSH ON THE IN VIVO AND IN VITRO RELEASE OF PROLACTIN AND LH IN THE RAT. O. Khorram* and S.M. McCann. Department of Physiology, University of Texas Health Science Center at Dallas, Dallas, Texas 75235.

The neurointermediate lobe tridecapeptide, α-MSH, has been localized throughout the brain, with the hypophysiotropic area of the hypothalamus containing some of the highest levels (O'Donohue et al., Brain Research 17: 101, 1979; Oliver and Porter, Endocrinology 102: 697, 1978). This finding along with its detection in the portal plasma of the rat suggests that α-MSH may act as a modulator of anterior pituitary function (Oliver et al., Endocrinology 101: 598, 1977). The following study was conducted to examine this hypothesis in relation to the secretion of LH and prolactin. Long-term ovariectomized (OVX) rats bearing chronically implanted third ventricular cannulae were employed. Blood was withdrawn from jugular cannulae in unanesthetized freely moving animals before and after intraventricular (IVT) injection of 2 µl of 0.9% NaCl, 100 nanograms or 2 micrograms of α-MSH dissolved in saline. No effect on plasma LH and prolactin levels was seen following injection of saline or the lower dose of MSH. The higher dose of α-MSH inhibited the release of prolactin and LH within ten minutes following its injection as indicated by a lowering of plasma levels of the hormones. The 2 µg dose was also effective in inhibiting prolactin release in OVX rats primed 72 hours earlier with 50 µg of estradiol benzoate (s.c.), although LH release was not affected in these animals. The inhibitory effect of α-MSH on prolactin release could be blocked by the intravenous (i.v.) infusion of the dopamine receptor blocker, spiroperidol (.1 mg/kg body wt.), 1 hour prior to the IVT injection of α-MSH. Direct i.v. injection of 5 µg α-MSH in 0.9% NaCl slightly inhibited LH release in OVX rats one hour after its injection while prolactin release was unaffected. Hemipituitaries from male and OVX female rats were incubated with various doses of α-MSH. The secretion of neither LH nor prolactin was affected in this system. In conclusion, we have demonstrated that IVT injection of α-MSH inhibits basal and stimulated prolactin release possibly via a dopaminergic mechanism. α-MSH also inhibits the release of LH; this effect was blocked by estrogen. The failure of α-MSH to affect the release of LH or prolactin in vitro implicates a central neural site of action for this melantropic peptide. Supported by NIH Grants AM-10073 and HD-09988 and the Ford Foundation.

108.2 MARKED CHANGES OF SOMATOSTATIN CONTENT IN DIFFERENT HYPOTHALAMIC AREAS OF THE RAT DURING PROESTROUS AND THEIR INVERSE RELATION TO SERUM PROLACTIN U.A.Knuth*,G.S. Sikand*,F.Casanueva*,V.Havlicek and H.G.Friesen* Deparment of Physiology, University of Manitoba, Winnipeg, Manitoba R3E 0W3

To investigate physiological changes in Somatostatin content of the hypothalamus on day of proestrous groups of 10 rats were decapitated every two hours from 8.00 to 18.00. The heads were subjected to microwave irradiation to prevent degradation of peptides. Anterior pituitary (13.3±0.33mg) and median eminence (0.53±0.33mg) were removed. Punch biopsies were obtained from the anterior (12.9±0.33mg) and posterior hypothalamus area (19.7±0.27mg) and the region of the arcuate nucleus (2.9±0.11mg). All samples were homogenized in 0.1N acetic acid by an ultrasound homogenizer and boiled for 10min. Somatostatin was measured by a specific RIA and is expressed as µg/g of tissue. Serum prolactin (ng/ml) was determined in samples obtained from trunk blood. The following table gives means and SEM of tissue concentration of Somatostatin and serum prolactin. Significant differences from the 8.00 values are indicated by *(p=0.01) and **(p=0.001). These values are derived by a SCHEFFE test after an analysis of variance was performed, which was highly significant for all areas except the median eminence.

daytime	8.00	10.00	12.00	14.00	16.00	18.00
pituitary	2.6 (0.93)	0.80 (0.80)	1.09 (0.24)	0.18 (0.02)	0.68 (0.27)	0.18 (0.18)
anterior hypothal.	10.9 (2.76)	7.63 (1.07)	5.28 (0.65)	0.87* (0.25)	2.12* (0.84)	0.53** (0.54)
posterior hypothal.	4.3 (1.00)	3.77 (0.55)	2.35 (0.48)	0.21** (0.04)	1.46 (0.65)	0.37* (0.11)
arcuate nucleus	20.9 (3.69)	13.28 (1.79)	20.11 (3.14)	7.07 (1.34)	12.14 (3.34)	9.86 (1.27)
median eminence	314 (34.1)	316 (94.1)	273 (44.5)	342 (30.6)	184 (20.0)	220 (13.4)
serum prolactin	9.6 (1.07)	23.3 (1o.6)	52.2* (18.9)	732** (169.)	834** (119.)	493** (33.6)

Somatostatin in anterior and posterior hypothalamus and arcuate nucleus was inversely correlated with prolactin levels (r=-.45, -.42 and -.35, respectively).

108.3 STUDIES OF PROLACTIN-RELEASING ACTIVITY IN PLASMA AND HYPOTHALAMIC EXTRACTS OF MAMMALIAN AND AVIAN SPECIES. M. Hochstrasser*, L-Y. Huang* and J. Rabii (SPON: C.H. Page). Department of Physiology and the Bureau of Biological Research, Rutgers University, New Brunswick, N.J. 08903.

It has become established that prolactin secretion is under tonic hypothalamic inhibition in mammalian species, whereas in birds, the influence of the hypothalamus appears to be primarily stimulatory. As prolactin-releasing activity (PRA) has been demonstrated in human and rat plasma, we undertook to examine the plasma of an avian species for similar activity. Young male domestic fowl (8-week-old) of the White Leghorn strain and adult male rats of the Sprague-Dawley strain were used in this study. The PRA of various extracts was tested in in vitro dispersed pituitary cell cultures. Both chicken and rat prolactin were measured by their respective radioimmunoassays. A dose-dependant PRA was shown to exist in methanol extracts of both chicken and rat plasma. Inter-species tests indicated that plasma extract from either rats or chickens was effective in inducing prolactin release from cell cultures of the opposite species. Chicken hypothalamic extract was markedly effective in releasing chicken prolactin both in vitro and in vivo. Chicken hypothalamic extract, however, was not as effective in releasing in vitro rat prolactin as it was chicken prolactin. The avian plasma PRA was further characterized by a number of physical tests. Using a cascade ultrafiltration experiment it was shown that the circulating PRA had a molecular weight between 1,000 and 10,000. Furthermore, the reconstituted extract was heated to 100°C for 10 minutes which showed that the PRA was only partially heat-stable. Finally, the reconstituted plasma extract was subjected to trypsin digestion and was observed to be resistent to proteolysis. In a separate study the PRA of plasma from young birds was compared with that from older animals (10-month-old). It was observed that the young birds had a significantly higher plasma PRA than the adults. These results suggest the presence of PRA in plasma of birds in larger apparent levels than the PRA of mammalian plasma. Furthermore, younger birds with high circulating prolactin levels have higher PRA than old birds with a low secretory rate of prolactin. Finally, this plasma PRA appears not to be species specific. (Supported by the Bureau of Biological Research)

108.4 TUBEROINFUNDIBULAR DOPAMINE NEURONS IN THE LACTATING RAT ARE NOT RESPONSIVE TO INCREASED PROLACTIN CONCENTRATIONS. K.T. Demarest, D.W. McKay*, G.D. Riegle* and K.E. Moore. Michigan State University, East Lansing, MI 48824.

The results of previous studies have demonstrated that tuberoinfundibular dopamine (TIDA) neurons are activated during periods of increased serum concentrations of prolactin (see Fed. Proc. 39: 2912, 1980). It was thus unexpected to observe that the increased release of prolactin during lactation was associated with decreased TIDA neuronal activity compared to values observed during diestrus. TIDA neuronal activity was estimated by the rate of DOPA accumulation in the median eminence 30 min following NSD 1015 (100 mg/kg, ip) and expressed as ng DOPA/mg protein/30 min. Serum prolactin was continuously elevated in lactating rats (Day 12 postpartum) in the presence of their pups (580±79 ng/ml); 4 hr following pup removal prolactin decreased (39±6 ng/ml) to values observed during diestrus and remained suppressed for at least 144 hr. DOPA accumulation in the median eminence of lactating rats in the presence of pups was significantly lower (7.0±0.8) than that observed during diestrus (15.7±0.9). In addition, the decrease in serum prolactin induced by pup removal was not associated with an increase in TIDA neuronal activity, but rather DOPA accumulation in the median eminence remained suppressed for at least 144 hr. It would appear the TIDA neurons are inhibited during lactation to allow serum prolactin to increase. Studies were undertaken to determine if TIDA neurons in the lactating rats are still responsive to prolactin. Lactating (Day 6-8 postpartum) and regularly cycling rats were implanted with cannula guides in the lateral cerebroventricle 4-7 days prior to sacrifice. Rat prolactin (RP-2-B; 10 µg/10 µl) or 0.9% saline vehicle was administered via intraventricular (icv) infusion 12 hr prior to sacrifice in 1) rats during diestrus, 2) lactating rats with pups and 3) lactating rats which had pups removed 12 hr prior to prolactin administration. Rates of DOPA accumulation in the median eminence of these rats were:

	Diestrus	Lactating (pups)	Lactating (no pups)
Vehicle	15.7±1.6	9.7±0.8	11.6±1.6
Prolactin	22.9±1.9*	11.0±0.8	11.9±1.4

DOPA accumulation in the median eminence of diestrus rats was significantly increased by the icv administration of prolactin. On the other hand, icv prolactin administration had no effect on DOPA accumulation in the median eminence of either of the groups of lactating rats. These results indicate that the prolactin feedback mechanism that normally activates TIDA neurons does not operate in the lactating rat. (Supported by NS09174 and a MSU-COM Research grant.)

108.5 HYPOTHALAMIC LHRH IN OVARIECTOMIZED RATS FOLLOWING IMMEDIATE OR DELAYED ESTROGEN TREATMENT: ANALYSIS BY RADIOIMMUNOASSAY (RIA) AND IMMUNOCYTOCHEMISTRY(ICC). D. A. Damassa*, J. C. King, and K. Elkind-Hirsch. Dept. of Anatomy, Tufts Med. Sch., Boston, MA.

To assess the influence of gonadal steroids on the form, quantity and distribution of LHRH within the hypothalamus, LHRH immunoreactivity was examined by RIA and ICC using characterized antisera. Female rats (CD) exhibiting regular estrous cycles were ovariectomized(ovxed) 2 days after vaginal estrus, and implanted with Silastic capsules (3.18mm o.d. X 1.57mm i.d. x 3 mm l) containing estradiol (E2):cholesterol (1:1) either at the time of ovariectomy (ovx) or 3 weeks after ovx. Control ovxed animals received empty capsules. One day or 2 weeks after implantation of the capsules, blood was collected by decapitation. The preoptic-hypothalamus was dissected into anterior (AH) and posterior(PH) regions, homogenized in cold 2N acetic acid, lyophilized and stored at -20°C. LHRH was assessed by RIA using antisera which require the entire decapeptide (TN-R42), the interior sequence(AA-743), or the C-terminal sequence(IJ-29) for high affinity binding. Each group consisted of at least 5 animals and results were analyzed using a 1-way ANOVA. All three antisera yielded the same results for LHRH immunoreactivity in the PH, therefore, only results with TN-R42 are shown. Animals ovxed and implanted immediately with E2 capsules had a high PH LHRH content(D-1: 4.14 ± .41 ng/region X ± SE, or D-14: 4.92 ± .30 ng/region) and low serum LH levels (D-1:37 ± 7 ng/ml RP-1 or D-14: 64 ± 23). Three weeks after ovx, PH LHRH was reduced to 2.24 ± .24 ng/region (ovx ctl) and was not increased by one day of E2 treatment (D-1: 2.48 ± .41 ng/ region) although serum LH was significantly reduced (ovx ctl: 636 ± 67 ng/ml vs D-1:320 ± 40). Two weeks of E2 treatment partially reversed the castration-induced changes in PH LHRH (3.76 ± .48 ng/region) and LH. In contrast to the PH, no differences in LHRH content of the AH were found following ovx or after immediate or delayed E2 therapy. LHRH immunoreactivity of the AH with IJ-29 was significantly lower than that measured with either AA-743 or TN-R42 for all treatment groups. To correlate RIA and ICC data, female rats were ovxed, treated with E2 as described above, and then perfused under Nembutal anesthesia. Fixed brains were removed, Vibratome sectioned (50μ) and incubated with antisera IJ-29 or AA-743 in the PAP technique. Immunoreactive LHRH in the PH was localized in the median eminence and the amount of reaction product correlated well with RIA values. (Supported by Grants HD 14356 to DAD and HD00352 and HD14092 to JCK).

108.6 EXPOSURE TO FEMALE ODOR ALTERS LEVELS OF LUTEINIZING HORMONE-RELEASING HORMONE AND LUTEINIZING HORMONE IN THE MALE GOLDEN HAMSTER. Heidi S. Phillips*, Fred L. Jackson*, and Beng T. Ho (SPON: P. Kralik). University of Texas Graduate School of Biomedical Sciences and Texas Research Institute of Mental Sciences, Houston, Texas 77030.

The odor of female hamster vaginal discharge elicits in male hamsters behavioral and endocrine responses which resemble the effects of systemically administered luteinizing hormone-releasing hormone (LHRH). The presence of LHRH-like immunoreactivity in olfactory pathways of the golden hamster suggests that LHRH may facilitate behavioral or endocrine responses to olfactory stimuli. The present study examined the effects in male hamsters of female odor on regional LHRH content and serum luteinizing hormone (LH) levels. Responses to female hamster vaginal discharge were compared to those elicited by peanut oil, an attractive but sexually neutral substance.

Sexually naive male golden hamsters were transferred from home colony cages into individual cages one hour prior to olfactory exposure. Cotton swabs saturated with female hamster vaginal discharge or peanut oil were placed in each cage and animals were decapitated at intervals from 2 to 45 minutes later. Acid extracts of hypothalamus, olfactory bulbs, and amygdala were prepared from each animal and assayed individually for LHRH by radioimmunoassay. Serum LH was also determined by radioimmunoassay.

Exposure to female odor transiently elevated serum LH, while peanut oil exposure produced no significant change. Serum LH was elevated threefold in female odor-treated animals vs. peanut oil-treated animals at 6 minutes post-exposure. By 12 minutes LH values did not differ between the two groups. Hypothalamic LHRH varied in an inverse manner with serum LH. Female odor decreased hypothalamic LHRH while peanut oil had no effect. Preliminary results indicate that LHRH in olfactory bulbs and amygdala does not respond differentially to the two odors investigated. The LHRH pattern observed in these extrahypothalamic sites following exposure to both odorous substances may be either a non-specific reaction to novel stimuli or a specific reaction to attractive olfactory stimuli. The results of this study demonstrate that in sexually naive male hamsters, female odor causes a decrease in hypothalamic LHRH and a concommitent increase in serum LH that is not produced by another novel, attractive odor.

108.7 THE EFFECT OF N-METHYL ASPARTIC ACID (NMA) AND MONOSODIUM GLUTAMATE (MSG) ON PLASMA LH LEVELS IN THE OVARIECTOMIZED ESTROGEN-PROGESTERONE PRIMED RAT. A.J. Carrillo and O. Alcantara*, Department of Anatomy, The University of Texas Health Science Center at San Antonio, San Antonio, Texas, 78284.

There exists considerable evidence that NMA stimulates the release of LH in male rats (Shainker & Cicero, Br. Res. 184:425, 1980). The present study was designed to examine the effect of NMA and MSG on LH release in the female rat. Adult female rats were ovariectomized; 4-6 weeks later they were anesthetized with ether and an i.v. cannula was permanently implanted into the jugular vein. At the same time the rats were injected (s.c.) with 50μg of estradiol benzoate (EB) and 25mg of progesterone (P). Three days after steroid priming the animals were injected subcutaneously or intravenously with NMA (25mg/kg) and MSG (1g/kg) dissolved in PBS or with PBS alone. Blood samples were obtained through the i.v. cannula from the freely moving awake rat at 0, 7.5, 15, 30, 45, and 60 min. post injection for plasma LH determination (RIA-NIAMDD). For comparison, intact 25 day old male rats were also injected (s.c.) with the same dosages of NMA and MSG as the females and decapitated 7.5 minutes post injection and trunk blood collected for plasma LH and FSH determination. A PBS injected and intact group served as controls. Subcutaneous injection of NMA and MSG in adult female rats had no significant effect on plasma LH levels when compared to the PBS group. In the 25 day old rats s.c. injection of NMA resulted in a significant ($p<0.01$) elevation of plasma LH but not FSH when compared to controls. MSG had no significant effect on gonadotropin secretion. Intravenous injection of NMA and MSG to ovariectomized EB-P primed rats resulted in a significant ($p<0.01$) elevation of plasma LH levels when compared to the PBS group. NMA injection produced a much greater ($p<0.05$) rise in plasma LH levels than MSG. Incubation of hemipituitaries from ovariectomized EB-P treated rats in medium 199 with NMA (5.0×10^{-3}M) resulted in a significantly ($p<0.05$) greater amount of LH released into the medium when compared to hemipituitaries in medium 199 alone. These data suggest that NMA is a more powerful stimulator of LH release than MSG in the young male as well as the adult female; however, NMA may in part stimulate the release of LH by acting directly on the pituitary gland. (Supported by USPHS Grant NS 14581 and 15454.)

108.8 FSH-RELEASING ACTIVITY IN THE POSTERIOR PORTION OF THE RAT MEDIAN EMINENCE. H. Mizunuma*, W.K. Samson*, M.D. Lumpkin* and S.M. McCann (SPON: M. Kaufman). Department of Physiology, University of Texas Health Science Center at Dallas, Dallas, Texas 75235.

Immunohistochemical studies have shown that LHRH nerve terminals are predominantly localized in the rostral portion of the median eminence (ME) in every species examined thus far. That these terminals are involved mainly in stimulating LH release from the pituitary gland has been confirmed by studies from our laboratory (Ojeda et al., 1977), in which greater LH than FSH release was observed after implantation of prostaglandin E_2 into this area of the rat brain. Furthermore, greater FSH than LH release followed implantation of the prostaglandin into the posterior portion of the ME suggesting the possibility that these implants released FSH-releasing factor. To test this hypothesis, the anterior half of the ME (aME) and the posterior half of the ME (pME) were removed separately from adult male rats and extracted in 0.2 N acetic acid. The content of immunoreactive (IR)-LHRH in the extracts was almost twice as high in the aME as in the pME (1942 pg/5 aME and 1038 pg/5 pME, respectively) in agreement with the immunohistochemical studies. IR-LH and FSH-releasing activities were measured in an in vitro incubation in which 8 hemipituitaries from adult male rats were incubated for 6 h at a dose of 5 tissue equivalents. The results were expressed as LH or FSH release (ng RP-1/ml/mg anterior pituitary wet weight). LH-releasing activity of the aME was 233.6 ± 11.3 which was significantly greater than that of the pME (179.8 ± 13.5) ($P<0.05$), whereas there were no differences in FSH-releasing activity (56.4 ± 3.5 and 57.5 ± 2.6). A significant dose-related increase in FSH release was noted in this system when 1 and 2 ng of synthetic LHRH were tested which indicates that the assay was sensitive to different amounts of LHRH with regard to FSH-releasing action. Therefore, the results demonstrate that the pME has greater FSH-releasing activity than can be accounted for by the LHRH content. The additional FSH-releasing activity is presumably due to a distinct FSH-releasing factor. Supported by NIH Grants AM-10073 and HD-09988 and the Ford Foundation.

108.9 SYNAPTIC MECHANISMS MEDIATING COPULATION-INDUCED OVULATION IN THE RAT. R.E. Leipheimer*, T.P. Condon* and J.J. Curry, Dept. of Physiology, The Ohio State Univ., Columbus, OH. 43210

The synaptic mechanisms involved in mediating copulation induced ovulation (CIO) in rats have never been specifically investigated. The present experiments were designed to: (1) investigate protocols influencing CIO in pentobarbital-blocked proestrus rats; and (2) investigate synaptic mechanisms involved in mediation of CIO by the administration of synaptic blocking agents. Only rats demonstrating two consecutive 4 day estrus cycles were used for these experiments. Rats were given an ovulation blocking dose of pentobarbital (35 mg/kg)ip at 1200 hrs on proestrus. That evening one of the following procedures was utilized to induce ovulation: limited mating (maximum of 30 mounts), all night mating, or glass rod stimulation of the vagina and cervix (2 or 5 min). Rats were examined the following morning for the presence of tubal ova. Results of this experiment demonstrated that all night mating was the most successful procedure utilized, and resulted in 100% ovulation in rats that mated. Glass rod stimulation was somewhat effective (25-50% ovulation), while limited mating was ineffective (0% ovulation). The next experiment examined neural synaptic mechanisms involved in mediating CIO. Pentobarbital-blocked proestrus rats were given a sc injection of one of the following synaptic blocking agents at 1700 hrs: phenoxybenzamine (20 mg/kg); propranolol (20 mg/kg); pimozide (2 mg/kg); methysergide (100 mg/kg) or atropine (70 mg/100g). Females were placed with males between 2000 and 2100 hrs and were allowed to mate all night. Females were separated from males at 0900 hrs, and presence of spermatozoa in the vaginal smears was taken as evidence of mating. Oviducts were subsequently removed and examined for presence of tubal ova. Results showed that administration of phenoxybenzamine, propranolol or pimozide did not interfere with CIO. Methysergide treatment was found to completely block CIO. Also, LHRH (200 ng) administration to methysergide treated rats resulted in ovulation. Atropine administration resulted in a loss of mating behavior and therefore, those animals did not ovulate. In addition, atropine also blocked glass rod induced ovulation. In summary, methysergide treatment completely blocked CIO, suggesting that serotonin may be important in mediating this response. And because LHRH administration to methysergide treated rats resulted in ovulation, a CNS site of action for methysergide was indicated. Because atropine administration blocked mating behavior and glass rod induced ovulation, acetylcholine was implicated to be important in the mediation of mating behavior and reflex ovulation.

108.10 THE LHRH NEURONAL SYSTEM IN FEMALE RATS: RELATION TO THE MEDIAL PREOPTIC NUCLEUS (MPN). Ei Terasawa and Gary A. Davis* Wis. Reg. Primate Research Ctr., Univ. of Wis., Madison, WI. 53706

Previously we have reported that in the preoptic and suprachiasmatic regions small lesions restricted to the MPN and the immediately adjacent area are effective to block spontaneous ovulation, to induce persistent vaginal estrus, and to abolish the progesterone-induced LH surge in female rats. Since the MPN is located immediately caudal to the organum vasculosum laminae terminalis (OVLT) where LHRH fibers are reported to be dense, these effects of MPN lesions may be due to interruption of the LHRH neuronal system whose axonal endings terminate in the median eminence (ME). In the present experiments LHRH neurons were immunocytochemically stained in spontaneous and MPN lesion-induced persistent estrous rats, as well as in intact proestrous and metestrous rats using Arimura's anti-LHRH (No. 734) and Sternberger-Meyer's peroxidase-antiperoxidase complex. Anesthetized animals were perfused with saline and Zamboni's fixative, and brains were cut on a Vibratome at 50 µm. Alternate sections were serially taken for immunocytochemical staining and for cresyl violet. The results were as follows: 1) Cell bodies of the LHRH neurons were found in small numbers in the area surrounding the OVLT and also in the preoptic area, diagonal bundle and septum. The distribution density of cell bodies in the MPN region was similar to that in the other preoptic regions, and the immunoreactive cells were located mostly in the lateral margins of the MPN or immediately lateral to the MPN. 2) More LHRH cells were found in proestrous than in metestrous rats, and in persistent estrous (both spontaneous and MPN lesion-induced) rats than in proestrous rats. 3) LHRH fibers were most concentrated in the ME, OVLT and ventromedial part of the arcuate nucleus (ARC). In the MPN some fibers were observed running along the ventricle or transverse to it, but there were more fibers in the area ventrolateral to the MPN. However, the fiber density of the MPN region was far less than that in the OVLT. 4) More fibers were found in the OVLT, ME and ARC in spontaneous persistent estrous rats than in any other animals. In MPN lesion-induced persistent estrous rats there were more fibers in the OVLT (rostral to lesions) and in the ARC than in those regions of proestrous rats, while in the ME the density seemed to be similar to that in proestrus animals.

LHRH staining, then, does not appear to be diminished in animals with MPN lesions, in spite of the fact that these lesions severely disrupt the phasic release of LH. These results do not support the idea that the effects of MPN lesions are due to a deficiency in the LHRH system, but are consistent with the possibility that this nucleus is critical for triggering LHRH release. (Supported by NIH grants RR00167, HD11355 and HD15433).

108.11 THE ENKEPHALIN ANALOG FK 33-824 POTENTLY INHIBITS LH SECRETION IN GONADECTOMIZED IMMATURE RATS. R. Bhanot* and M. Wilkinson. Dept. Physiol. Biophys. and Obst. Gynecol., Fac. Med., Dalhousie University, Halifax, NS, Canada B3H 4H7.

Endogenous opiates are implicated in the regulation of gonadotropin secretion in man and in laboratory animals and may also exert an important influence during development (Blank et al. Science 203 1129 (79). We have sought to probe a hypothalamic involvement of opiates in sexual maturation with the aid of the synthetic enkephalin FK 33-824 (FK) which potently inhibits LH in man and which does not cross the blood-brain barrier (Grossman et al., Clin. Endocr. 14 41 (81).

In female rats (26 days; 48h post-ovx) FK completely inhibits the post-ovx elevation in LH (347±78 ng/ml to 29±4 ng/ml, $p<0.005$; 1 mg/kg). Approximately 50% inhibition was observed with a 10-fold lower dose. Comparable results were obtained with male rats of the same age. A 1.0 mg/kg of FK reduced post-ovx serum FSH levels of immature female rats (26 days) from 1317±64 ng/ml to 1108±35 ng/ml. This response of FSH contrasts strongly with that of LH in that the analog did not bring FSH levels to those in intact animals (192±28 ng/ml). However, we have found naloxone to increase FSH levels in both ovx and intact immature animals. The effects of FK on gonadotropin release could be prevented by simultaneous injection of naloxone (0.5 mg/kg).

Opiate regulation of gonadotropin secretion does not appear to occur directly at the pituitary gland. Pituitary halves from immature male rats pre-incubated with FK (2h; 10^{-10} - 10^{-6} M) responded normally, in terms of LH release, to GnRH (1.7×10^{-9} M). Similarly, naloxone (10^{-6} M) failed to modify either LH or FSH release from hemipituitaries of ovx or intact immature rats.

In view of the profound effects exerted by FK on LH secretion we have investigated the influence of daily FK or naloxone injections (1 mg/kg; from day 24) on the timing of vaginal opening (V.O.) in immature female rats. Neither drug had any effect on the age at V.O., body weight, ovarian or uterine weight or number of ova released at first ovulation when compared with age-matched vehicle-treated controls. Similar experiments with a regimen of increasing daily dosages (1-4 mg/kg FK or naloxone) also had no effect on puberty.

Although our studies with FK and naloxone provide further evidence of an opiate component in the control of gonadotropin secretion in immature rats, they failed to reveal opiate involvement in sexual maturation. More recently however, we have observed age-related decreases in the ability of FK to suppress LH secretion in maturing gonadectomized rats. (Supported by MRC grant to M.W.).

108.12 COMPARISON OF MORPHINE'S EFFECT ON UNIT ACTIVITY IN THE VENTROMEDIAL NUCLEUS (VMN) FOLLOWING SYSTEMIC OR INTRACRANIAL ADMINISTRATION. J.M. Lakoski and G.F. Gebhart. Department of Pharmacology, University of Iowa, Iowa City, Iow 52242.

The extensive anatomical interconnections of extrahypothalamic areas to the VMN have been demonstrated to modulate the secretion of luteinizing hormone (LH). Extrahypothalamic areas, including the amygdala (AMYG) and the periaqueductal central gray (PAG), have also been demonstrated to mediate the morphine-induced depression of LH. We have further investigated the role of the AMYG and PAG by comparing the effects of systemic vs intracranial administration of morphine (MOR) on single unit extracellular activity in the VMN. Unit activity in the VMN (7.8-8.4 mm ventral to the dura) was recorded in urethane-anesthetized (1.5 gm/kg, ip) adult male Sprague Dawley rats using conventional electrophysiological techniques; only one unit per animal was tested for responses to morphine administration and all sites were subsequently confirmed histologically. Opiate sensitive units (n=33, 72% of units tested) were identified in intact and chronically castrated animals; castration resulted in a significant enhancement of spontaneous unit activity compared with intact animals (4.6 ± 0.5 and 2.9 ± 0.4 spikes/sec, respectively). MOR SO_4 administered iv in log intervals produced a marked decrease in firing (> 75%) with cumulative doses (as MOR base) ranging from 0.8-6.4 mg/kg ($\bar{X}$ = 3.2 mg/kg). Naloxone administered in log intervals (0.4-.16 mg/kg) reliably reversed the MOR-induced depression of firing. The stereospecificity of this response was confirmed by the inability of dextorphan (cumulative dose to 1.6 mg/kg iv) to alter VMN activity. In animals previously prepared with cannulae implanted bilaterally in the AMYG or PAG, microinjection of MOR (5.0 µg/0.5 µl CSF/site) also produced a significant inhibition of unit activity in the VMN; this depression was reversed by the systemic administration of naloxone. Microinjection of dextorphan (5.0 µg/0.5 µl CSF/site) into these sites failed to alter unit activity in the VMN. The time course of maximal depression of firing induced by MOR differed depending on the route of drug administration. Thus, these data demonstrate the ability of morphine to influence the neuronal activity of the VMN whether administered systemically or intracranially into discrete extrahypothalamic sites. These data also provide further insight into the mechanism whereby extrahypothalamic sites, such as the AMYG and PAG, with demonstrated high levels of specific opiate receptor binding and known interconnections with the hypothalamus may mediate the morphine-induced depression of LH.

Supported by NS-12114 and the Graduate College, Univ. of Iowa.

108.13 ESTROGEN ELICITS LH AND FSH SURGES IN ACYCLIC FEMALE RATS BEARING PROLACTIN(PRL)/GROWTH HORMONE(GH)-SECRETING TUMORS.
Thomas E. Nass*, John K.H. Lu* and Howard L. Judd* (SPON: P.C.K. Leung). Depts of Obstetrics/Gynecology and Anatomy and Lab of Neuroendocrinology, Brain Res Institute, University of California Los Angeles School of Medicine, Los Angeles, CA 90024.

Subcutaneous(sc) transplantation of PRL/GH-secreting tumors (Furth,MtT.W15) into regularly cyclic female rats results in a rapid cessation of estrous cycles associated with persistent diestrous vaginal smears. To date, the mechanisms by which abnormal secretion of PRL and/or GH inhibit normal reproductive cycles are unknown. The present study was undertaken to determine if the stimulatory feedback action of estrogen on gonadotropin secretion is present in acyclic female rats (Wistar) bearing MtT.W15 tumors. Blood samples were obtained before and after the tumor transplantation at 2-3 day intervals for about one month, and sera were assayed for FSH, LH and PRL concs. by RIAs. Tumor transplantation resulted in a large increase in PRL secretion within 5-10 days and a cessation of estrous cyclicity. However, serum concs. of both LH and FSH during the prolonged acyclic state were comparable to basal values in regularly cyclic females. After a chronic (8 wks) elevation of circulating PRL, 5 acyclic females were each injected sc with 20 μg estradiol benzoate(EB) at 1230h. On the following day all animals showed significant increases in serum LH (893 ± 71 vs 65 ± 6 ng/ml, mean ± SEM, $p<0.01$) and FSH (1471 ± 377 vs 239 ± 17 ng/ml, $p<0.01$) at 1700h. A second injection of EB 4 days later also elicited LH and FSH surges. In a second group (n=5) of acyclic females bearing MtT.W15 tumors for 3 wks, a priming dose of 1 μg EB was followed 21h later by a 50 μg EB injection. EB administration resulted in significant increases in both serum LH (303 ± 142 vs 57 ± 7 ng/ml, $p<0.05$) and FSH (574 ±138 vs 254 ± 34 ng/ml, $p<0.05$) between 1600 -1800h on the next day. Similarly, a second test with EB administration 9 days later also induced both LH and FSH surges. These results demonstrate that, in the presence of high circulating PRL/GH, acyclic female rats maintained normal basal secretion of LH and FSH and responded to estrogen administration by increasing gonadotropin release. These findings provide evidence that the stimulatory feedback mechanism of estrogen on gonadotropin secretion is present, but not functioning, in female rats during the acyclic state associated with high circulating concs. of PRL/GH, and suggest that the cessation of estrous cyclicity may be related to a lack of estrogen stimulation to the central nervous system (Supported in part by NIH research grants AG01512 and CA23093).

108.14 ULTRASTRUCTURAL CHANGES IN LH GONADOTROPES EFFECTED BY ETHANOL AND VITAMIN A INTERACTION. J.P.Hagedoorn*, G.G.Gordon* and Ch.S.Lieber*(SPON: A.B.Rothballer). Depts.of Anat.,Medicine,New York Med.College, Valhalla,N.Y.,10595;Alc.Res.Treatm.Center,V.A.Med. Center,Bronx,N.Y.,10468,Mt.SinaiSchool of Medicine, New York,N.Y.,10029.

Young male Sprague-Dawley rats were pair-fed liquid isocaloric diets in which 36% of the carbohydrate caloric intake was substituted by ethanol. Each diet was either Vitamin A replete- or deficient (100 grams/day, resp. 0.33 grams/day). Dietary treatment lasted 70 days.At sacrifice bloodsamples were taken for serum analysis of testosterone and luteinizing hormone. Testes, hypophysis and hypothalamus were resected and routinely fixed, dehydrated and embedded in Epon for ultrastructural examination. The LH producing cells of the adenohypophysisshowed a marked reduction in granular contents,extensive vacualization, lamellar formation and abundant RER in cisternal profiles in the ethanol-fed Vitamin A deficient animal when compared to the ethanol-fed Vitamin A replete rat. Mere Vitamin A deficiency without alcohol treatment did not significantly affect the secretory granular contents of the LH gonadotropes when compared to these pituitary cells of the Vitamin A replete rats. Low serum testosterone levels of the alcohol-fed Vitamin A deficient animals was consistent with impaired spermatogenesis, as observed on ultrastructural analysis of the seminiferous tubules. Our data show morphologically, that the hypogonadal effects of alcohol, intensified by Vitamin A deficiency are concomitant with a diminished granule maturation in the LH cell of the anterior pituitary gland. High Vitamin A supplements will minimize these endocrine effects of alcohol on the hypophyseal-gonadal axis.

108.15 SELECTIVE ENDOCRINE EFFECTS ON IN VIVO TRYPTOPHAN HYDROXYLASE ACTIVITY AND SEROTONIN LEVELS IN DISCRETE RAT BRAIN NUCLEI.
J. B. Long*, W. W. Youngblood*, and J. S. Kizer* (SPON: G. H. Greeley). Biol. Sci. Res. Ctr., Depts. of Medicine and Pharmacology, Univ. of N. C. Sch. of Medicine, Chapel Hill, N. C. 27514.

To identify endocrine-responsive serotonergic neurons in the rat brain, in vivo tryptophan hydroxylase activities (THA) and serotonin (5HT) levels were measured in discrete brain nuclei after gonadectomy (2 wks.), adrenalectomy (10 days), or administration (4 wks.) of propylthiouracil (PTU) to male rats. THA was determined by measurement of the accumulation of 5 hydroxytryptophan (5HTP) following L-aromatic amino acid decarboxylase inhibition by Ro4-4602/1. 5HTP and 5HT were separated by liquid cation exchange and subsequently measured by a sensitive radioenzymatic assay. This procedure is specific and sensitive to 10 pg of 5HTP or 5HT. 5HTP accumulation and 5HT levels were measured in the lateral septal nucleus (LS), suprachiasmatic nucleus (SCN), anterior hypothalamic nucleus (AH), median eminence (ME), central amygdaloid nucleus (AG), dorsal raphe nucleus (DR), and central superior nucleus of the raphe (NCS) following endocrine manipulations. Gonadectomy significantly ($p<0.05$) decreased THA in the SCN, DR, and NCS by 19.2, 18.4, and 11.7% respectively but was without effect on the THA of other nuclei examined. 5HT levels in all nuclei were unchanged following gonadectomy. Administration of testosterone propionate (500 ug/kg b.wt., s.c. daily) for 6 days to gonadectomized males did not reverse the gonadectomy-induced decrease in THA in the SCN, DR, and NCS, but did significantly decrease THA in the LS and AG. Adrenalectomy significantly increased THA in the ME by 58.5% and the NCS by 34.7%. while significantly increasing 5HT levels in the ME by 35.2%. Administration of corticosterone (2.5 mg/animal, s.c. daily) for 4 days to adrenalectomized rats did not reverse these increases. PTU administration (0.05% in drinking water) significantly decreased THA in the SCN by 15.5% and NCS by 16.1%, while significantly increasing 5HT levels in the LS, AH, AG, and DR by 28.3, 19.6, 26.0, and 25.7% respectively. These findings indicate the presence of CNS serotonergic neurons which selectively respond to altered endocrine states, and also demonstrate a dissociation of the effects of altered endocrine states on 5HTP synthesis and 5HT levels measured in these nuclei.

108.16 VALIDATION OF A PERIFUSION SYSTEM FOR AUTOMATED PULSATILE DELIVERY OF HORMONES TO PITUITARY CELLS.
Jeanne E. Martin, George J. O'Neil*, and Carol Sattler*, Departments of Pharmacology and Ob.-Gyn., Washington U. Med. Sch., St. Louis, MO 63110.

A perifusion system has been developed for automated, long-term pulsatile delivery of hormones to dispersed anterior pituitary cells. Rat anterior pituitary glands are dissociated with collagenase and hyaluronidase and incubated overnight. The next day, the cells are washed once and placed into perifusion chambers. The chambers are 25-mm stainless steel Swinnex filter holders with luer fittings (Millipore) modified by the addition of a second inlet (for LHRH) oblique to the central inlet (for basal medium). Each chamber contains a stainless steel screen supporting cellulose acetate and glass fiber filters which retain the cells. The medium is MEM with Hank's salts and contains 25 mM HEPES, 14 mM glucose and antibiotic-antimycotic (HMEM). HMEM is supplemented with 10% newborn calf serum (NBCS-HMEM) for basal perifusion and with 1% BSA for LHRH medium and the corresponding control. Media are delivered from reservoirs at 0°C through the chambers by 2 peristaltic pumps regulated by timers to operate alternately. Both pulse length and pulse interval can be varied over a wide range. With the use of multi-head pumps, 4 chambers can be perifused simultaneously.

Cells ($4-5x10^6$/chamber) obtained from castrated female rats were perifused (0.2 ml/min) with NBCS-HMEM for 110/120 min and with LHRH (0.1-100 nM) or control medium for 10/120 min for up to 48h. Ten-min effluent fractions were collected with the use of a multi-channel fraction collector. LH concentrations were measured by RIA. Medium LH rapidly declined within several fractions to 50-200 ng/ml and, in the controls, remained at this level for the duration of the experiment. Pulsatile LHRH at all concentrations produced discreet peaks of LH which returned to the control level between pulses. Peak magnitude was dose-related and maximal by 10 nM LHRH. Pulsatile release continued for at least 48h. At the end of perifusion, both LHRH-treated and control cells responded robustly and equivalently to 1 μM LHRH. This finding indicates that the control cells were still viable and responsive and the LHRH-treated cells apparently were neither highly sensitized nor de-sensitized by pulsatile exposure to LHRH. The simplicity, ease of operation, and relative inexpense of this automated perifusion system make it a useful technique for study of long-term pulsatile treatment of cells with hormones or other agents. (Supported by NIH Grant HD-11854.)

108.17 STRESSORS ELEVATE PITUITARY CYCLIC AMP IN THE RAT. G.J. Kant, B.N. Bunnell, R.H. Lenox, L.L. Pennington*, D.R. Collins*, E.H. Mougey* and J.L. Meyerhoff. Dept. Med. Neurosciences, Walter Reed Army Institute of Research, Washington, D.C. 20012 and Dept. Psychiatry, Univ. of Vermont, Burlington VT 05405.

Cyclic AMP may be involved in the release or synthesis of pituitary hormones. Incubation of pituitaries in vitro with cyclic AMP analogues increases the release of hormones into the medium and various neurotransmitters and releasing factors have been reported to increase pituitary cyclic AMP levels in vitro. In previous studies we have found that pituitary cyclic AMP levels in vivo are responsive to dopaminergic, cholinergic, and adrenergic agonists and that immobilization also increases pituitary cyclic AMP. We hypothesized that other stressors might also increase pituitary cyclic AMP in vivo, possibly by central release of neurotransmitters or releasing factors into the pituitary through the portal circulation. Pituitary cyclic AMP could then link stress-activated central circuits with pituitary hormonal output.

Male rats were handled for two weeks prior to the experiment to minimize non-specific stress effects. Six stressors (cold, forced running, saline injection (i.p.), formalin injection (s.c.), immobilization and electric foot shock) were tested. Six animals were sacrificed for each stressor at each of three time points (15, 30 and 60 minute stress duration). In addition 12 control "non-stressed" rats were sacrificed immediately upon removal from the home cage.

All animals were sacrificed by high-power microwave irradiation for 5 sec. Plasma prolactin, corticosterone, and growth hormone and pituitary cyclic AMP were measured by radioimmunoassays.

All stressors except saline increased pituitary cyclic AMP, prolactin and corticosterone; growth hormone was variable but generally decreased in the stress groups. Pituitary cyclic AMP levels after each stressor are given below in pmoles cyclic AMP/mg wet weight ± S.E.M.: control levels were 1.06±.09.

STRESSOR	15 MIN	30 MIN	60 MIN
saline (i.p.)	1.01 ± .06	1.01 ± .05	.86 ± .11
cold	1.33 ± .20	1.78 ± .51	1.63 ± .18
running	1.55 ± .23	4.12 ± 2.71	2.73 ± .55
formalin s.c.	6.96 ± 2.58	5.42 ± 1.34	3.36 ± .62
immobilization	5.12 ± 1.40	5.50 ± 1.56	3.25 ± .57
shock	10.08 ± 2.31	13.61 ± 3.23	10.17 ± 2.04

Prolactin levels increased 60 fold from the least stressed to the most stressed (shock) groups, the more moderate stressors produced intermediate prolactin levels. Corticosterone levels were roughly 5 times higher in all stress groups compared to controls.

108.18 PAIN STIMULI MODIFY THE ACTH RESPONSE TO HEMORRHAGE. D.A. Bereiter, P.M. Plotsky and D.S. Gann. Division of Biology and Medicine, Brown Univ., RI Hospital, Providence, RI 02902.

To assess the interaction of pain and blood loss on the activation of the release of ACTH, acutely prepared cats were hemorrhaged (H) in the presence or absence of tooth pulp (TP) stimulation. Adult cats of either sex were anesthetized with α-chloralose/urethane (40/200 mg/kg, iv). Catheters were placed in the inferior vena cava above the liver for blood sampling, in the descending aorta for monitoring blood pressure and heart rate, and in the femoral artery for hemorrhaging. A bipolar electrode was positioned in the upper incisor for TP stimulation (100-500 μA, 0.2 msec, 3Hz). Stimulations lasted 3 min for TP, and 10 ml/kg of blood was withdrawn over 1 min and reinfused at 3 min for H. Each cat received H, H + TP and TP in a randomized sequence over a 6 h period.

Table I. Mean % Change in ACTH from baseline

	During Stimulation	Post Stim. (+ 2 min)	Post Stim. (+ 7 min)	Post Stim. (+ 12 min)
H alone	+ 0.8	+ 68.0	+ 31.6	- 0.8
H + TP	+ 47.5	+ 332.6	+ 100.9	+ 221.7
TP alone	- 10.4	- 32.2	- 34.9	- 28.9

As shown in Table I, H alone led to a small transient increase in ACTH by 2 min post H. TP alone led to a decrease in ACTH in all cats (n = 5) over the entire sampling period ($P<.01$). In contrast, H + TP led to a larger and more sustained increase in ACTH (interaction, $P<0.05$) relative to either H or TP alone. The apparent facilitation of TP on the ACTH response to H could not be accounted for by a difference in the hemodynamic response, since changes in mean blood pressure and heart rate to H and H + TP were equivalent. Furthermore, H and H + TP led to equivalent increases in plasma glucose and osmolality. Clearly, these results suggest that H and TP do not activate separate afferent neural pathways for the release of ACTH, but must interact at some common neural loci proximal to the site of CRF release. Preliminary neurophysiological experiments suggest that such interaction may exist at the brain stem level.

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108.19 *IDENTIFICATION OF THE "TUBEROINFUNDIBULAR" SYSTEM OF THE RAT* USING IMMUNOHISTOCHEMICAL LOCALIZATION OF RETROGRADELY TRANSPORTED WHEAT GERM AGGLUTININ(WGA). R.M. Lechan, J.L. Nestler and S. Jacobson*(SPON:S. Reichlin). Endocrine Div. and Dept. of Anatomy, Tufts-New England Medical Center, Boston, MA 02111

The median eminence (ME) of the hypothalamus is an important neurovascular structure which has a critical role in the neuroendocrine regulation of the anterior pituitary gland. To determine the origin of neuronal cell bodies which project to the ME, we extend our previous observations which employed HRP (Lechan et al, Brain Res.,195,13,'80) by using the more sensitive retrograde marker protein, WGA.

The ME of adult, male, albino, CD rats was exposed by the retropharyngeal approach and under the dissecting microscope, WGA was injected into the ME (0.5-1.5 ul of a 5% soln. dissolved in TBS, pH 8.1). In other animals, WGA was suspended in poly(vinyl alcohol) (PVA), adopting methods described by Dr. M. Moskowitz (Brain Res., in press), and allowed to diffuse into the ME after being applied directly to its surface. After survival times of 18-24 hrs., the animals were perfused through the aorta with Bouin's soln. and the brains sectioned coronally at 60 u on a vibratome. The resulting free floating tissue sections were incubated for 24 hrs. with antiserum to WGA (E-Y Lab.,Inc.) at a titre of 1:800, diluted in TBS containing 0.3% Triton X-100. Following the peroxidase-antiperoxidase technique, the final reaction product was developed with 0.05% DAB and 0.01% H_2O_2.

Retrogradely transported WGA was present in bipolar and multipolar perikarya in the dorsal and lateral portions of the arcuate nucleus, the periventricular nucleus and the parvocellular division of the paraventricular nucleus, excluding the dorsal and lateral subdivisions and portions of the medial subdivision. Unipolar and bipolar cells were also labeled in the medial preoptic nucleus, lamina terminalis, diagonal band of Broca and medial septum. Fewer immunoreactive cells were identified in the dorsomedial nucleus, anterior hypothalamus, and in the nucleus ambiguus of the brain stem. Even with this more sensitive technique, however, we failed to identify any neuronal input from the amygdala and detected only rare cells in the ventromedial and suprachiasmatic nuclei. Reaction product within magnocellular nuclei of the hypothalamic-hypophysial system was apparent after WGA injections but reduced in the supraoptic nucleus, accessory magnocellular nuclei and portions of the paraventricular nucleus, when allowed to diffuse into the ME from the PVA-suspension.

These results confirm many of our earlier observations but also identify projections to the ME from neurons outside of the hypothalamus and indicate that the concept of a "tuberoinfundibular" system must be expanded.

108.20 PULSATILE SECRETION OF LH IN MALE RATS: VARIATION WITHIN AND AMONG INDIVIDUALS. Gary B. Ellis* and Claude Desjardins* (SPON: R.V.Smith). Institute of Reproductive Biology, Dept. of Zoology, University of Texas, Austin, Texas, 78712.

In examining moment-to-moment changes in circulating LH levels in individual male rats [Biol. Reprod., 24(Suppl.1)], we observed a spectrum of hormonal profiles among individuals in the population. Most male rats exhibited well-defined episodes of LH release, typically characterized by a 5-7 fold increase in plasma LH levels within a 5-minute interval and a decline through the next 60-75 minutes. These LH pulses occurred singly or in sequences of 2 or 3. Other males exhibited less-well-defined episodes of LH secretion, or highly variable LH levels with no apparent pulses. No distinct day/night distribution of secretory bouts was seen, regardless of the pattern of LH release. In the present study, we expand our perspective of episodic hormone secretion and the diversity among individual hormonal profiles by evaluating the pattern of LH secretion of a single animal at different times.

Each of four rats (age 29 weeks; 595-697 g) was fitted with an indwelling atrial cannula, and loosely tethered to a valve mounted outside of his cage. Blood was withdrawn every 5 minutes for 8 hours (96 samples @ 0.2 ml), on three occasions (at 5, 10, and 15 days post-cannulation). Withdrawn blood was replaced by a mixture containing 45% rat red blood cells and 2.5% human plasma proteins in Krebs-Ringer solution. Blood sampling was completed during the light portion of an LD 14:10 photoperiod. Rats appeared unperturbed during the sampling period, eating and drinking on occasion.

Unambiguous episodes of LH secretion, as defined above, occurred in all animals. During 8-hour sampling periods, rats exhibited from 0 to 6 distinct pulses. When sequences of 2-4 pulses occurred, they were often regularly spaced at intervals of 65-85 minutes. Within a particular animal, on different days, LH pulses varied in number; amplitude; and timing, relative to adjacent pulses and to absolute clock time. Thus, a particular LH secretory profile did not serve as a consistent hormonal "signature" for an individual rat.

Discrete, short-term pulses in LH release are now recognized as a fundamental aspect of the pattern of tonic LH secretion in normal male rats. The inter-individual variation in the degree and nature of pulsatile LH activity is most likely a manifestation of the considerable intra-individual variation through time.

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SYMPOSIUM

109 CELLULAR MECHANISMS OF EPILEPTOGENESIS. D.A. Prince (Chairman; Stanford), R. Wong (Louisiana State Univ.), W. Crill (Univ. of Washington, R.A. Nicoll (UCSF), E. Dudek (Tulane).

A complex array of neuronal events interact to produce epileptogenesis and the contribution of each of these may vary in different neuronal populations.

1) Voltage-dependent slow depolarizations and burst generation: In hippocampal pyramidal neurons (HPCs) and dentate granule cells (GCs) the degree of involvement in convulsant-induced epileptiform discharge is related to the capacity to generate intrinsic Ca^{++} dependent slow depolarizations and bursts. CA3 HPCs tend to burst normally, and also serve as pacemakers during epileptogenesis, while GCs have poorly developed voltage-dependent depolarizations and are only minimally involved in epileptogenesis in vitro. In spinal motoneurones prolonged depolarizations produced by penicillin are associated with enhancement of a persistent inward current, probably carried by Ca^{++}, and a decrease in outward current due to a depolarizing shift in E_K, perhaps related to the significant increases in $[K^+]_o$ that occur during epileptogenesis. Voltage-dependent conductances for K^+ and probably Ca^{++} in HPCs are under control of neuromodulatory substances such as ACh and norepinephrine, which act tonically to influence baseline membrane properties and burst generation.

2) Disinhibition: GABA-mediated chloride dependent IPSPs normally suppress intrinsic burst firing in hippocampal pyramidal somata and dendrites. GABA antagonists block IPSPs, lengthen EPSPs, and lead to intrinsic spontaneous depolarizations and bursts which increase neuronal output and excitatory synaptic coupling between neurons. A Ca^{++} activated K^+ conductance also functions as an inhibitory event in HPCs. Orthodromically evoked EPSPs are followed by a GABA-mediated IPSP and a longer duration hyperpolarization due to increased gK, presumably a consequence of Ca^{++} entry during the EPSP. The latter hyperpolarization, which is increased as IPSPs are blocked and bursts develop, serves to limit burst duration and frequency. Blockade of this event can lead to prolonged depolarization and repetitive firing.

3) Enhanced EPSPs: These serve to trigger intrinsic bursts, synchronize aggregates of neurons, and, in some instances (e.g., neocortical neurons), contribute significantly to the slow envelope of depolarization shifts during epileptogenesis. Although chemical synaptic coupling is a major event underlying intercellular synchronization during epileptogenesis, anatomic and physiologic evidence shows that some hippocampal and neocortical neurons are electrotonically coupled. This may contribute in part to the neuronal synchrony observed during epileptogenesis.

WORKSHOP

110 QUANTITATIVE MICROSCOPY: THEORY, METHODS AND APPLICATIONS. L.M. Smith (Chairman; Brown Univ.), E.E. Underwood* (Georgia Inst. Tech.), J.J.Norden (Vanderbilt Univ.), E.R. Macagno (Columbia Univ.), M.M. Salpeter (Cornell Univ.).

The last few years have witnessed a dramatic increase in the number of quantitative morphological studies of neural systems. This workshop is intended to help Neuroscientists become aware of---and be able to use---powerful, yet mathematically simple methods of analyzing quantitative and structural information from light and E.M. images.

Dr. Underwood will begin the workshop by giving a brief history of the field of stereology, the three-dimensional interpretation of two-dimensional images. He will present the basic definitions and equations used in stereology and morphometry and explain how these are used to estimate the proportion of some volume of tissue occupied by some element, the number of some element in a volume of tissue, and the real size distribution of microscopic profiles.

Dr. Norden will then demonstrate how to use planimetry and point counting methods to estimate volumetric density of neurons and nuclei from light images. She will illustrate how such techniques can be used to measure changes in both the number and size distribution of neurons in the presence of tissue volume changes following denervation. Possible applications of these methods to histo- and immunocytochemical studies will be presented.

Dr. Smith will discuss stereological methods used to extract quantitative and structural information from E.M. images. Topics such as sampling procedures, sources of experimental bias, estimates of profile size, shape and volume fraction, and semi-automatic image analyzers will be covered. She will demonstrate how to calculate the number of synapses and boutons in a volume of normal and denervated nervous tissue.

Dr. Macagno will discuss computer graphics techniques developed in the past decade which can be applied to the analysis of serially sectioned tissue. Such techniques are used for the reconstruction in three dimensions of individual neurons and neuronal assemblies. These methods allow the visualization of the complexity of neuronal branching patterns and the determination of the distribution of contacts among neurons.

Dr. Salpeter will discuss how the limited resolution of E.M. autoradiography can cause problems in localizing sources of radioactivity in tissues with complex geometry. Examples will be given, and the statistical procedures for solving problems will be illustrated.

Handouts with guidelines for the practical application of each of these methods will be provided.

111.1 CALCIUM INDEPENDENT RELEASE OF GABA FROM ISOLATED HORIZONTAL CELLS OF THE TOAD RETINA. E. A. Schwartz* (SPON: V. Holcombe). Dept. of Pharmacological and Physiological Sciences, University of Chicago, Chicago, IL 60637.

When toad (Bufo marinus) retinae were incubated first with veratrine, then with antibodies that reacted with the outer segments of photoreceptors, and finally with complement, horizontal cells survived and most other neurons died. This preparation of "isolated" horizontal cells accumulated radioactive GABA from the incubation medium. The subsequent release of radioactive GABA could then be measured. The efflux of GABA was increased by depolarization or by adding GABA to the external medium. An increase in GABA efflux produced by an elevated potassium concentration (41.5 mM) was unaffected when calcium in the external medium was replaced with cobalt and when sodium was replaced with either choline or lithium. An increase in GABA efflux produced by glutamate (100 μM) was unaffected when calcium was replaced with cobalt and when sodium was replaced with lithium, but was inhibited when sodium was replaced with choline. An increase in GABA efflux produced by external GABA (100 μM) was unaffected when calcium was replaced with cobalt but required sodium; neither choline nor lithium would substitute for sodium. After a high concentration of GABA (2-20 mM) had produced a maximal increase in GABA efflux, the addition of glutamate (2 mM) produced no further effect. Conversely, after a high concentration of glutamate (2-20 mM) had produced a maximal increase in efflux, the addition of external GABA (2 mM) produced only a small further increase. Superfusing with either 2 mM glutamate, or 2 mM GABA, or 2 mM glutamate + 2 mM GABA each increased efflux approximately ten fold. These results could occur if GABA efflux were mediated by a carrier system which could be activated by either depolarization or homoexchange.

(Supported in part by NIH grant EY-02440.)

111.2 A NEW APPROACH TO MEASURING THE TURNOVER RATE OF GABA C. M. Forchetti* and J. L. Meek. Lab. Preclin. Pharmacol., NIMH, St. Elizabeths Hosp., Washington, D.C. 20032.

Measurement of GABA turnover in rat brain has proven difficult whether using pharmacological blockade of synthesis or degradation or isotopic incorporation methods. Approaches involving peripheral injection of drugs to block GABA synthesis or breakdown have met with limited success: some drugs (isoniazid) are convulsants, others (aminooxyacetic acid) affect both the synthesis and breakdown of GABA, while the action of certain specific drugs (γ-vinyl GABA) is slow in onset, making it impossible to determine initial rates for accurate estimation of GABA turnover. In studying GABA in the median raphe (MR) and substantia nigra (SN), we made stereotaxic injections into the MR or SN of isoniazid (50μg) to block glutamate decarboxylase or gabaculine (80 ng) to block GABA-transaminase. The method provides a rapid onset of inhibition and a high local concentration of drugs without producing convulsions or sedation. The drugs were injected in 1 μl of saline during 5 min. The GABA content was measured by HPLC in tissue punched from frozen 400 μm sections.

Isoniazid injection caused a decrease in GABA content of the median raphe that was rapid in onset (30% decrease by 7 min) but not exponential and short in duration (the content returned to near control values by 90 min). The difficulty in achieving a prolonged inhibition of GAD and the high concentraton of isoniazid required (50 mg/ml) limits the usefulness of this approach.

Gabaculine induced an increase in GABA content that was rapid in onset and linear for 60-90 min, and reached a content 5 times that of controls by 8 hr. The estimated rates of turnover were 37 and 60 nmol/mg prot/hr in the MR and SN, respectively. These turnover rates may be low if the accumulated GABA can affect its own synthesis. To establish if the GABA accumulation reflects turnover, we destroyed the major GABA-ergic input to the SN by hemitransections. The nigral content of GABA decreased by 80% 7 days after surgery; the gabaculine induced GABA accumulation was also decreased by 80%. In the median raphe, we examined if local injection of substance P (4μg) would affect GABA turnover. Substance P increased the gabaculine induced accumulation of GABA by 30%.

In summary, GABA turnover can be estimated by measuring the initial rate of accumulation of GABA after local injection of the GABA-transaminase inhibitor gabaculine. This approach has the advantage of linearity in initial rates, low toxicity, and apparently high specificity.

111.3 DECREASE OF GLUTAMATE DECARBOXYLASE IN VENTRAL GLOBUS PALLIDUS AFTER ELECTROLYTIC LESIONS OF THE NUCLEUS ACCUMBENS. G. T. Murata*, L. L. Butcher, and P. H. Kelly. (Spon: D.F. Lindsley). Dept. of Physiology and Biophysics, University of Southern California Sch. Med., Los Angeles, CA 90033, and (L.L.B.)Dept. Psychology, UCLA, Los Angeles, CA 90024.

The main efferent projections of the nucleus accumbens are to the substantia nigra and a subcommissural region which has been termed "ventral globus pallidus" (Heimer & Wilson, 1975; Nauta, Smith, Faull, & Domesick, 1978). There also appear to be sparser projections to parts of the globus pallidus itself and to the ventral tegmental area. We have investigated if these projections are cholinergic or GABA-ergic by examining if lesions of the nucleus accumbens result in decreases of the neurotransmitter-synthesizing enzymes, choline acetyltransferase (ChAT) and glutamate decarboxylase (GAD), in these regions.

Unilateral electrolytic lesions of the nucleus accumbens were made in adult Sprague-Dawley rats. Seven to nine days later the rats were killed by decapitation and their brains frozen and sectioned in a cryostat. Fifty micron sections were taken for verification of lesion sites and 200-300 micron sections for dissections of globus pallidus, ventral globus pallidus, substantia nigra, and ventral tegmental area. On the lesioned side GAD was reduced to 75 + 11% of control ($p<0.05$) in the ventral globus pallidus. Smaller decreases in the globus pallidus proper (by 13%) and ventral tegmentum (by 6%) were not statistically significant. GAD was unchanged in the substantia nigra. ChAT was not significantly altered in any of the regions studied. These results suggest that at least some of the neurons whose axons project from the nucleus accumbens to the ventral globus pallidus use GABA as a transmitter. (Supported by USPHS grants NS 16175 (to P.H.K.) and NS 10928 (to L.L.B.).

111.4 THE CHOLINERGIC SYSTEM AND THE ANTINOCICEPTIVE EFFECT OF GABA AGONISTS. D.A. Kendall,* M. Browner* and S.J. Enna. Depts. Pharmacology and Neurobiology, Univ. Texas Med. Sch., Houston, Texas 77025.

The antinociceptive action of three classes of GABA agonists was examined in mice using hot-plate and tail-immersion tests. A significant increase in reaction time was noted in the hot-plate test following treatment with the direct-acting GABA receptor agonists, kojic amine or THIP, with γ-vinyl GABA, an inhibitor of GABA degradation, or with nipecotic acid ethyl ester, an inhibitor of high affinity GABA transport. Studies with naloxone indicated that the increase in pain threshold was not mediated through the brain opiate system. However, it was possible to reverse the antinociceptive effect of these drugs with atropine, a cholinergic muscarinic receptor antagonist. Receptor binding experiments indicated that, except for the ethyl ester of nipecotic acid, the GABA agonists had little affinity for the cholinergic muscarinic receptor site. In addition, atropine, at a dose that completely blocked the antinociceptive action of kojic amine, was unable to attenuate the sedative effects of this drug. These findings suggest that, regardless of their mechanism, all classes of GABA agonists are capable of inducing an antinociceptive response in mice and that this action is apparantly secondary to a GABA-mediated increase in brain cholinergic function. The discovery that the sedative and antinociceptive effects are functionally unrelated indicates that it may be possible to develop nonsedating antinociceptive GABA agonists. (Supported in part by USPHS grants NS-13803 and NS-00335).

111.5 A STUDY OF PLASMA ASPARTIC ACID LEVELS AND PATHOLOGY IN THE ARCUATE NUCLEUS FOLLOWING ASPARTAME AND SODIUM ASPARTATE ADMINISTRATION. E. W. Powell, Terry Sims, Jean Linebarger* and Bradley Diner*. Departments of Anatomy and Pathology, University of Arkansas for Medical Sciences, Little Rock, AR 72205.

The arcuate nucleus is susceptible to damage when exogenous aspartic acid is administered in high doses to neonatal mice (Olney et al, 1971). Utilizing light microscopic and electron-microscopic examinations and enzymatic biochemical determinations, this study correlates the increase in aspartic acid plasma levels with damage of the arcuate nucleus following the administration of oral doses of L-aspartyl-L-phenylalanine methyl ester (aspartame) and subcutaneous injections of sodium aspartate. Plasma aspartic acid concentrations were measured in 6 to 8 day old mice following oral doses of physiological saline, 2 mg/gm (body weight), 3 mg/gm of aspartame and .5 mg/gm subcutaneous injections of sodium aspartate. Following an oral 2 mg/gm dose of aspartame, plasma aspartic acid levels had a mean peak value of 0.80 ± .10 (SEM) μ mol/ml at 1 hour as compared to a control value of .05 ± .004 μ mol/ml. No pathology was observed in the arcuate nucleus in animals orally dosed with 2 mg/gm aspartame. However, oral doses of 3 mg/gm aspartame and subcutaneous injections of 0.5 mg/gm sodium aspartate resulted in a similar degree of frank neuronal death in all of the brains examined. In subjects treated with 3 mg/gm of aspartame, the aspartic acid plasma levels reached a mean peak value of .65 ± .11 μ mol/ml at one hour post gavage; however, they remained elevated above the levels of the 2 mg/gm dose subjects between the 2nd and 6th hour post gavage intervals. The fact that the plasma concentration peak at 1 hour post gavage was lower for the 3 mg/gm dose than it was for the 2 mg/gm dose may have been due to physical factors associated with a larger bolus of aspartame. Following subcutaneous injections of 0.5 mg/gm of sodium aspartate, plasma aspartic acid levels reached a mean value of 3.9 ± .98 μ mol/ml in five minutes and fell to 3.0 ± .36 μ mol/ml at the next interval sampled at 15 minutes.

These results suggest that the duration of elevated plasma aspartic acid levels as well as the peak concentration determines whether pathology will occur in the arcuate nucleus of the 6 to 8 day old mouse. These results show that following subcutaneous injections of aspartic acid, the plasma must be sampled within 5 minutes in order to determine the peak plasma amino acid values.

111.6 EXCITATORY AMINO ACID RECEPTOR MEDIATED RELEASE OF ^{3}H-ACETYLCHOLINE FROM STRIATAL SLICES OF THE RAT : MAGNESIUM SENSITIVITY AND PHARMACOLOGICAL CHARACTERISTICS. Bernard Scatton and John Lehmann Synthélabo-L.E.R.S., Biology Department, 92220 Bagneux, France.

Application of L-glutamate, L-aspartate and excitatory amino acid receptor agonists to superfused striatal slices previously incubated with ^{3}H-choline evoked the release of ^{3}H-acetylcholine (ACh). In medium containing magnesium (1.2 mM), application of L-glutamate, L-aspartate, kainate, ibotenate, N-methyl-DL-aspartate (NMA), L-cysteate, quisqualate, and the recently developed glutamate agonist RS-α-amino-3-hydroxy-5-methyl-4-isoazolepropionic acid (AMPA) (0.1 mM to 1 mM) produced an increase in the efflux of ^{3}H-ACh which was abolished by removal of calcium from the medium. Under these conditions, the maximal effects and potencies of agonists were similar.

However, when magnesium was removed from the medium, a six-fold increase in the release evoked by application of L-glutamate was observed. Removal of magnesium also potentiated the response elicited by NMA, L-aspartate, AMPA, ibotenate, and L-cysteate, but not that elicited by kainate and quisqualate. In the absence of magnesium, the excitatory amino acid agonists assumed a clear rank order of potency :

NMA > ibotenate > AMPA = L-glutamate ≥ L-aspartate > L-cysteate

> kainate = quisqualate

In magnesium free medium, both DL-2-amino-4-phosphonobutyrate (APB) and glutamate diethylester (GDEE) were capable of completely blocking the release of ^{3}H-ACh evoked by L-glutamate, APB being significantly more potent than GDEE. In contrast, DL-α-amino adipate was almost totally inactive. APB was also capable of completely blocking the release of ^{3}H-ACh evoked by L-aspartate, kainate, quisqualate and L-cysteate.

The concentrations of excitatory amino acid receptor agonists found to be effective in eliciting release of ^{3}H-ACh are similar to those effective in electrophysiological studies. Striatal cholinergic neurons are thought to receive a direct excitatory amino acid input from the cerebral cortex. The excitatory amino acid receptor thought to mediate this action has been characterized by measuring the release of ^{3}H-ACh from striatal slices. This represents a simple model to study the direct action of amino acid neurotransmitters on a population of neurochemically defined target cells and their biological response.

111.7 STRYCHNINE: BRAINSTEM AND SPINAL CORD MEDIATION OF EXCITATORY EFFECTS ON ACOUSTIC STARTLE. J.H. Kehne*, D.W. Gallager, and M. Davis. Dept. Psychiat., Yale Univ. Sch. Med., New Haven, CT 06508

The acoustic startle response has proven to be a sensitive measure for evaluating the effects of drugs on behavioral reactivity to sensory stimulation. In the present study, the effects of the glycine antagonist strychnine (STRY) were measured on acoustic startle. STRY (0.25 - 2.0 mg/kg, i.p.) produced a powerful, dose-dependent increase in startle amplitude that reached its greatest magnitude within 10-15 minutes after injection. These doses did not produce convulsions or behavioral activation and did not increase movement in the absence of startle stimuli. Furthermore, enhanced startle amplitude does not appear to be a general property of convulsant drugs, since i.p. administration of picrotoxin, a GABA antagonist, produces a dose-dependent inhibition of acoustic startle.

In an attempt to localize the site of action of this excitatory STRY effect, rats were implanted with catheters in the lumbar region of the spinal cord (intrathecal implantation), in the cisterna magna, or in the lateral ventricle. One to two days later they were tested for startle after microinjections of STRY. Dose-dependent excitatory effects on acoustic startle were found when STRY was injected onto the spinal cord (3.1 - 12.5 μg) or into the cisterna magna (6.2 - 25 μg), whereas infusion into the lateral ventricle produced a dose-dependent inhibition (6.2 - 25 μg). The excitatory effects were not attributable to convulsions or behavioral excitation. The maximal increases produced by 25 μg STRY intracisternally (144%) and 6.2 μg intrathecally (164%) were approximately equivalent to the peak effect seen following systemic administration (160%).

Thus, the excitatory effect produced by systemic injection of STRY can be attributed to its action in the spinal cord and brainstem. These results are consistent with studies suggesting that glycine neurons and receptors are primarily localized in the caudal regions of the central nervous system.

The inhibitory effect of intraventricularly-administered STRY was somewhat surprising, since there is little evidence for glycine or glycine receptors in the forebrain. However, high concentrations of STRY have been reported to have GABA antagonist properties. Since intraventricular injection of picrotoxin produces a potent inhibition of startle, it is possible that the inhibition accompanying intraventricular STRY infusion resulted from a blockade of GABA receptors.

The present results suggest that glycine exerts a tonic inhibitory effect on acoustic startle. The possible relation of such a system to phenomena that involve reduction in startle amplitude (e.g., habituation, pre-pulse inhibition) is discussed.

111.8 EXCITATORY AMINO ACIDS EVOKE A VOLTAGE-DEPENDENT DECREASE IN THE CONDUCTANCE OF CULTURED MURINE NEURONS. John F. MacDonald and J. Martin Wojtowicz. Playfair Neuroscience Unit, Dept. Pharmacology, U. of Toronto, Toronto, Ont. M5T 2S8.

Neurons derived from foetal mouse spinal cord or brain were grown, dissociated, in tissue culture. Intracellular recordings were then performed with either a single or with two independent microelectrodes (3M KCl). Membrane conductance (G) was assayed by passing: 1) constant current pulses via the recording electrode 2) constant current pulses via a second graphite shielded electrode 3) constant voltage pulses during a single electrode voltage-clamp 4) constant voltage pulses during a conventional two electrode voltage-clamp. Results were qualitatively similar with each method of measurement. Drugs were applied by pressure microperfusion or by microiontophoresis. In addition TTX (1 to 3 μM) was added to the bathing medium (Hank's) in order to block TTX-sensitive action potentials and to prevent synaptically mediated intercellular effects of excitatory amino acids. L-glutamic, L-aspartic, DL-homocysteic and N-methyl-D-aspartic acids (but not Kainic acid) depolarized neurons from resting values in association with a decrease in G (inward current). However, when membrane potential was lowered towards 0 mV by steady-state current injection this decrease gradually converted to a net increase in G. A distinct reversal potential (-10 mV) could be attributed to this increase in G whereas a second reversal potential (-80 to -85 mV) was linked to the decrease. The decrease of G was therefore likely due to a reduction of G_{K+} and is similar to the reported action of DL-homocysteic acid on in vivo cat motoneurons (Engberg, et al, J. Physiol. 288:227-261, 1979). Extracellular applications of G_{K+} blockers such as TEA, 4-aminopyridine, Co^{2+} Mn^{2+}, Cd^{2+}, Mg^{2+} and EGTA were unable to mimic this decrease of G. In addition, acetylcholine (up to 5 mM) was unable to evoke such a decrease. Ba^{2+} (5 mM) consistently decreased G but the voltage-dependency differed in that the magnitude of the decrease was augemented with depolarization rather than diminished as was seen with excitatory amino acids. These results demonstrate that excitatory amino acids both decrease and increase G of cultured neurons. The decrease is likely due to reduction of a resting G_{K+} distinct from that underlying delayed rectification or an analogous M-current reported in sympathetic neurons (Constanti, et al., Brain Res. 206:244-250, 1981). (supported by the MRC of Canada and by the Dystonia Research Foundation).

111.9 THE ACTION OF GLUTAMATE ON CA1 PYRAMIDAL NEURONS. J. J. Hablitz and I. A. Langmoen*. Sect. of Neurophysiology, Dept. of Neurology, Baylor College of Medicine, Houston, TX 77030.

Iontophoretically applied glutamate (glu) is a potent excitant of hippocampal CA1 pyramidal neurons. We used the guinea-pig in vitro hippocampal-slice preparation to investigate the mechanism by which glutamate excites these neurons. Intracellular recordings were obtained from CA1 pyramids while glu (1 M, pH = 8) was iontophoresed onto the proximal apical dendrites.

Glu reliably depolarized and excited all neurons tested. The glu response was highly localized (±15 μM) and dose dependent. Two types of response were seen. The most typical, occurring in 85% of cells, was characterized by a steadily increasing cellular firing, superimposed on a depolarization that seldom exceeded 20 mV. The other, obtained with increased glutamate doses, was a large depolarization that reached a plateau 35-45 mV positive to rest. The limiting slope of log-log dose-response curves for the first type of response varied from 1.5 to 5.0, indicating that more than one glu molecule was involved in the response. The depolarization and cellular firing induced by moderate glu-ejection currents (10-40 nA) showed no appreciable desensitization during 30-sec applications.

The effect of glu on input resistance (R_{in}), as measured by 100-300 msec hyperpolarizing-current pulses, was dose dependent. Small doses of glu produced either no change or an apparent increase in R_{in}. With larger doses, R_{in} invariably decreased. Increases in R_{in} were observed also with passive depolarization of the membrane. This suggested that the increase in R_{in} seen with glu resulted from conductance changes after the depolarization instead of a direct effect of glu. To study the direct effect of glu on R_{in}, we used slices in which all regenerative activity had been blocked by bath application of TTX (1 μg/ml) and $MnCl_2$ (4 mM). Here, glu produced a dose-dependent depolarization and decrease in R_{in}.

The glu-response amplitude was membrane-potential dependent and had a reversal potential (E_{glu}) near 0 mV (Langmoen and Hablitz, Neurosci. Lett., 1980, 23:61), suggesting that Na ions play a prominent role in producing the glu depolarization. Replacement of 75% of the $[Na]_o$ with TRIS reduced the slope of the glu dose-response curve, decreased the maximum response, and shifted E_{glu} to -38 mV.

We suggest that glu increases the permeability of the postsynaptic membrane primarily to sodium. Because E_{glu} in normal saline is close to 0 mV, the inward Na^+ current must be contaminated by an outward current that most likely is carried by K^+, since Cl^- injection does not alter the glu response.

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111.10 RESPONSE OF HIPPOCAMPAL SYNAPTIC FIELDS TO ANALOGUES OF ACIDIC AMINO ACIDS. J. F. Koerner* and C. W. Cotman (SPON: R. L. Purple). Dept. of Biochemistry, Univ. of Minnesota, Minneapolis, MN 55455, and Dept. of Psychobiology, Univ. of California, Irvine, CA 92717.

Analogues of the putative excitatory transmitters aspartic acid and glutamic acid were tested for antagonism against stimulus-evoked activation of Schaffer collateral-CA1 pyramidal cell synapses and perforant path-granule cell synapses in slices of rat hippocampus. Responses to the analogues, applied via the superfusing medium, were measured by their effects on the extracellular synaptic field potential. Compounds examined included higher homologues of D- and L-α-aminodicarboxylic acids, diaminodicarboxylic acids, phosphonate analogues of acidic amino acids, γ-D-glutamylglycine, and cis-2,3-piperidine dicarboxylic acid. Many of the compounds were chosen for their potent antagonism for the N-methyl-D-aspartate receptor of the dorsal-ventral root excitatory pathway of the spinal cord. Against Schaffer-CA1 pyramidal cell synapses, all analogues showed relatively low and similar potency with less than 15% inhibition at 0.5 mM and at least 40% inhibition at 5 mM. Most also evoked extracellular responses, including population spikes, abnormally prolonged times of inhibition and recovery, and anomalous dose-response curves, attributable to weak agonist responses of CA1 pyramidal cells. Thus for this synaptic field there are presently no known potent antagonists, and no conclusions can be drawn about this postsynaptic receptor from structure-function relationships, such as it being aspartate- or glutamate-preferring, or with specificity for L- versus D-amino acids. These results strongly contrast with those obtained for perforant path-granule cell synapses. In particular, the portion of the perforant path derived from the lateral entorhinal cortex is selectively inhibited by micromolar L-2-amino-4-phosphonobutyric acid, a structural analogue of L-glutamic acid. (Koerner, J.F., and Cotman, C.W., Brain Res., 217: in press). The extracellular responses of this pathway to this drug are those expected for inhibition by a pure antagonist. The hippocampal data also contrast with the responses documented by others for the dorsal-ventral root excitatory pathway of the spinal cord (Watkins, J.C. and Evans, R.H., Annu. Rev. Pharmacol. Toxicol., 21:165, 1981). In that system, the higher homologues tested were the most potent antagonists, and the D-isomers were more potent than the L-isomers. Thus these three excitatory pathways of the CNS each show a unique pattern of pharmacological response to this series of drugs. (Supported by NIH grants NS 08957 and MH 19691 and the Minnesota Medical Foundation).

111.11 PHOSPHOLIPID METHYLATION, A POSSIBLE MOLECULAR EVENT INVOLVED IN THE ACTION OF BENZODIAZEPINES. G. Toffano, G. Calderini*, A. Leon* and A. Battistella*. Dpt. of Biochemistry, Fidia Research Laboratories, Abano Terme, Italy.

The participation of postsynaptic GABAergic mechanisms in the action of benzodiazepines has been evidentiated by electrophysiological, pharmacological, biochemical, histological and behavioural observations. However the intimate molecular mechanisms by which benzodiazepines facilitate GABAergic transmission is still hidden in the complexity of GABA receptor.This supramolecular unit includes CL^- channel GABA recognition sites, endogenous GABA binding modulator (s), benzodiazepine recognition sites and putative ligand(s) for the benzodiazepine receptor sites (for a review see Costa, E. and Guidotti, A., Ann. Rev. Pharmacol., 19:531-545, 1979).

Recently it has been reported that benzodiazepines increase membrane fluidification and phospholipid methylation in C_6 glioma cells, which however contain benzodiazepine recognition sites of "peripheral-type" (Strittmatter et al., Nature, 282:857-859, 1979). As phospholipid methylation alters a variety of membrane functions (Hirata, F. and Axelrod, J., Science, 209:1082-1090, 1980) we investigated whether also the stimulation of benzodiazepine recognition sites of "central-type" enhances phospholipid methylation. Crude synaptic plasma membranes from rat cerebellum were incubated with 0.7 μM [^{3}H]-S-adenosylmethionine ([^{3}H] -SAM) in the absence or presence of 10^{-7} M diazepam.

The incorporation of [^{3}H]-methyl-group into phospholipidic fraction increases in the presence of Diazepam and conversely the binding of [^{3}H]-Diazepam is enhanced in membrane preparation preincubated with SAM. The effect is dose dependent. On the other hand in fresh membrane preparation the addition of the methyl donor produces a significant decrease of the KD for [^{3}H]-GABA binding (from 130 to 40 nM).

From these data, one could infer that the occupancy of benzodiazepine recognition sites stimulates phospholipid methylation, and that, in turn, increased membrane fluidity allows for a change of GABA receptor responsiveness.

111.12 DEPLETION OF GABA AND SEIZURES PRODUCED BY ISONIAZID AFTER IRREVERSIBLE INHIBITION OF GABA-TRANSAMINASE. R.F. Keating* and K. Gale (SPON: A. Raines), Department of Pharmacology, Georgetown University, Schools of Medicine and Dentistry, Washington, D.C., 20007.

The rate of decline in GABA after inhibition of GABA synthesis (by isoniazid, INH) was measured in several brain regions of rats which had been pretreated with gamma-vinyl-GABA (GVG), an irreversible inhibitor of GABA-transaminase (GABA-T). After GVG alone (1800 mg/kg i.p.), GABA-T activity was inhibited by 80-85%. GABA levels reached a plateau by 12 hr, remaining constant (3-4 fold over control) beyond 18hr. In rats which were treated with GVG, (1800 mg/kg i.p.) 12 hr before, INH (600 mg/kg i.p.) caused a net decrease in GABA at 45 min which was equivalent to the decrease observed in controls receiving INH alone. To determine the rate of decline in GABA after INH, rats which had been pretreated (12 hr) with GVG were killed at 20, 45, 70, 90, and 120 min after INH. The rate of decline after INH was highest in superior colliculus and substantia nigra (50-60 nmol/mg prot/hr) and lowest in cerebellar cortex and caudate nucleus (20 nmol/mg prot/hr). These rates were found to be similar to those obtained in control rats treated with INH alone (20 and 45 min before killing). In rats receiving GVG + INH, seizures were frequently observed after a latency of 45 min following INH.

The direct intracerebral microinjection of GVG (10ug into superior colliculus and 5ug into substantia nigra) resulted in a virtually complete (> 95%) inhibition of GABA-T and a significantly greater elevation of GABA than had been observed after systemic treatment. Under these conditions, there was a significant attenuation of the decline in GABA induced by systemic INH.

These data indicate that a normal rate of elimination of GABA can be maintained despite marked irreversible inhibition of GABA-T. We have previously demonstrated that the elevation of GABA measured 12 hr after GVG was associated with compartments other than nerve terminals. The decline in GABA induced by INH 12 hr after GVG, may therefore be due to depletion of nerve-terminal GABA. On the other hand, direct application of GVG into brain results in a rapid increase in nerve-terminal associated GABA and prevents the depletion of GABA induced by INH. Nerve terminals may therefore represent a critical site for the degradation of GABA in circumstances in which there is less than complete inhibition of GABA-T.

112.1 MICROIONTOPHORETIC APPLICATION OF 5HT BLOCKS RESPONSES TO NOXIOUS STIMULI IN SINGLE UNITS IN THE PARAFASCICULARIS OF THE RAT. E. Andersen and N. Dafny, University of Texas Health Science Center, Dept. of Neurobiology and Anatomy, Houston, TX.

The raphe nuclei of the midbrain are known to be involved in a pain modulation system. They contain a high consentration of serotonergic (5HT) cell bodies and pharmacological manipulation of 5HT has been shown to alter the effects of morphine and other types of analgesia. Electrical stimulation of the raphe/central grey region causes behavioral analgesia and causes changes in neurons in the spinal cord which respond to noxious stimuli. Noxious stimuli cause changes in many forebrain structures as well, and it is likely that the raphe nuclei and 5HT modify responses to pain not only in the spinal cord, but in other specific areas of the CNS.

Our laboratory has previously shown that an intralaminar thalamic nucleus of the rat (parafascicularis, PF) like similar nuclei in other species, is particularly responsive to noxious stimuli. Furthermore, electrical stimulation of the dorsal raphe nucleus (DR) blocks the increase in firing rates caused by noxious stimuli in single units of the PF.

In order to test 5HT as a possible neurotransmitter in this system, 5HT was microiontophoresed directly onto spontaneously active units in the PF of urethane-anesthetised rats. Doses of 20, 50, and 80 nAmps were given for two minutes each with recovery periods between each dose. 5HT caused a decrease in the firing rates of spontanouesly active neurons in a dose dependent manner. Noxious tail pinch, in contrast, increased firing rates. The application of 5HT onto units excited by tail pinch caused a decrease in firing rates.

Microiontophoretically applied 5HT therefore has a similar effect as DR stimulation in inhibiting the response of PF neurons to noxious stimuli. This indicates that 5HT may be a neurotransmitter used by the DR in a direct, ascending pathway to the PF whose function is to modify responses to painful input at this central level.

112.2 DOES NORADRENALINE MEDIATE THE MODULATORY EFFECTS OF THE LOCUS COERULEUS ON LUMBAR DORSAL HORN INTERNEURONS? J. Franck, C. J. Hodge, Jr., A. Apkarian, R. Stevens, G. Peca-Vogelsang, and J. Wisnicki. Dept. of Neurosurgery, Upstate Medical Center, Syracuse NY 13210.

Stimulation of the locus coeruleus (LC) results in potent inhibition of responses of lumbar lamina IV and V neurons to peripheral stimulation. Since LC is rich in noradrenaline (NA) containing neurons, and since noradrenergic projections from the brain stem to the spinal cord have been implicated in spinal cord antinociception, it has been proposed that NA is the principal mediator of direct coeruleo-spinal modulatory effects. We report anatomical and physiological evidence to the contrary.

Evan's Blue (EB) was injected into the lumbar spinal cord of cats. After 3 days, fluorescent microscopy was used to examine the brain stem, which had been processed with glyoxylic acid. This allowed identification of cells containing retrogradely transported EB as well as cells containing catecholamines (CA). Most of the cells in LC that project to the lumbar cord do not contain NA.

Responses of lumbar lamina IV and V cells, activated by either peripheral innocuous or noxious stimuli, were studied in cats. Stimulation of the dorsolateral pons with monopolar electrodes was used to locate points where stimulation was effective in inhibiting the responses of lumbar dorsal horn cells to somatic stimulation. The minimal stimulation thresholds (10 uA) for inhibition were in LC. The latency to initial inhibition of lumbar unit responses following LC stimulation was 30-50 msec. This data implies that the conduction velocity is at least 10 m/sec., which is faster than any reported conduction velocity for central NA containing neurons.

After IV or direct topical cord administration of phenoxybenzamine, an alpha-blocker, or propranolol, a beta-blocker, potent dorsal horn inhibition could still be evoked at low thresholds (less than 50 uA). Intrathecal 6-hydroxydopamine was used to induce depletion of lumbar cord NA. Adequate depletion was confirmed by fluorescent microscopy and chromatography. There was no apparent difference between these cats and untreated cats in either the electrical thresholds or loci of stimulation capable of causing lumbar dorsal horn cell inhibition.

We conclude that the coeruleo-spinal inhibition is not principally mediated by NA-containing LC neurons that project to lumbar spinal cord.

112.3 SERUM MORPHINE LEVELS DURING SUPPRESSION OF DORSAL HORN WDR NEURONS BY SPINALLY ADMINISTERED MORPHINE. J.G. Collins, J. Bach*, E. Homma*, L.M. Kitahata, Dept. Anesthesiol. and Dept. Lab. Medicine, Yale Univ. Sch. Med., New Haven, CT. 06510

The peridural administration of opioids can produce profound analgesia in both man and experimental animals. We have recently demonstrated that the spinal administration of morphine causes a dose dependent, partially naloxone reversible suppression of WDR neurons in the dorsal horn of the spinal cord. The use of spinal narcotic analgesia raises a question as to the degree of systemic uptake of the drug. Systemic uptake could explain part or all of the analgesia and/or neurophysiological effects seen following spinal morphine administration. The present study was carried out in order to determine serum morphine levels at the time of suppression of neuronal activity in the dorsal horn of the spinal cord following the spinal administration of morphine.

Cats, ranging in weight from 2.5 to 5.0 kg, were prepared for electrophysiological recording as previously described.[1] Blood samples were drawn prior to surgical preparation of the animal (to control for possible release of endogenous opioids during surgery), prior to drug administration and 10, 20, and 30 min after the spinal administration of morphine (0.1 or 0.25 mg.). Samples were centrifuged and serum was separated and frozen until assayed. Morphine levels were determined using a commercially available RIA Kit (Roche Diagnostics "Abuscreen") calibrated with morphine standards prepared in cat serum. The assay detects both free morphine and morphine glucuronide. No other drugs administered to the animals were known to react with the assay.

Both time and dose response effects were seen following the spinal administration of morphine. At the time of maximum suppression of neuronal activity (30 min.) the average serum levels following 0.25 mg of spinally administered morphine were below 20 ng/ml. The 0.1 mg dose of morphine produced even lower serum levels.

The present study indicates that there is systemic uptake following the spinal administration of morphine but that the serum levels are relatively low at the time of maximum neuronal suppression. They are much lower than the levels reported in humans at the time of adequate analgesia (50 ng/ml)[2]. Studies are being conducted to determine the serum levels of free morphine at the 30 min. time point.

1. Namiki, A., Collins, J.G., Kitahata, L.M. et al.: Anesthesiology 53:475-480, 1980.
2. Berkowitz, B.A., Ngai, S.H., Yang, S.C. et al.: Clin. Pharmacol. Ther. 17:629-635, 1975.

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112.4 SPINAL MORPHINE ADMINISTRATION SUPPRESSES NOXIOUSLY EVOKED ACTIVITY OF DORSAL HORN WDR NEURONS. E. Homma*, J.G. Collins, L.M. Kitahata, Dept. of Anesthesiol., Yale Univ. Sch. of Med., New Haven, CT. 06510

Studies of the neuropharmacology and neurophysiology of the spinal cord have led to an appreciation of the importance of spinal sites in the production of analgesia. Such studies are responsible for the present interest in the technique of spinal narcotic analgesia, a technique which has been shown to produce profound analgesia in both man and experimental animals. The present study was carried out in order to determine if the spinal administration of morphine is capable of producing a dose dependent, naloxone reversible suppression of noxiously evoked activity of wide dynamic range neurons in the dorsal horn of the spinal cord.

Extracellular single unit recordings were obtained from physiologically identified wide dynamic range neurons in decerebrate, spinal cord transected (L-1) cats. The animals had been surgically prepared (tracheal, carotid artery, jugular vein cannulation and lumbar laminectomiy L-7, L-4) under halothane, nitrous oxide-oxygen anesthesia but at least 4 hours passed between the end of anesthetic administration and the start of drug studies. Physiologic parameters were monitored and maintained within normal limits. Spontaneous and stimulus driven activity (radiant heat stimulus of 51°C, 8 sec. on the peripheral receptive field located on the footpad of the hindpaw) were studied both during the control situation and following the spinal administration of either 0.1 or 0.25 mg of morphine.

Although 0.1 mg of spinally administered morphine was found to produce small reductions in both stimulus driven and spontaneous activity, 0.25 mg produced a significant reduction in both stimulus driven and spontaneous activity with stimulus driven activity being suppressed to a greater extent. The spinal administration of naloxone (0.2 or 0.4 mg) produced some reversal of the spinal morphine effect on the WDR neuronal activity. Subsequent intravenous administration of 0.2 or 0.4 mg of naloxone produced further reversal of the narcotic effect. Complete naloxone reversal was not seen.

This, to the authors knowledge, is the first report of the ability of spinally administered narcotics to block neuronal activity elicited by noxious stimuli at the periphery. It is further evidence to support both the importance of spinal sites for the production of analgesia and the ability of spinally administered narcotics to block the afferent input of information following the presentation of a noxious stimulus at the periphery. (Supported by NIH Grant NS-09871)

112.5 NORADRENERGIC UPTAKE BLOCKERS ANTAGONIZE TONIC DESCENDING INHIBITION OF CAT SPINAL CORD NOCICEPTOR-DRIVEN NEURONES. P.J. Soja* and J.G. Sinclair. Div. of Pharmacology & Toxicology, Faculty of Pharmaceutical Sciences, University of British Columbia, Vancouver, B.C. V6T 1W5 Canada

The biogenic amines noradrenaline (NA) and serotonin (5-HT) have been implicated in antinociception and inhibition of spinal cord nociceptor-driven neurones by stimulation of certain brainstem nuclei. Furthermore it has been suggested that opiates produce analgesia in part by activating (a) descending NA and/or 5-HT pathway(s). However it is not known what synaptic transmitter(s) is (are) involved in mediating the powerful tonic descending inhibition of nociceptor-driven neurones from supraspinal structures. Recent studies (Soja and Sinclair, Brain Res. 199: 225-230, 1980 and Griersmith et al., Brain Res. 204: 147-158, 1981) have failed to implicate 5-HT in tonic descending inhibition. Therefore the present experiments were initiated to determine whether NA is involved in this tonic inhibitory impingement. In chloralose-anaesthetized cats a thermoelectric cooling device positioned at the L_1 spinal cord segment was employed to reversibly cold block the cord. Extracellular unit activity was recorded from wide-dynamic-range (WDR) neurones in the lumbar cord. Noxious radiant heat (with feedback control, 47-55°C, 10-15 s, 2 min intervals) was used to activate these units. The degree of tonic inhibition, as judged by comparing the noxious heat-evoked response in the normal vs. cold blocked state of the spinal cord, was determined for each neurone. With the cord in the normal state nisoxetine (6 mg/kg i.v.), a specific NA uptake blocker, enhanced the noxious heat evoked unit responses in 5 animals and decreased them in one. Desipramine (6 mg/kg i.v.) produced similar results on 6 other WDR neurones. Only one cell was tested per animal. Responses obtained during the cold block state after the drugs were administered were the same as those obtained in the control cold block state suggesting that these agents did not affect primary afferent input of these cells. This also implies that these agents did not exert their effects directly on the recorded cells but rather were dependent on the tonic descending inhibition. If NA mediates tonic descending inhibition then these agents which enhance NA synaptic activity should have decreased the heat evoked responses in the cells tested. However the results suggest that a noradrenergic system participates in antagonizing the tonic descending inhibition of spinal cord nociceptor-driven neurones.

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112.6 THE ROLE OF ACETYLCHOLINE IN INTERACTION BETWEEN THE PERIAQUEDUCTAL GRAY AND NUCLEUS RAPHE MAGNUS. M.M. Behbehani and C.E. Mack*. U. Cincinnati Coll. of Med., Dept. of Physiol., Cincinnati, OH 45267

The involvement of the nucleus raphe magnus (NRM) in inhibition of pain has been clearly established. Physiological and anatomical methods have shown that the NRM receives diverse afferents from all regions of the brain. Of particular importance are the afferents from the periaqueductal gray (PAG). Although the afferents to the NRM have been studied by histochemical methods, the neurotransmitter(s) involved in activation of NRM cells is not known. The experiments reported here were designed to examine the role of acetylcholine in the function of NRM neurons and to determine whether this neurotransmitter is the one which mediates the excitation of NRM neurons by the PAG. Rats were anesthetized with 1.2 g/kg of urethane; after relevant surgical procedures, a monopolar stimulating electrode was placed in the central part of the PAG. A glass recording electrode filled with pontamine blue and glued to a 5-barrel electrode was used to record single unit activity of the NRM Ach (500mM), scopolamine (200mM) and gallamine (200mM) were used in these experiments. One electrode filled with 3M NaCl was used as a balance electrode. The pH of the drugs ranged between 4.5 and 5.5. Of the 53 cells tested that responded to PAG stimulation, 36 responded by excitation and 13 by inhibition. For each cell, after the response to PAG stimulation before and after scopolamine application was determined, the response to iontophoretically applied Ach (80-130 nA, 10 or 20 seconds) was examined. Of the 53 cells, 33 were excited, one was inhibited and 21 did not respond to Ach. In order to determine the type of Ach receptor present in the NRM, the effect of muscarinic and nicotinic antagonists were examined. Of the 32 cells tested, application of scopolamine for 1 minute totally or partially blocked the response to Ach in 18 cells and in 14 cells Ach response was not affected by scopolamine. Response to gallamine was tested in 15 cells. In 11 cells, gallamine alone caused a significant increase in the firing rate of the cells. The response to Ach was not affected by gallamine in any cells. A strong correlation between excitatory response to PAG stimulation and to Ach was observed. However, since a large number of cells were excited by Ach but inhibited by PAG stimulation, the fact that several cells excited by PAG did not respond to Ach and the observation that the response to PAG stimulation could not be blocked by scopolamine provide evidence that Ach is not the transmitter that mediates the interaction between the PAG and the NRM. The fact that the response to Ach could be reversed by scopolamine and not by gallamine indicates that the Ach receptor present in the NRM is muscarinic. Supported by NIDA DA02282.

112.7 SIMULTANEOUS SUPPRESSION OF PAIN, ARTERIAL BLOOD PRESSURE AND HEART RATE BY CLONIDINE IN THE CAT: LACK OF OPIATE RECEPTOR INVOLVEMENT. Samuel H.H. Chan and Yih-Huey Chen. Dept. of Life Sciences, Indiana State University, Terre Haute, IN 47809.

There are recent evidences that implicated an inter-relationship between the regulation of pain and blood pressure. For example, pain threshold appears to be elevated in hypertensive animals or patients. The antihypertensive agent clonidine is now also known to be antinociceptive, and the analgesic action of this imidazoline compound is potentiated by morphine. Furthermore, cross-tolerance exists between clonidine and the opiate in the production of pain suppression. In light of these findings, the present study was carried out to investigate the simultaneous effects of clonidine on pain, arterial blood pressure (ABP) and heart rate (HR), as well as the possible involvement of opiate receptors in these processes.

Experiments were performed on adult cats that were anesthetized with α-chloralose and urethane (40 and 350 mg/kg, i.p.). The jaw-opening reflex (JOR), quantified as the averaged EMG signals recorded from the digastric muscle in response to dental-pulp stimulation, was taken as the experimental pain index. It was monitored alongside the ABP and HR throughout the experiment. Drugs were administered via a cannulated vertebral artery.

Intravertebral injection of clonidine (2, 4 and 10 μg/kg) induced a dose-dependent suppression of the JOR, ABP and HR. The degree of analgesia induced by any dose, however, was greater than the elicited hypotension or bradycardia. Such clonidine-promoted antinociceptive, vasodepressive and cardioinhibitory effects were not reversed by a subsequent injection of naloxone (0.2, 0.4 and 1.0 mg/kg), an opiate antagonist.

Reversal of the drug-administration sequence produced similar results. Initial injection of naloxone did not result in any significant changes in the JOR, ABP and HR. However, clonidine that followed still elicited appreciable analgesia, hypotension and bradycardia, the degrees of which were comparable to those observed in the previous series.

These data demonstrated that in addition to its hypotensive and cardioinhibitory effects, clonidine also possesses an antinociceptive action. However, there appears to be a dose-difference in the production of analgesia, vasodepression and bradycardia by the imidazoline compound. Furthermore, the failure of naloxone to reverse the clonidine effects implicated that it is unlikely for the opiate receptors to be involved in these pain and cardiovascular suppressive processes.

(Supported in part by the American Heart Association, Indiana Affiliate and the Research Committee, Indiana State University)

112.8

WITHDRAWN

112.9 EFFECT OF N. RAPHE ALATUS (NRA) & PERIAQUEDUCTAL GRAY (PAG) LESIONS ON FOOTSHOCK INDUCED ANALGESIA (FSIA) & CLASSICALLY CONDITIONED ANALGESIA. L.R. Watkins*, E.G. Young*, I.B. Kinscheck* & D.J. Mayer. Dept. of Physiology, MCV/VCU, Richmond, VA 23298.

It has recently been shown that front paw FSIA & classically conditioned analgesia involve endogenous opiates since they are antagonized by systemic naloxone, spinal naloxone & morphine tolerance. In contrast, hind paw FSIA involves non-opiate systems for it fails to be reduced by these manipulations. Each of these phenomena appears to be neurally mediated since none are attenuated by hypophysectomy or adrenalectomy. In addition, all are dependent upon activation of supraspinal sites which inhibit pain via descending pathways in the dorsolateral funiculus of the spinal cord.

A major source of these descending axons is the NRA, which consists of the combined cell populations of the n. raphe magnus (NRM) & the n. reticularis paragigantocellularis (NRPgc). This area has previously been implicated in morphine analgesia (MA) & stimulation-produced analgesia (Br Res, 181:1, 1980). NRA, NRM & NRPgc are also involved in front paw FSIA. When rats are exposed to 90 sec front paw shock, the resultant analgesia (as measured by the tail flick test) is greatly reduced in NRA, NRM & NRPgc lesioned animals compared to sham operated controls. When these rats were tested for both systemic MA and front paw FSIA, a highly significant correlation was found between lesion-induced reductions in both forms of opiate analgesia. Preliminary studies indicate that at least NRPgc is involved in classically conditioned (opiate) analgesia. Using the non-electrified grid as the conditioned stimulus (CS), 90 sec hind paw shock as the unconditioned stimulus (UCS) & analgesia as the unconditioned response, 3 CS-UCS pairings result in analgesia being produced following exposure to the CS alone. This classically conditioned analgesia was reduced in rats with NRPgc lesions. In contrast, non-opiate FSIA produced by 90 sec hind paw shock is not blocked by these lesions. These results provide the first anatomical evidence that opiate & non-opiate analgesia may be mediated by independent supraspinal systems.

In addition, the effects of rostral & caudal PAG lesions were tested for FSIA & classically conditioned analgesia. Neither lesion had any effect on front paw FSIA or hind paw FSIA. However, caudal lesions reduced classically conditioned analgesia. Since the PAG has been implicated in affect, the observations that PAG lesions and librium both reduce classically condtioned analgesia suggests that an affective state, such as fear, is involved. This research was supported by Grant DA-00576 to DJM.

112.10 NON-ENDORPHINERGIC PATHWAYS SUPPRESS PAIN IN HUMANS. J.B. WALKER* AND R.L. KATZ (SPON: M.H. CHASE). Department of Anesthesiology, UCLA School of Medicine, Los Angeles, California 90024.

Subcutaneous electrical stimulation (20HZ) of median, radial, and saphenous nerves produces prolonged analgesia. This effect of subcutaneous nerve stimulation (SCNS) is not mediated by opiate receptors (1) there is no cross-tolerance with opiates, and SCNS suppresses pain in patients who have received chronic administration and meperidine every two hours for months (2) patients unresponsive to morphine benefit from SCNS (3) there is no tolerance to SCNS, and (4) SCNS, can be administered with oral analgesics (both opiate and non-opiate) to increase patient comfort. These observations suggest that non-endorphinergic pathways produce powerful analgesia.

Although the mode of SCNS is unknown, it decreases the excitability of spinal reflexes. In a double blind study, SCNS suppresses clonus in spastic patients for three hours. The effect is contralateral so that each patient can serve as his own control. Suppression is not mediated by opiate receptors because it is not mimicked by opiates nor is it naloxone-reversible. The neurochemical basis for long duration of SCNS-induced alterations in neural excitability are currently under investigation.

112.11 DIFFERENTIAL EFFECTS OF FENTANYL AND DIAZEPAM ON PAIN SENSATIONS AND PSYCHOPHYSICAL PERFORMANCE. R.H. Gracely*, P.J. Wolskee*, W.R. Deeter* and R. Dubner (SPON: R. Dionne). Neurobiology and Anesthesiology Branch, NIDR, NIH, Bethesda, MD 20205.

Functional measurement divides psychophysical assessment into perceptual, cognitive-integrative and response stages. This technique requires subjects to integrate pairs of stimuli from two independent stimulus sets. In this study, subjects rated both pain produced by electrical stimulation of the tooth pulp and pain symbolized by a word. This procedure produces measures of each stimulus set and a measure of cognitive integration performance. Eighty-two subjects were presented all possible pairs of 5 tooth pulp stimuli (a blank and 4 current intensities ranging from pain threshold to pain tolerance) and 5 verbal descriptors of pain intensity (no sensation, weak, moderate, strong, very intense). Subjects used a 15 point category scale to rate the average of the pain intensity experienced and the pain symbolized by the descriptor. The 25 pairs were presented twice in random order before and after the double blind intravenous administration of 1) 0.66 mg/kg diazepam or saline placebo, followed immediately by 2) 0.001 mg/kg fentanyl or saline placebo. Seven subjects could not perform the task and were excluded from the analysis. Two-way analyses of variance (fentanyl effect X diazepam effect) assessed the effects of the drugs on the two stimulus sets and on a nonparametric index of integration error. The intensity of the tooth pulp sensations was reduced significantly after fentanyl in comparison to placebo ($F(1,64)=8.79$, $p<.005$). Diazepam had no effect. The magnitude of pain intensity implied by the verbal descriptors was not altered by either drug. The ability to perform the stimulus integration task was reduced significantly by diazepam in comparison to placebo ($F(1,64)=7.63$, $p<.01$) and not altered by fentanyl. Significant interactions were not observed for any analysis.

These results show that pain perception was altered only by fentanyl, that stimulus integration was altered only by diazepam, and that the use of the category scales and the meanings of the words were not altered by either drug. These findings suggest that most subjects can perform an integration task involving physical sensations and words and that functional measurement techniques can distinguish between the effects of pharmacological manipulations on pain sensations and psychophysical performance.

112.12 SIMULTANEOUS RECORDINGS FROM HUMANS OF PAIN SENSATION AND NOCICEPTOR ACTIVITY BEFORE AND AFTER CUTANEOUS HYPERALGESIA C.J. Robinson, H.E. Torebjork* and R.H. LaMotte. Dept. of Anesthesiology, Yale Univ. Med. Schl., New Haven, Ct. 06510

Our aim was to study the functional relationship between pain sensations and activity in single cutaneous nociceptors. Brief heat stimuli of 38 to 51^{0}C were delivered to the foot or leg before and at varying intervals of time after a conditioning stimulus (CS) of prolonged noxious heat. Fourteen humans, each of whom gave informed consent to an approved protocol, made continuous ratings of the magnitude of pain sensations evoked by the heat stimuli. Simultaneously, percutaneous recordings were made of the evoked responses of single C-fiber polymodal nociceptive afferents in the common peroneal nerve.

Prior to the CS, the magnitude of neuronal response (NR), defined as the number of impulses evoked by each stimulus, increased linearly with stimulus temperature. In contrast, the magnitude ratings (MRs) of pain increased as a positively accelerating function of temperature. Following the CS, threshold temperatures required to evoke a minimal MR or NR were initially elevated. But, by 10 minutes after the CS, MR thresholds of most subjects were lower than pre-CS values (hyperalgesia) as were the NR thresholds of about half of the nociceptors (sensitization).

The correlation between each subject's MRs and the NRs of the nociceptor under study was weak. This relation between the MR and NR was monotonic but nonlinear before the CS; and typically nonmonotonic after the CS due to a saturation of the NR at the higher temperatures. In contrast, a closer correlation,and one with a greater degree of linearity,was obtained between pain sensation and nociceptor response when the MRs of each subject were related to the average NRs of all nociceptors studied. These observations indicate that the central summation of activity across the population of active nociceptors is an important determinant of the magnitude of pain sensation in man.

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113.1 ACTIVITY OF DOPAMINE-CONTAINING SUBSTANTIA NIGRA UNITS IN FREELY MOVING CATS. Michael E. Trulson, Donald W. Preussler and Gailyn A. Howell. Dept. Psychol., Univ. of Texas at Dallas, P.O. Box 688, Richardson, TX 75080.

Fluorescence histochemical mapping studies have revealed that the substantia nigra is densely populated with dopamine-containing neuronal perikarya. These neurons diffusely innervate the striatum, which is intimately involved in the control of movement. The nigral-striatal dopamine system is so diffuse, in fact, that it has been estimated that each dopamine neuron gives rise to approximately 500,000 synaptic contacts in the striatum, and that virtually every neuron in the striatum receives a dopaminergic input (Anden et al, Acta Physiol. Scand. 67: 306-312, 1966). While the anatomy of the nigral-striatal dopamine system has been well-described, the physiological role of this system remains unknown, despite intensive research efforts involving pharmacological and electrophysiological techniques. Although these latter studies have greatly increased our knowledge of the nigral-striatal system, one common disadvantage of these previous studies is the fact that they were carried out in animals that were immobilized and often anesthetized. Therefore, in the present study, we recorded the activity of dopamine-containing substantia nigra units in awake, freely moving cats. Nigral unit activity was recorded by means of movable 32 or 62 u dia insulated nichrome wires. Dopamine-containing nigral units were initially identified on-line by their unusually wide action potential (2-5 msec) and characteristic triphasic wave form (Guyenet and Aghajanian, Brain Res. 150: 69-84, 1978). This identity was later confirmed by the nearly total inhibition of unit activity by low doses of apomorphine (0.5 mg/kg, i.p.), and by subsequent histological analysis, i.e., all units were in the densely dopaminergic pars compacta of the substantia nigra. During quiet waking (i.e., no overt movement) nigral units displayed a slow, somewhat irregular activity ($\bar{X}$= 4.1 spikes/sec). Unit activity showed no significant change from quiet waking during slow-wave sleep ($\bar{X}$= 3.4 spikes/sec) or REM sleep ($\bar{X}$= 3.6 spikes/sec). These results are in sharp contrast to the other monoamine-containing neurons, since the activity of both serotonin- and norepinephrine-containing neurons have been shown to decrease dramatically during REM sleep. While nigral unit activity was somewhat higher during active waking ($\bar{X}$= 5.2 spikes/sec), i.e., when overt behaviors such as walking, grooming or eating were occurring, there was no apparent relationship to phasic movement. This is interesting in view of the fact that we have recently found that many striatal units show bursts of activity in relation to phasic movement (Trulson and Jacobs, Neuropharm. 18: 735-738, 1979). Therefore, dopamine-containing nigral units apparently exert some modulatory influence in the striatum, but are not involved in phasic changes in the activity of striatal neurons.

113.2 UNIT ACTIVITY OF DOPAMINERGIC NEURONS IN FREELY MOVING CATS. George F. Steinfels, James Heym, and Barry L. Jacobs. Prog. in Neurosci., Dept. Psychol., Princeton Univ., Princeton, NJ 08544.

The role of brain dopamine in various behaviors and motor processes has been studied by a variety of methods. Single unit studies of dopamine-containing neurons in chloral hydrate anesthetized rats have contributed much to our understanding of this system. However, the use of anesthetized animals precludes studying, for example, sleep-waking behaviors or motor related functions. Thus, we have attempted to identify and characterize single unit activity of dopaminergic neurons in the substantia nigra (SN) in freely moving cats. Since the majority of previous single unit studies of dopamine neurons was conducted in chloral hydrate anesthetized rats, we initially conducted pilot studies which examined unit activity in the pars compacta of the SN in chloral hydrate anesthetized cats. These neurons in the cat shared several distinctive characteristics with known dopaminergic cells studied in chloral hydrate anesthetized rats: action potentials of long duration (2-4ms) relative to other neurons (< 2ms) in the area; firing in bursts with successive decreases in spike amplitudes; firing rates of 4-10 spikes/sec; and suppression by i.v. apomorphine. In a second series of studies, cats were prepared for chronic unit recordings as previously described (Brain Res. 163: 135-150, 1979). The coordinates for the area studied in the SN were A 3.0-6.0, L 1.8-3.8, and H (-)4.0-(-)6.0. Ninety cells were studied from 9 cats. Of these, 20 cells were found which had the following characteristics: action potential durations of 2-4ms; firing rates of 2-6 spikes/sec; single discharges along with periods of bursts which displayed successive decreases in spike amplitude. Initial observations indicate that the activity of these neurons is neither heavily state-dependent nor motor-related. Eight of the twenty cells were held long enough for pharmacologic manipulations to be performed. Apomorphine (0.35-1.0 mg/kg, i.p.) caused decreases in firing rates of 60-100%, whereas, haloperidol (0.5-0.75 mg/kg, i.p.) caused increases of 30-60% in firing rates. The remaining 70 cells had action potential durations of 0.5-2.0ms and firing rates of 5-40 spikes/sec. Their activity was often heavily motor-related. In 25 of these cells, administration of apomorphine (0.35-1.0 mg/kg, i.p.) caused either no change or an increase in firing rates. Therefore, based upon physiological and pharmacological evidence, the unit activity of neurons in the area of pars compacta in the freely moving cat is similar to that of known dopaminergic cells in anesthetized rats and cats. Thus, this study is the first to report recording from presumed dopaminergic neurons in freely moving animals. (Supported by NIMH Grant MH 23433).

113.3 DOPAMINERGIC UNIT ACTIVITY IN THE BEHAVING RAT. J.D. Miller, M.K. Sanghera and D.C. German. Depts. of Physiology and Psychiatry, Univ. of Texas Health Science Center, Dallas, TX. 75235.

Mesencephalic dopaminergic (DA) neurons have been implicated in a number of behavioral processes, including intracranial self-stimulation, attentional mechanisms, motoric function, and a variety of pathological states such as schizophrenia and Parkinsonism. Single DA cells have been recorded in the substantia nigra zona compacta (nucleus A9) and ventral tegmental area (nucleus A10) both in the anesthetized and paralyzed-unanesthetized rat. These cells have a characteristic long duration action potential (> 2 msec), and typically fire in bursts at a rather low rate (3-10 Hz). A number of investigators have studied nigral single unit activity in the awake behaving monkey and rat; however, it is not clear whether DA cells were among the units recorded. It is a matter of some importance to understand the function of DA neurons in the unanesthetized, behaving animal. The goal of the research reported here was to develop methods which would allow recording of DA cells in behaving rats. Male albino rats (Sprague-Dawley) between 225-275g were used in these experiments. The animals were operantly conditioned on either a DRL-10 sec or a classical conditioning task with chocolate milk as the reinforcer. A stainless steel recording well was stereotaxically positioned over the DA cell region and glass coated tungsten microelectrodes were driven into the DA cell region while the animals were performing the operant task. A total of 183 tracks were run in 19 awake, behaving rats; 105 of these tracks were run in A10 (< 1 mm from midline), and 78 in A9 (1-1.5 mm from midline). In four out of 96 tracks DA cells were observed. In these four tracks, six DA cells were found. These six cells were encountered in the classical conditioning task, in animals who were behaviorally inactive. In rats tested with low doses of haloperidol (HALO), however, 49 DA cells were observed in 87 tracks. The majority of the cells observed under HALO were found during classical conditioning. Histological examination of microlesions showed that recording sites were in DA cell regions in both untreated and HALO-treated animals. The DA cells exhibited long spike durations (> 2 msec) and typically exhibited a characteristic bursting firing pattern with spikes of decreasing amplitude. There was little relationship between cell firing pattern and performance of the operant tasks, although some few A10 units gave a burst of activity to the conditioning stimulus. These results suggest that the incidence of A10 and medial A9 DA neurons is reduced during active behavioral states, whereas when the animals are behaviorally calmed (e.g. with HALO) cell incidence is higher. (This research was supported by NIMH grant MH-27574).

113.4 NIGRAL DOPAMINERGIC NEURONS: SINGLE UNIT ACTIVITY IN THE AWAKE, UNRESTRAINED RAT. L. T. Meltzer* and B. S. Bunney. Depts. Psychiatry & Pharmacology, Yale Univ. Sch. Med., New Haven, CT 06510

Central dopamine (DA) systems have been implicated in the modulation or mediation of both natural and drug-induced behaviors. However, most of the evidence linking DA systems with behavior is indirect as the activity of DA systems has not been directly assessed at the same time that the behavior is occurring. To directly correlate the activity of DA systems with behavior, we have begun to record the extracellular single-unit activity of substantia nigra (SN) zona compacta (ZC) neurons in the awake, unrestrained rat.

For these experiments, a stainless steel sleeve was implanted on the skull of male rats (380-480 gm) above the SN and a microdrive and field-effect-transistor inserted into the sleeve. Cell activity was recorded through glass insulated tungsten electrodes (1 u tip diameter). Jugular vein cannulae for drug administration in the freely moving animal were also implanted. Recording was begun one week after surgery.

Abundant spontaneously active putative DA neurons were found. Three sets of criteria were used to determine that DA neurons were being recorded. (1) Neurophysiological characteristics: The putative DA neurons had action-potential characteristics identical to those of identified DA neurons in the anesthetized and paralyzed rat. These included a wide action-potential (3-4 msec duration), a notch in the initial positive phase, and often a bursting pattern with spikes of successively decreasing amplitude within the burst. As is true in the anesthetized preparation, these characteristics are different from those of non-DA neurons seen in brain regions adjacent to the ZC. Firing rates were between 1.0 and 4.5 spikes/second. (2) Pharmacological responsiveness: The activity of these putative DA neurons was decreased by the DA agonist apomorphine and increased by the DA antagonist haloperidol. In addition, there appears to be a population of these neurons that are tonically inhibited, since more appear to be firing after haloperidol treatment. (3) Histological verification: Following recording, current was passed through the electrode resulting in a small lesion at the recording site. Brains were processed for histology utilizing both thionin staining and catecholamine fluorescence techniques. Recording sites of putative DA cells were histologically verified to be within the ZC of the SN. In conclusion, single unit recordings were obtained in the awake, unrestrained rat from neurons in the ZC of the SN. They had neurophysiological characteristics and pharmacological responses similar to identified DA neurons and, therefore, are presumed to be dopaminergic. (Supported by USPHS grants MH-28849, MH-25642 and the State of Connecticut.)

113.5 UNIT ACTIVITY OF MEDULLARY SEROTONIN-CONTAINING NEURONS IN FREELY MOVING CATS. James Heym, Geroge F. Steinfels and Barry L. Jacobs. Prog. in Neurosci., Dept. of Psychol., Princeton Univ., Princeton NJ 08544.

A great deal has been learned about the activity of serotonin-containing neurons in the dorsal raphe nucleus (DRN), and the factors influencing the discharge rate of these cells. On the other hand, little is known about the activity of serotonergic neurons in raphe nuclei other than the DRN. Nucleus raphe pallidus (NRP), on the midline of the medulla, contains a relatively high proportion of serotonin-containing neurons. The present study examined the activity of serotonergic and non-serotonergic cells in the region of the NRP in adult freely moving cats. Single unit activity was recorded utilizing a methodology previously described in detail (Brain Res.: 163, 135-150, 1979). Briefly, movable electrode bundles consisting of 32 and 62μ diameter, insulated, nichrome wires were implanted 2 mm above the NRP at an angle 15° posterior to vertical. Stereotaxic coordinates for the NRP target were P -10.0, L 0.0, H -9.5. Electrodes were also implanted for recording the EEG, EOG, and dorsal neck EMG. Serotonergic neurons were initially identified on-line by their slow and regular spontaneous activity which was similar to that previously reported for serotonergic cells in the DRN. Discharge rates of medullary serotonergic neurons were highest during waking (4.3 ± 0.5 spikes/sec, mean ± sem, n = 12), decreased somewhat during slow wave sleep (3.3 ± 0.5 spikes/sec), and were slowest during REM sleep (0.8 ± 0.3 spikes/sec). These cells responded to administration of the specific serotonin agonist 5-methoxy-N, N-dimethyltryptamine (5-MeoDMT, 250 μg/kg i.m.) with a mean decrease in activity of approximately 80%. Despite these general similarities to serotonergic neurons of the DRN, the activity of medullary raphe neurons also showed some marked differences. The mean discharge rate of medullary serotonergic neurons was higher across all behavioral states and the activity of these neurons was not as strongly related to behavioral state or arousal. In addition, the activity of medullary serotonergic neurons was unaffected by auditory or visual stimuli, appeared unrelated to EEG spindles, and was less responsive to systemic administration of 5-MeoDMT. Control cells recorded from the NRP region had more irregular spontaneous activities, often firing with high frequency bursts, did not exhibit their slowest firing rate during REM sleep, and did not show a large decrease in activity after 5-MeoDMT. These initial data indicate that the activity of serotonergic neurons in the medulla bear strong similarities to identified serotonergic neurons in the DRN of the midbrain. However, clear differences also exist which may relate to functional distinctions.

113.6 RAPHE UNIT DISCHARGE IN FREELY MOVING CATS: DEPENDENCE ON CENTRAL MOTOR ACTIVITY. Barry L. Jacobs, George F. Steinfels and James Heym. Prog. in Neurosci., Dept. Psychol., Princeton Univ., Princeton, N.J. 08544.

We have previously reported that the activity of serotonergic (raphe) neurons in freely moving cats is grossly correlated with the level of behavioral arousal, or tonic motor activity (Brain Res. 163: 135-150, 1979). Neurons within the dorsal raphe nucleus of the cat discharge at a high level (approx. 3-4 spikes/sec) during active waking or arousal, and display a monotonic decrease in activity in conjunction with decreasing levels of behavioral arousal or tonic motor activity, becoming virtually silent (approx. 0.1 spikes/sec) during REM sleep. In a subsequent study (Soc. Neurosci. Abst. 6: 235, 1980; Brain Res., in press), we found that this decrease during REM sleep was primarily attributable to the profound atonia that characterizes this state. Cats with bilateral lesions in the pontine tegmentum display REM sleep without atonia. During these REM sleep periods, the discharge rate of raphe neurons in those animals displaying the greatest amount of motor activity was only 40% below that seen in these neurons during waking. The present series of studies continues this analysis, employing two different approaches. Unilateral injections of the cholinomimetic agent, carbachol (6 μg/0.37 μl), into the pontine tegmentum (P -4.0, L 2.0, H -6.0) of cats produces complete antigravity muscle atonia within 4-20 min. During these periods of drug-induced atonia, the activity of serotonergic neurons within the dorsal raphe nucleus was almost completely suppressed, in spite of the fact that the animals were unambiguously awake and responsive (see Brain Res. 163: 135-150, 1979 for complete details of the unit recording procedure). On some occasions the carbachol injections did not produce atonia, and in these cases no decrease in raphe unit activity was observed. To distinguish between central and peripheral induced atonia, we administered intravenous injections of succinylcholine, a peripherally acting paralytic agent, to cats that were artificially respirated. In this case, in spite of atonia to the point of depressing natural respiration, there was no effect upon raphe unit activity. Finally, we administered high doses of either a centrally acting muscle relaxant (mephenesin 50-200 mg/kg i.p.) or a peripherally acting muscle relaxant (dantrolene 100 mg/kg i.p.). Confirming our previous results, a large decrease in raphe unit activity was seen following the mephenesin-induced atonia, whereas no change in unit activity accompanied the dantrolene-induced atonia. In conclusion, the results of the present experiments, taken in conjunction with our previous data, indicate that the discharge of serotonergic neurons in the dorsal raphe nucleus is heavily dependent on central motor activity.

113.7 AN ANALYSIS OF NEURONAL ELEMENTS WITHIN THE NUCLEUS MEDIANUS RAPHE AND BEHAVIOR. K.E. Asin and H.C. Fibiger. Dept. Neurol. Sci, Univ. British Columbia, Vancouver, B.C. V6T 1W5.

In past years, we (Asin et al. Neur.Absts. 2-6; Wirtshafter et al. Neur.Absts. 5-6) have demonstrated that electrolytic lesions of the median nucleus of the raphe (MR) produce behavioral changes which may be likened to those seen following damage to certain limbic structures-the septum and hippocampus, in particular. Although these data support limbic-midbrain interactions in behavior, the question arises as to which neuronal elements within or near the MR produce the behaviors described, since this area of the midbrain tegmentum contains both serotonergic (5HT) and non-5HT cell bodies, as well as fibers of passage. This study sought to clarify which elements, when damaged, lead to the behavioral changes seen following electrolytic MR lesions.

Six groups of rats, matched for body weight, were operated. Following pretreatment with desmethylimipramine, one group received an intra-MR injection of 5,7-dihydroxytryptamine (DHT) and another group received a vehicle injection. Another group of rats was given an intra-MR injection of ibotinic acid (IBO)(which destroys cell bodies) and another group received the vehicle. The final groups were either sham operated or were given an electrolytic lesion of the MR (ELEC).

During the two week post-operative recovery period, DHT and IBO rats lost equivalent amounts of weight which were greater than that lost by controls, but the ELEC group surpassed all others in terms of the duration of weight loss; the body weight of this group remained depressed throughout the two weeks.

When tested in the open field, the IBO group was more active than both the controls and DHT's, but their hyperactivity was far less than that shown by ELEC rats. Also, both DHT and controls showed significant spontaneous alternation in a T maze, whereas IBO injection reduced alternation but failed to produce the perseveration shown by ELEC's. In photocell cages, both IBO and ELEC groups were hyperactive prior to and following an injection of 1 mg/kg d-amphetamine.

Finally, all rats were trained on a T-maze learned alternation task. The behavior of DHT and control groups was equivalent; although IBO rats were impaired on the task, they did not perform as poorly as ELEC rats.

These results suggest that the destruction of 5HT elements within the MR are not responsible for many of the effects pro produced by electrolytic damage to the nucleus. However, the destruction of non-5HT cell bodies and, especially, fibers of passage, does produce patterns of behavior similar to those seen following destruction of certain limbic nuclei. (KEA is supported by Post-doc. Fellowship #1 F32 NS06399-01 from NINCDS.)

113.8 EVIDENCE FOR DOPAMINERGIC INVOLVEMENT IN THE HYPERACTIVITY PRODUCED BY ELECTROLYTIC MEDIAN RAPHE LESIONS. D. Wirtshafter and K.E. Asin. Dept.Psychology, University of Illinois at Chicago Circle, Chicago, Il 60680 and Kinsmen Laboratory of Neurological Research, University of British Columbia,Vancouver, B.C., Canada.

It is well known that electrolytic lesions of the median raphe nucleus produce a dramatic increase in locomotor activity which must reflect the release of other structures from some sort of inhibitory influence. In an attempt to identify which neural systems might play a role in raphe lesion-induced hyperactivity, the current study examined the effects of several drugs on photocell cage activity in animals with median raphe lesions. All testing was conducted 6 to 8 weeks after surgery.

Haloperidol (0.15,0.30 mg/kg) produced a dose dependent decrease in the activity of both lesioned and sham operated control animals. The reduction was significantly larger in lesioned than in control rats with the effect that, at a dose of 0.3 mg/kg, activity in the two groups was indistinguishable. This result suggests that dopaminergic mechanisms play some role in lesion-induced hyperactivity. In contrast, the α-adrenergic antagonist phenoxybenzamine (20 mg/kg) produced a parallel reduction in the activity of lesioned and control rats. In intact animals phenoxybenzamine depressed activity to about the same extent as did haloperidol (0.15 mg/kg), but it was much less effective than haloperidol in lesioned animals. These results suggest that α-adrenergic mechanisms do not play an important role in raphe lesion-induced hyperactivity. Naloxone (1,10 mg/kg) was without effect on activity in either group. The GABA antagonist picrotoxin (1,2 mg/kg) reduced activity in both lesioned and sham operated rats, but was unable to eliminate the relative hyperactivity of the lesioned subjects.

In order to investigate whether dopaminergic mechanisms play a causal or merely a permissive role in the hyperactivity, control and lesioned rats were injected with reserpine (2.5mg/kg) and 6 hours later activity in response to apomorphine (0.5mg/kg) was measured. If the role of dopamine in hyperactivity was merely permissive, one would expect that lesioned animals would show a larger locomotor response to apomorphine than controls. In fact, the opposite pattern of results was obtained with lesioned animals tending to show a smaller response than shams. The simplest explanation of these findings is that median raphe lesions produce an increase in dopamine turnover which leads to both hyperactivity and to hyposensitivity of dopamine receptors. This possibility is in agreement with the results of studies showing increased dopamine turnover following raphe lesions.

113.9 NORADRENALINE, EXTINCTION, AND SPATIAL ALTERNATION LEARNING. M. Pisa, M.T. Martin-Iverson, and H.C. Fibiger. Div.Neurol.Sci., Dept. Psychiatry, Univ. British Columbia, Vancouver, B.C. V6T 1W5

It has been proposed (Mason, S.T. and Iversen, S.D., Brain Res. Rev. 1:107, 1979) that a deficit of selective attention could best account for the impairments of discrimination learning, discrimination reversal, and spatial alternation learning, and for the facilitation of nonreversal shift, of rats with 6-OHDA lesions of the dorsal noradrenergic bundle (DNB). However, we were recently unable to replicate any of these behavioral findings (Pisa, M. and Fibiger, H.C., Neurosci. Abstr. 6:724, 1980). The most robust behavioral effect of DNB lesions reported by Mason and his colleagues is resistance to extinction of continuously reinforced responses--the dorsal bundle extinction effect (DBEE). To evaluate whether our previous failure to replicate the impairment of spatial alternation learning resulted from an inability to produce behaviorally effective noradrenaline depletions--depletions, that is, sufficiently severe to induce the robust DBEE--we examined the behavior of 13 rats with bilateral injections of 6-OHDA (4 µg in 2 µl of ascorbate-saline solution) in the DNB during both extinction of continuously reinforced lever pressing and spatial alternation learning.

One month after surgery, the rats were magazine trained and then given 12 sessions of lever-pressing acquisition and three sessions of extinction, each session lasting 15 min. All 12 control rats learned to press the lever, three rats with lesions failed to do so. The remaining 10 rats with lesions did not differ from the controls in acquisition. Also, they did not perform significantly more responses than the controls in the extinction sessions; however, they took longer than the controls to reach the criterion of two minutes of no responding in the first extinction session. Some support was therefore found for a DBEE. The rats were then trained in a conventional T maze. After one session with five trials of spontaneous alternation, and one session of free exploration and adaptation to the food pellets, the rats were given daily sessions of five trials of food-reinforced, left-right alternation, with an intertrial interval of 15 sec spent outside the maze. After 22 such sessions, no significant difference in performance emerged among DNB-lesioned and control rats. Thus, DNB lesions that produced a DBEE failed to impair spatial alternation learning. Taken together with our previous study, these observations indicate that, although DNB lesions increase resistance to extinction according to one, albeit arbitrary criterion, there is at present no reliable behavioral evidence in direct support of Mason's attentional hypothesis.

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113.10 EFFECTS OF DORSAL NORADRENERGIC BUNDLE LESIONS ON MEASURES OF CONFLICT, LEARNING AND LOCOMOTION. D.R. Britton, C. Ksir, and G. Koob. Behav. Neurobiol. Lab., The Salk Institute, La Jolla, CA 92138 and Department of Physiology, University of N.M., School of Medicine, Albuquerque, N.M. 87131

The dorsal noradrenergic bundle (DNB) has been implicated in a number of behavioral processes including learning locomotor activity and the expression of anxiety. We investigated evidence for such involvement in the rat. Male Sprague-Dawley albino rats were given bilateral 6-hydroxydopamine lesions to the dorsal bundle. These lesions resulted in substantial (90%) depletion of norepinephrine (NE) in cortex and hippocampus. There was also a significant decrease in NE in hypothalamus and caudate. Dopamine was decreased by 15% in caudate and not significantly effected in other regions.

Following a recovery period of 10-12 days animals were fasted for 24 hrs. and tested in an open field paradigm previously shown to be sensitive to anxiolytic drugs (Britton and Britton, Pharm., Biochem and Behav., in press). In this test, lesioned animals show behavior which is in the opposite direction of that produced by anxiolytic drugs. The lesioned group showed a significant decrease in the $\bar{x}$ amount of food eaten per approach to the food pedistal (located in the center of the novel open field) and a decrease in the total amount eaten during the 15 min. duration of the test. These measures have previously been shown to be increased in animals treated with anxiolytic drugs. Lesioned animals, fasted for 24 hr. and tested for food consumption in their home cages, ate the same amount as sham lesioned controls. The decreased food consumption and decreased amount eaten per approach to the food pedistal appear, therefore, to reflect an increased neophobia in lesioned animals. This is consistant with other reports of DNB lesion-induced neophobia (S. Mason, et. al., Physiol & Bhv, 1978) and does not support the hypothesis that anxiolytics act by decreasing forebrain NE functioning. These same animals were subsequently tested for acquisition and extinction in an operant task. Legioned animals showed no differences between the two groups in locomotor activity measured by photocell activation in single cages over a 2 hr. period.

113.11 ELECTROPHYSIOLOGICAL AND BEHAVIORAL EVIDENCE OF INTERACTION OF DOPAMINERGIC AND GUSTATORY PROJECTIONS TO THE AMYGDALA. G. J. Mogenson and M. Wu*. Dept. of Physiology, University of Western Ontario, London, Canada, N6A 5C1.

The amygdala receives gustatory afferents which have been implicated in taste-motivated behavior. Lesions of the amygdala alter the intake of salt solutions and change the preference for novel foods. The central nucleus of amygdala (CNA), which is the main target of taste afferents, also receives dopaminergic afferents. The present experiment was undertaken to study the possibility that these dopaminergic afferent fibers interact with taste afferents and contribute to taste-motivated behavior.

Extracellular single unit recordings were obtained from neurons in the CNA of rats anaesthetized with urethane using glass micropipettes filled with 0.5M sodium acetate. The effects of electrical stimulation of the pontine taste area (PTA) or the ventral tegmental area (VTA) which contain gustatory and dopaminergic neurons respectively, were investigated. 73% of the CNA neurons were affected by PTA stimulation and 51% by VTA stimulation, with 41% of CNA neurons receiving convergent inputs from PTA and VTA.

In two-bottle taste preference tests, the intracerebral injection of either apomorphine (DA agonist) or spiroperidol (DA blocker) into the CNA produced a significant increase in consumption of 1.5% NaCl solution with no change in water intake after overnight deprivation of water. The administration of procaine also significantly increased the consumption of 1.5% NaCl, but, with simultaneous injections of both apomorphine and spiroperidol the intake of water and 1.5% NaCl were the same as during control tests. As an additional control for anaesthetic or other non-specific effects of apomorphine and spiroperidol, these drugs were injected via chronic cannulae into the nucleus accumbens and locomotor activity measured in an open-field test. Locomotor activity was increased by injections of apomorphine into the nucleus accumbens but injecting spiroperidol along with apomorphine reduced this effect in a dose-related manner.

These observations suggest that the CNA receives converging dopaminergic afferents from VTA and gustatory afferents from PTA which may play a role in taste-motivated behavior.

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113.12 **DIFFERENTIAL ANTAGONISM OF d-AMPHETAMINE EFFECTS ON MOTOR ACTIVITY AND AGONISTIC BEHAVIOR IN MICE.** **Klaus A. Miczek. Dept. Psychology, Tufts Univ., Medford, MA 02155.**

Catecholamines (CA) appear to be critically involved in motor activity as well as aggressive, defensive and flight behavior. An ethological and pharmacological analysis was performed to examine the effects of CA agonists and antagonists on several elements of fighting behavior and motor activities, as they were engendered in resident-intruder confrontations in mice. Resident mice typically show the attack pattern, whereas intruders react with escapes, defensive postures, and vocalizations. The drugs were administered i.p. either to the resident male Swiss-Webster mice or to the intruder. Videorecords of 5-min confrontations were viewed by experimenters who measured with the aid of a computerized data collection system frequency and temporal pattern of all essential acts, postures, displays and movements characteristic of attack, threat, pursuit, defense, flight, grooming as well as walking and rearing. d-Amphetamine (0.63-5 mg/kg) (1) decreased attack and threat behavior at the higher doses, (2) increased defensive postures, (3) left grooming and vocalizations unaltered, (4) increased monotonically walking. Haloperidol (0.125- 1 mg/kg), phenoxybenzamine (5-15 mg/kg), propranolol (10-20 mg/kg), and methysergide (1-30 mg/kg) decreased attacks and threats as part of their general activity-reducing effect; by contrast, escapes, defensive upright postures and vocalizations remained largely unaffected by these drugs, although walking and rearing were reduced. These results indicated that defensive and flight reactions are least affected by the CA drugs, whereas basic motor functions such as walking across the cage are very sensitive to these drugs. Combined administration of d-amphetamine and one of the antagonists showed that (1) the motor activity-enhancing effects of d-amphetamine were effectively blocked by haloperidol, phenoxybenzamine, propranolol, but not by methysergide, (2) the aggression-reducing effects of d-amphetamine were not antagonized, but intensified by haloperidol, phenoxybenzamine, propranolol and methysergide. These results suggest different CA mechanisms underlying motor activities and aggressive behavior. Peripheral effects of the antagonists may have masked central actions of the CA drugs and may have contributed to the behavioral effects.

114.1

WITHDRAWN

114.2 FUNCTION OF CHEMICALLY-MODIFIED ACETYLCHOLINE RECEPTOR. M. G. McNamee, J. W. Walker* and R. J. Lukas. Dept. of Biochem. and Biophys., Univ. of Calif. at Davis, Davis, CA 95616.

Chemical modification of membrane-bound Torpedo californica acetylcholine receptor by the disulfide reducing agent dithiothreitol has two major effects on receptor function: (1) it shifts the dose-response curve for carbamoylcholine-induced increases in $^{22}Na^{+}$ permeability to 10-fold higher concentrations, and (2) it decreases the binding affinity of the receptor for the same agonist about 6-fold. Despite the quantitative changes in agonist binding and flux response, dithiothreitol-treated membranes display all other functional properties expected of the receptor. The flux response is blocked by preincubation of the membranes with carbamoylcholine, a phenomenon known as desensitization. In parallel, the receptor undergoes a carbamoylcholine-induced shift from a low-affinity to a high-affinity binding state for the same agonist. All of the effects of dithiothreitol are reversed by the oxidizing agent 5,5'-dithiobis(2-nitrobenzoic acid). Alkylation of the membranes with N-ethylmaleimide after dithiothreitol reduction results in complete inhibition of the flux response, and the effect is not reversed by the reoxidation treatment. The N-ethylmaleimide also shifts the receptor into a very low-affinity binding state for carbamoylcholine that is shifted to only a slightly higher affinity by preincubation with carbamoylcholine. Prior to reduction, N-ethylmaleimide has no effect on receptor binding or flux properties. Detailed binding studies on membranes affinity alkylated at one-half of the agonist binding sites indicate that the α-neurotoxin binding site not occupied by the affinity label displays all the same properties as unlabeled membranes, including the dithiothreitol and N-ethylmaleimide effects. The results will be discussed in the context of several hypotheses previously proposed to account for the diverse effects of thio-group modifications on the acetylcholine receptor.

114.3 SULPHYDRYL MODIFICATION OF ACETYLCHOLINE RECEPTOR KINETICS, A. Steinacker and †D.C. Zuazaga, Biophysics, The Rockefeller University, New York, N.Y. 10021, †Lab. of Neurobiology, U. of Puerto Rico, San Juan, P.R. 00901.

Disulfide groups are known to be critical determinants of acetylcholine receptor (AChR) function. Treatment of the AChR of the intercostal muscle at the lizard neuromuscular junction with specific reagents which modify disulfide groups on the receptor molecule produces an increase in the decay time of miniature endplate current (mepc) which cannot be accounted for by changes in single channel open times as measured by noise fluctuations. The decay of the mepc and the noise spectra show only a single exponential. A small increase in rise time of the mepc is also seen. Control experiments using carbachol and an anticholinesterase were done to determine that the change in mepc decay was not due to an action on disulfide bonds of the endogenous cholinesterase. These data are interpreted in terms of a three-state kinetic model ($A + R \underset{k_2}{\overset{\alpha}{\rightleftharpoons}} AR \underset{\beta}{\overset{k_2}{\rightleftharpoons}} AR^*$) in which time spent in the intermediate bound but not open state of the receptors is increased. This could be due to an increase in affinity of the receptor brought about by chemical modification of the receptor. An affinity increase should be reflected in a change in β or k_2 of the three-state kinetic model Experiments under way are designed to determine these constants. Supported by Muscular Dystrophy Association and NIH NS15956 (AS) and NIH NS07464 (DC.Z.)

114.4 LOCATION OF THE ACETYLCHOLINE RECEPTOR KINASE IN THE POST-SYNAPTIC MEMBRANE. C.G. Davis*, D. Milfay*, I. Diamond, and A.S. Gordon. Dept. of Neurology, Univ. of Calif., San Francisco, CA 94143.

We have previously reported that the AChR is phosphorylated in situ by an endogenous protein kinase. This reaction is reversible; endogenous phosphatase activity dephosphorylates the AChR. The presence of such a rapid, reversible enzymatic system capable of mediating changes in the structure of the receptor protein suggests that it may play a role in receptor function. As a step in understanding the physiological significance of AChR phosphorylation, we have undertaken to determine whether the kinase is restricted to one side of the plasma membrane, and, if so, which side.

Sealed right-side-out membrane vesicles were prepared from Torpedo californica electroplax. Incubation of these vesicles with [γ-^{32}P]-ATP for one minute did not result in significant phosphorylation of AChR subunits. However, if the vesicles were lysed by hypoosmotic shock, phosphorylation of four bands of MW 36K, 40K, 60K and 65K was greatly enhanced. The degree of enhancement correlated with the strength of the osmotic shock. The possibility that the effect of hypoosmotic shock on phosphorylation might be due to solubilization of the kinase or its substrates was considered. To test this possibility, lysed vesicles were resealed before the addition of γ-^{32}P-ATP. Phosphorylation of the 4 bands was reduced by 70-85% as compared to that observed in untreated lysed vesicles. Furthermore, if lysed vesicles were centrifuged and the supernatant incubated with intact vesicles, no enhancement was observed. Together, these results strongly indicate that hypoosmotic shock increases phosphorylation by making ATP accessible to the inside of vesicles and not by release of kinase or substrate from the membranes.

Other methods of membrane lysis were also tested for effect on AChR phosphorylation. Triton X-100 at a concentration of .02% caused a significant increase in phosphorylation of the same four polypeptides. Digitonin, a detergent specific for cholesterol, had an identical effect at the same concentration.

Since no significant phosphorylation was observed in intact right-side-out vesicles, while three different methods of membrane lysis resulted in greatly increased phosphorylation of AChR subunits, it can be concluded that the AChR kinase is restricted to the cytoplasmic side of the post-synaptic membrane.

114.5 KINETICS OF HISTRIONICOTOXIN BINDING TO TORPEDO NICOTINIC POST-SYNAPTIC MEMBRANES. Dan C. Medynski* and Jonathan B. Cohen. Dept. of Pharmacology, Harvard Medical School, Boston, MA 02115.

Rapid-mixing and ultrafiltration techniques have been used to measure the kinetics of binding of [^{3}H]-perhydrohistrionicotoxin ([^{3}H]-HTX) to nicotinic post-synaptic membranes isolated from Torpedo electric tissue. In control experiments at 20°C, both in the absence of agonist and with acetylcholine (Ach) sites occupied by carbamylcholine (Carb), the equilibrium binding function (K_{eq} = 0.25 μM, 0.48 ± .09 sites per α-bungarotoxin site) was the same whether determined by ultrafiltration or ultracentrifugation and was also the same as that at 4°C in the presence of carb. Despite the identity of K_{eq} under these different conditions, the kinetic parameters defining that equilibrium binding indicate the existence of significant differences: 1) Kinetics of dissociation of [^{3}H]-HTX at equilibrium was measured as the rate of exchange for H_8-HTX or meproadifen. For ^{3}H-HTX concentrations resulting in occupancy of 2 to 80% of the binding sites, when Ach sites were occupied by agonist or antagonists, the dissociation kinetics were characterized by a single exponential ($T_{1/2}$ = 10 min at 20°C and 180 min at 4°C) for greater than 75% of the exchange reaction. 2) With Ach sites unoccupied at 20°C in the presence of 2-20 μM H_8-HTX, $T_{1/2}$ = 30 min. In the presence of meproadifen, the observed dissociation rate depended upon the meproadifen concentration, presumably because meproadifen binds to the Ach sites as well as to the anesthetic site. 3) When membranes were equilibrated at 20°C with [^{3}H]-HTX in the absence of cholinergic ligands and then exchange measured by the addition of non-radioactive anesthetic simultaneously with agonist, the rates were increased. For carb concentrations of 0,10, and 500 μM, the $T_{1/2}$'s were 30, 0.5, and 0.5 min respectively. This fact indicates that the occupied HTX site can be converted by agonist to a transient conformation characterized by rapid dissociation, a conformation distinct from that stabilized by HTX (-carb) or by carb at equilibrium. 4) Analysis of [^{3}H]-HTX association kinetics at 20°C in the absence of cholinergic ligands has provided evidence that the association reaction is limited by a slow conformational transition; however, there is no evidence of preferential binding to the 20% of the receptors which pre-exist in a conformation binding Ach with high affinity (Biochemistry 19:5354,1980). The parameters characterizing the kinetics of binding of HTX will be related to the known conformational transitions of the membrane-bound Torpedo receptor.

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114.6 ACETYLCHOLINE RECEPTOR REGULATION IN THREE MAMMALIAN MUSCLE CELL LINES. Jeffrey Boone Miller* and Zach W. Hall. Dept. of Physiology, University of California, San Francisco, CA 94143

As one step in investigating the biochemical and genetic mechanisms underlying acetycholine receptor (AChR) metabolism in muscles, we have examined AChR clustering, degradation rate, and immunological properties in three muscle cell lines: BC_3H-1, derived from a mouse tumor and similar to smooth muscle; C_2, derived from adult mouse skeletal muscle; and L6, derived from rat skeletal muscle. All three lines can be induced to synthesize AChR, but only C_2 and L6 cells fuse to form myotubes.

The distribution of AChR labeled with ^{125}I-α-bungarotoxin (^{125}I-Butx) was examined by autoradiography. Clusters, which were similar to those reported on myotubes in primary cultures, were seen only on C_2 myotubes. The clusters had a longest dimension of 10-30μm, and were found as early as one day after fusion began, although they became more frequent on older myotubes.

Degradation of the ^{125}I-Butx labeled AChR on C_2 myotubes showed complex kinetics that could not be described by a single exponential. In addition, the halftime for degradation increased from 8h on day two in fusion medium to 15h on day four. Such complex AChR degradation kinetics have also been reported with cultured primary myotubes (1). The degradation rate of clustered AChR was measured by autoradiography and found to be no different than the degradation rate of the total AChR population. Thus, the complex kinetics of C_2 AChR degradation can not be explained by the presence of clustered and non-clustered AChRs - a conclusion reinforced by the finding that the AChRs in L6, which never cluster, have a similarly complex pattern of degradation. In contrast, BC_3H-1 AChRs have an 8h degradation halftime (2) which we found is unchanged by prolonged culture.

The immunological properties of AChRs in extracts were determined by precipitating ^{125}I-Butx-AChR complexes with a myasthenic serum that distinguishes junctional from extra-junctional receptors (EJR). The AChRs in each cell line were immunologically indistinguishable from EJR of the animal from which the cell line was derived.

Thus, in the three cell lines examined, the properties of AChRs in C_2 most closely resemble those seen in embryonic myotubes. C_2 cells may, therefore, serve as useful models in which to study the mechanisms regulating receptor distribution.

(1) Miskin et al. (1978) Cell 15, 1287-1300
(2) Patrick et al. (1977) J. Biol. Chem. 252, 2143-2153

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115.1 Granule Cell Behavior in Dissociated Cultures of Weaver (wv) Mutant Cerebellum. M. Willinger,* D. M. Margolis* and R. L. Sidman, Depts. of Neuropathology, Harvard Med. School, and Neuroscience, Children's Hospital Medical Center, Boston, MA 02115

The behaviors of granule cell neurons in dissociated monolayer cultures of cerebellum from seven-day-old normal (+/+), heterozygous weaver (+/wv) and homozygous weaver (wv/wv) mice were examined by phase contrast time lapse cinematography. The mixed cell population was plated at 1.5 x 10^4 cells/mm^2 onto poly-L-lysine-coated glass surfaces. The cell cultures were photographed between 20 and 60 hours in vitro at a rate of 2 - 6 frames per minute. We observe a mutant gene dose-dependent effect on the behavior of granule cell neurons in vitro. Neurite elongation proceeds in a noncontinuous fashion in normal cells. Neurite retraction is rarely observed. Cell body and intranuclear migration are each unidirectional. Cell death was rare. The features that distinguish +/wv cells from +/+ cells are the following: partial neurite retraction, reversal of migrational direction and moderate cell death. The most striking features of wv/wv granule cell behavior are: frequent neurite initiation followed by complete retraction, cell body and intranuclear migration in more than one direction, and a high percentage of cell death. Homozygous weaver neurites rarely exceeded 10 - 15 μm, and are different in morphology from +/+ and +/wv neurites. The growth cones are phase-dark and thick; the neuritic shafts are constantly active, bearing membrane blebs and microspikes.

Quantitation of both the percentage of cells having neurites of a given length class and of the exact lengths of neurites in fixed cultures showed a mutant gene dose-dependent impairment of neurite growth over the first week in vitro (Willinger, Margolis and Sidman, J. Supramol. Struc., 1981 in press). Preliminary quantitative analyses of the time-lapse films show a mutant gene effect on rates of growth cone elongation. The average rates of neurite growth are 196 μm/day for +/+ neurons and 5 μm/day for wv/wv neurons. The mean maximum rates of growth cone elongation are 938 and 339 μm/day for +/+ and wv/wv neurons, respectively.

In conclusion, the weaver mutant gene affects neurite outgrowth by impairing neurite elongation and maintenance, rather than neurite initiation. The effect is gene dose-dependent and probably represents, at least in part, the in vivo weaver phenotype.

115.2 THE RELATIONSHIP OF MELANIN DISTRIBUTION TO CELLULAR DEGENERATION IN THE DEVELOPING MAMMALIAN EYE. A.C. Strongin and R.W. Guillery. Dept. Pharmacol. Physiol. Sci., The University of Chicago, Chicago, IL 60637.

Melanin appears early in the development of the mammalian eye cup, long before it can perform any of its known adult functions. Studies on individuals with abnormally low amounts of melanin in the retinal pigment epithelium show a chiasmatic misrouting of some retinofugal fibers; because a variety of genes may produce such abnormalities by a variety of cellular mechanisms, it seems likely that melanin itself is involved in producing the pathway abnormality. The appearance of melanin correlates temporally with the closure of the optic fissure. Silver and Hughes (J. Morph., 140:159, 1973) have shown that extensive cellular degeneration occurs normally in the region of the optic fissure while it is in the process of closure; however, no pigment cells near this region show any signs of degeneration. Melanin does not even appear in the region of the fissure until closure and associated degenerative changes are complete. This "avoidance" of a region of cellular degeneration by pigment cells has led to this study of the distribution of melanin as it relates to zones of degeneration throughout the eye cup and stalk in fetal hamsters, mice, ferrets, and humans.

Although melanin is not associated with degenerative changes near the fissure, there is a region, where the eye cup joins the dorsal stalk, which contains melanin that is closely related to degenerative changes within the melanocytes. Here, pigment granules form dense clusters which are associated with basophilic areas of cytoplasm. These clusters are most prominent for a short period when axons are invading the ventral part of the eye stalk. Electron microscopic observations thus far indicate that these clusters are lysosomal in nature; acid phosphatase studies in progress should clarify this problem. Because of its specific localization within the developing eye cup and stalk, and its close temporal relationship to axonal outgrowth, the degradation of melanin may have a significant role in the organization of retinofugal pathways, especially in the ordering of the chiasmatic course of the fibers. The above results are consistently found in all four species studied. Slightly more pigment degeneration is seen in the ferret and human, which do have relatively larger uncrossed retinofugal components than the hamster or mouse.

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115.3 IMMUNOCYTOCHEMICAL IDENTIFICATION OF MACROPHAGES IN THE CORPUS CALLOSUM OF THE NEONATAL RAT. K.L. Valentino and E.G. Jones Dept. of Anatomy and Neurobiology and McDonnell Center for the Study of Higher Brain Functions, Washington University School of Medicine, St. Louis, Missouri 63110.

Cells identified as macrophages on morphological grounds have been seen in the fetal and neonatal rat corpus callosum, but definitive proof of their character and their origins remain unclear. We wished to determine whether phagocytic cells that invade the corpus callosum during its formation in the fetal brain were true macrophages and, thus, of nonneural origin. An immunocytochemical study was carried out using a monoclonal antibody specific for macrophage surface polypeptides, MAC-1 (Springer et al., Eur. J. Immunol. 9:301, 1979).

Brains of neonatal rats were fixed by perfusion with 4% formaldehyde and .01% glutaraldehyde. The brains were then frozen in liquid nitrogen and sections cut with a cryostat. Primary antibodies and controls included MAC-1, thy-1, S-100, rat IgG and normal rabbit serum. Secondary antibodies were fluorescein conjugated goat anti-rat IgG or F $(ab')_2$ fragments.

Specific staining of macrophages in and around the corpus callosum was seen with MAC-1, but also with control rat IgG, and with the secondary antibody alone, when the secondary antibody was the whole anti-rat IgG. No other cells in the brain were labelled. This pattern of staining is indicative of Fc receptor binding, itself indirect evidence that the cells are macrophages. Fc receptor binding could be eliminated by using F $(ab')_2$ fragments as the secondary antibody. This treatment results in specific staining of the cells with MAC-1. Though the phagocytic callosal cells, like macrophages elsewhere, are autofluorescent, this can be reduced to the red end of the spectrum by a pre-treatment with Evans blue. Thus, cells with a surface marker specific to macrophages have been identified in the developing rat brain.

Stained macrophages were found in the same locations as described in normal electron microscopic studies: in the corpus callosum dorsal to the lateral ventricle, in the cavum septi pellucidi and where the corpus callosum pierces the ventricular zone of the hemisphere. Staining with thy-1 was negative, as was staining with S-100 at early ages. The callosal macrophages stained with S-100 in older animals in which astrocytes were also stained. The staining of macrophages with S-100 may indicate phagocytosis of glial elements during formation of the corpus callosum. Whether the macrophages play any active role in guiding the callosal axons across the midline and towards their targets is still unclear.

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115.4 **INHIBITION OF NEURITE EXTENSION BY FLUORODEOXYURIDINE IS AGE-DEPENDENT, DOSE DEPENDENT AND REVERSIBLE.** Vincent Argiro* and Mary Johnson (SPON: R.P. Bunge). Dept. Anat. & Neurobiol., Wash. Univ. Sch. Med., St. Louis, MO 63110.

Fluorodeoxyuridine (FdU) is used as an anti-mitotic agent in tissue culture studies and in cancer chemotherapy in humans. Its effect on post-mitotic cells such as neurons has not been extensively studied. In our studies of the kinetics of neurite growth of autonomic neurons in culture, we initially used FdU to suppress non-neuronal cell proliferation, but it became apparent that the drug had an inhibitory effect on neurite extension.

Explants of superior cervical ganglion were taken from rats ranging in age from embryonic day 15 (E15) to postnatal day 279 (P279), cultured on a fibrous collagen substrate and fed a medium containing serum, embryo extract and NGF. When included, the standard dose of FdU was 10^{-5} molar. Neurite lengths were determined by tracing the circumference of the neurite halo at 5-8 invervals over several weeks. Average neurite lengths and linear regression growth rates were calculated with the aid of a computerized morphometry system.

Age Dependence: The standard dose of FdU was given throughout the culturing period. The inhibitory effect is greatest for adult explants and embryonic explants and least for perinatal explants. At two weeks in culture the mean neurite lengths relative to untreated cultures were: E15-66%, E21-77%, P2-74%, P16-72%, P279-36%. At one week in culture the growth rates relative to untreated cultures were: E15-52%, E21-65%, P2-62%, P16-66%, P279-45%.

Dose Dependence: FdU doses were 10^{-6}, $3x10^{-6}$, 10^{-5}, $3x10^{-5}$, and 10^{-4} molar. E21 explants were minimally effected at all doses and throughout the culturing period. E18 explants were most affected after two weeks in culture. Growth rates ranged between 88% of control for the smallest dose and 49% for the largest dose at 12 days in culture; the dose curve was flat at about 80% at 3.6 days. The adult (P43) explants were most severely affected in the first few days of culture. Growth rates relative to controls ranged from 79% to 14% at 3.6 days, and from 100% to 72% at 12 days.

Reversibility: Cultures were either maintained on the control medium, maintained on the medium containing 10^{-5} molar FdU, fed control medium for three days and then fed FdU-containing medium, or fed FdU-containing medium for three days follwed by control medium. For all ages tested, cultures taken off the drug increased their growth rate and those placed on the drug after three days showed a delayed inhibition of growth.

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115.5 PREFERRED AXON PATHS IN THE WING OF DROSOPHILA. Marjorie A. Murray‡ Margrit Schubiger‡ and John Palka. Dept. of Zoology, Univ. Washington, Seattle, WA 98195

The axons of sensory neurons in the transparent wings of Drosophila melanogaster can be seen clearly with methylene blue or silver staining. They normally form bundles which travel through particular veins to reach the base of the wing. We have used this system to test hypotheses about factors which establish the specific axon paths seen in adult tissue.

One plausible hypothesis is based on the observation that epidermal cells in the anterior and posterior regions of the wing form two discrete, non-intermingling populations which meet at a straight boundary just anterior to the fourth longitudinal vein (L4). We have asked, do the mechanisms (thus far unknown) which segregate the epidermal cells into two compartments also prevent the growth of axons from one compartment into the other?

In wild type flies all sensory axons develop in the anterior compartment, so axonal behavior at the compartment boundary cannot be studied. Hairy wing mutants, however, carry supernumerary receptors in both compartments. Their axons are observed to converge on vein L3 of the anterior compartment. This is true both for axons originating from receptor cells in the posterior compartment and for axons from supernumerary anterior receptors. Thus, the compartment boundary has no obvious influence on the paths taken by adult axons, but vein L3 appears to have some special properties.

Why is vein L3 so attractive to growing axons? Perhaps it carries pioneer axons such as have been seen during early development in a variety of insect appendages. Nerve strands have been described in everted wing discs of Drosophila as early as 6-9 hours after pupariation, long before the differentiation of adult sensory neurons (Waddington, C.H., J. Genet., 41:75-139, 1940). With Nomarski optics we have seen distinctive cells at the tips of the wings of somewhat older pupae, and are looking with transmission electron microscopy to determine whether these are indeed pioneer neurons. Our electron micrographs of the strands described by Waddington in the younger pupae, however, show that they are composed primarily of tracheae, and correlated observations with Nomarski optics reveal that tracheae run only in veins which will later carry nerves. If pioneer axons do not guide sensory fibers in the developing wing, other structures such as tracheae may act as guiding substrates.

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115.6 MULTIPLE PIONEER NEURONS ESTABLISH (IN VIVO AND IN EMBRYO CULTURE) MAJOR NERVE TRUNKS FOLLOWED PREFERENTIALLY BY IDENTIFIED NEURONS. H. Keshishian and D. Bentley. Neurobiology Group and Zoology Dept. University of California, Berkeley, Ca. 94720.

The role of pioneer neurons in establishing the numbers and routes of peripheral nerve branches was examined in the metathoracic leg of embryonic grasshoppers, using Lucifer yellow dye-fills, silver impregnations and cobalt back-fills. It was previously shown that the first pioneer neuron cell-pair to originate in the leg, cells PN1, establish a stereotyped axonal trajectory that serves as the substrate for the axonal fasciculation and development of nerve 5b1 (Keshishian '80, Dev. Biol. 80, 388). It is now shown that a second pair of pioneer neurons, cells PN2, recapitulate in the tarsal and tibial segments of the leg the ontogeny and stereotyped axonal projection seen for the PN1 cell-pair in the tibia and femur. The PN2 cell-pair project their axons in a stereotyped route across the tibia with the characteristic cross-over morphology seen with the PN1 axons in the femur. The PN2 axons join the PN1 axons at the proximal femur, and they collectively project into the central nervous system.

Utilizing double staining methods, the two pioneer neuron cell-pairs were found to serve as the guidance substrate for subsequently differentiated afferent and efferent axons. Lucifer yellow dye-filled pioneer neuron axons were found running within silver impregnated nerve trunks, with the axons serving as a nucleus for axonal fasciculation. Thus, nerve 5b1 arises through the initial axonal projection of cells PN1 and nerve 5b2 through the projections of the PN2 cell pair.

The guidance preferences of identifiable neuronal projections were examined. A group of tarsal sensory neurons, the TC cells, establish their initial projection along the segmental apodeme (tendon) until their axons contact the PN2 axons. At that point the TC axons show a preference for the pioneer neurons over the apodeme for their subsequent guidance. Similar behavior was observed for axons of the femoral chordotonal organ. Other neurons, such as the SR1 (a putative femoral stretch receptor), used the pioneer axons as the sole guidance substrate from the site of axonogenesis to the CNS.

The axonal projections to the femoral and tibial muscle groups were observed to grow peripherally along the PN-established nerve trunks to the borders of their target muscles, where they diverged to form novel projections. Thus, these efferent projections are not established by axons that adopt the shortest available routes across the limb-bud.

In embryos cultured in vitro from the 30% to 40% stages of development, the pioneer neurons differentiated and established a normal axon trajectory to the CNS.

115.7 THE GROWTH CONES OF TWO IDENTIFIED SIBLING NEURONS DIVERGE AT A PARTICULAR CHOICE POINT DURING GRASSHOPPER EMBRYOGENESIS. Jonathan A. Raper and Corey S. Goodman. Dept. of Biol. Sci., Stanford University, Stanford, CA 94305

We have been studying the development of two identified neurons,'G' and 'C', during grasshopper embryogenesis. These identified interneurons are siblings that arise from an identified precursor cell, neuroblast 7-4. The cell bodies of G and C assume a characteristic and highly accessible position before axonogenesis. In most egg clusters G's cell body is slightly anterior and lateral to C's on the dorsal surface.

G initiates axonal outgrowth a few hours before C. G's growth cone traverses the posterior commissure, a pathway pioneered by descendants of the same neuroblast that produce G and C. Upon reaching the lateral edge of the contralateral neuropil, G's growth cone elongates anteriorly upon an anterior-posterior running axonal pathway. C's growth cone follows G across the commissure and elongates posteriorly along the same axonal pathway.

This pathway upon which G and C diverge is formed by the axons of three neurons. One axon originates from a cell body which is ipsilateral to the pathway and in the same ganglion of origin as G and C. Its process runs posteriorly. The other two axons originate from two adjacent cell bodies contralateral to the pathway in the next posterior ganglion from that containing G and C. Their processes run anteriorly.

Correlating the relative times of arrival and elongation of these identified axons in the lateral pathway suggests a working hypothesis whereby (i) G's growth cone recognizes and follows the anterior running axons originating in the next posterior ganglion; and (ii) C's growth cone recognizes and follows the posterior running axon originating in the same ganglion. We plan to test this 'guide fiber' hypothesis by determining if G and C can make their appropriate choices in the absence of any one of these identified axonal processes.

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115.8 A MONOCLONAL ANTIBODY WHICH STAINS PIONEER NEURONS AND EARLY AXONAL PATHWAYS IN GRASSHOPER EMBRYOS. S. Chang*, R. Ho*, J.A. Raper and C.S. Goodman. (SPON: D. Edgington). Dept. of Biol. Sci., Stanford University, Stanford, CA 94305.

During the embryonic development of the grasshopper nervous system, growth cones of neurons make specific and stereotyped choices; the interactions of these cells with their environment is undoubtedly important to their further development. In an effort to identify the molecules which are mediating such interactions, we have made monoclonal antibodies to the grasshopper nervous system. The antibodies were produced by injecting mice with adult nervous tissue and screening successful hybridomas on 40% grasshopper embryos. One of the antibodies produced in this manner, designated I-5, may be recognizing an antigen which is important during embryogenesis. Staining of the embryo by I-5 first appears on the central and peripheral pioneers. The first cells to establish a particular axonal pathway are called pioneer neurons; their growth cones take stereotyped routes while navigating on the basement membrane. Later developing cells use the pioneer pathways to navigate upon. The pioneer cell bodies stain with the I-5 antibody shortly after their final cell division; subsequently, their growth cones and axonal processes stain. With time the cell body staining declines whereas the processes remain darkly stained. The antibody shows striking specificity. Of the central pioneers, certain ones consistently stain darker than others. Furthermore, many later developing axons do not stain at all. The antibody also selectively stains certain non-neuronal tissue. For example, a thin muscle over each segmental ganglion stains with the antibody, although muscles in general do not.

We are now investigating the molecular nature of the antigen which I-5 recognizes, and using the antibody in embryo cultures to ascertain the functional importance of the antigen.

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115.9 MONOCLONAL ANTIBODIES REVEAL THE DEVELOPMENT OF PERIPHERAL PIONEER NEURONS AND AXONAL PATHWAYS IN GRASSHOPPER EMBRYOS. R. Ho*, S. Chang*, and C.S. Goodman. Dept. of Biol.Sci., Stanford University, Stanford, CA 94305

In grasshopper embryos, Bate(1976) and Keshishian(1980) described pairs of cells in the periphery, called peripheral pioneer neurons, which establish the first peripheral axonal pathways in the antennae and limb buds. The growth cones of the pioneers take a stereotyped pathway along the basement membrane on the inside of the ectoderm. We have been making monoclonal antibodies to the grasshopper nervous system. One of the antibodies,designated I-5, and described in detail in another abstract (Chang et al 1981),stains the peripheral and central pioneer neurons. The advantage of the monoclonal antibody technique is that all of the pioneer cells throughout the embryo can be reproducibly and reliably stained in whole mount preparations using an HRP conjugated second antibody. Furthermore, their relationship to other peripheral cells and to the peripherally growing axons of central neurons can also be seen.

The cell bodies of the pioneer neurons first stain with the I-5 antibody just after their final cell division; the antibody stains their growth cones and axons throughout their development. We have described pairs of peripheral pioneer neurons throughout the head, thorax, and abdomen including the antennae, mouth parts, limb buds, and body wall.

In the antennae, two pairs of distal pioneers establish the two axonal pathways in the antennae itself, however, there is another pair of pioneers at the base which first establishes the pathway from the base of the antennae to the CNS. At the same time, growth cones from central cells extend distally making other pathways toward the base of the antennae. A similar sequence occurs in the limb buds, with pairs of pioneers at the base establishing connections with the CNS and the distal pioneers establishing the pathway in the limb bud. Growth cones from central cells also contribute to these pathways. Thus, the peripheral pathways in the appendages and between the appendages and the CNS are established by a series of peripheral pioneers and central growth cones.

Because it is so easy and reliable to stain all of the pioneers with the I-5 antibody, we now plan to manipulate the pioneers and their environment in embryo culture in order to understand the mechanisms by which they establish their stereotyped pathways.

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115.10 LACK OF EFFECT OF NERVE GROWTH FACTOR (NGF) AND NGF-ANTIBODIES INJECTED INTO THE CYTOPLASM OF TARGET CELLS. R. Heumann* and H. Thoenen (SPON: F. Hefti). Max-Planck-Institute for Psychiatry, Department of Neurochemistry, D-8033 Martinsried, FRG.

Nerve growth factor is taken up by the terminals of its target neurons by a selective, saturable mechanism and subsequently is transported retrogradely, in membrane-confined compartments, to the perikaryon where it evokes its characteristic biological effects. Previous electron-microscopic studies (quantitative autoradiography and histochemical localization of NGF covalently coupled to horseradish peroxidase) provided no evidence that NGF leaves the membrane-confined compartments. However, it could not be excluded that very small, but physiologically significant, quantities of NGF reach the cytoplasm and subsequently also the cell nucleus. Therefore, we studied the effect of NGF or of NGF-antibodies introduced directly into the cytoplasm of target cells. Loaded guinea pig erythrocyte-ghosts were fused with pheochromocytoma cells (PC12) or with neurons isolated from embryonic chick dorsal root ganglia. Taking into account the multiplicity of ghost to cell fusion and the degradation of injected ^{125}I-NGF in the cytoplasm, the quantity of intact NGF present at the end of the observation period exceeded by several fold that accumulated in the cells by receptor-mediated endocytosis when ^{125}I-NGF (100 ng/ml) was added to the culture medium. NGF injected into the target cells by means of erythrocyte ghosts distributed equally throughout the cytoplasm and the nuclear chromatin. However, it did not evoke the characteristic effects of fiber outgrowth or maintenance of survival observed when NGF is added to the culture medium and acts via plasma membrane receptors. Conversely, NGF-antibodies injected into the cytoplasm did not prevent the effects of NGF mediated by membrane receptors. It is concluded that cytoskeletal elements or the chromatin of the nucleus are not direct physiological targets of NGF and that both fiber outgrowth and the maintenance of survival must be mediated by a second messenger mechanism subsequent to the interaction of NGF with membrane receptors.

115.11 A HEPARAN SULFATE PROTEOGLYCAN INDUCES RAPID NEURITE OUTGROWTH IN VITRO. A. D. Lander*, D. K. Fujii*, D. Gospodarowicz*, and L. F. Reichardt. Department of Physiology and Cancer Research Institute, Univ. of California, San Francisco, CA 94143.

The bovine corneal endothelial cell produces, in culture, a basement membrane-like extracellular matrix. When dissociated rat sympathetic ganglion cells were plated on this matrix, rapid and extensive neurite outgrowth was seen in less than 4 hours. Outgrowth occurred in the absence of nerve growth factor (NGF) and was unaffected by antiserum to NGF. Rat sensory and sympathetic neurons also displayed rapid neurite outgrowth when plated onto polylysine-coated dishes that had been preincubated with serum-free medium conditioned by corneal endothelial cells (CM_{SF}). This effect required direct contact of neurons with the treated surface; when only half of a dish was pretreated with CM_{SF}, only neurons on that half responded. A good response did not require the presence of serum in the culture medium.

The activity of CM_{SF} was destroyed by trypsin, by incubation at pH 1.6 or 12.7, and by heating to 80°C, but was stable to heating to 60°C, collagenase, DNase, and neuraminidase. When CM_{SF} was fractionated on Sepharose 6B, a single broad peak of activity was found just behind the void volume. On non-dissociative equilibrium cesium chloride gradients, CM_{SF} activity banded in the density range of 1.3-1.4. The profile of activity coincided with that of a major peak of ^{35}S found when CM_{SF} metabolically labelled with $Na_2[^{35}S]O_4$ was fractionated in this way.

A large molecular size, a density greater than protein, the presence of incorporated sulfate, and tight binding to polycations are characteristics of proteoglycans, macromolecules composed of protein to which sulfated glycosaminoglycan (GAG) chains have been added. It was found that the activity of CsCl-gradient-purified CM_{SF} could be abolished by crude heparinase, an enzyme that degrades heparan sulfate (HeS), the major GAG produced by the bovine corneal endothelial cell in vitro. Chondroitinase ABC, which degrades the other GAGs produced by this cell type, had no effect. However, neither HeS alone, nor any other purified GAG, had neurite outgrowth promoting activity. This, along with the results with trypsin and heparinase, suggests that the activity of the proteoglycan factor requires both its protein and GAG parts.

The observation that a HeS-containing proteoglycan can direct neurite outgrowth in vitro suggests that HeS proteoglycans, which are major constituents of neuronal and target tissues, may serve a related in vivo role.

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115.12 CONDITIONING FACTOR(S) FOR NEURITE OUTGROWTH: MOLLUSCAN NEURONS IN CULTURE, Richard G. Wong*, Robert D. Hadley, Stanley B. Kater, Garry C. Hauser*, Elaine C. Martel*. Dept. of Zoology, Univ. of Iowa, Iowa City, IA 52242.

Previous work in our laboratory has shown that well characterized identified neurons of the buccal ganglia of the snail Helisoma are capable of neuronal plasticity often involving neurite outgrowth.[1-5] As an approach to the problem of mechanisms of initiation of neurite outgrowth and axonal elongation in this preparation we have developed in vitro culture procedures for studying outgrowth from isolated neurons.

Neurons from central ganglia were isolated by enzymatic and mechanical dissociation and cultured in vitro in modified Liebowitz L-15 medium. Isolated neurons maintained electrical excitability but remained spherical in defined medium throughout culture durations up to 2 weeks. Extensive neurite outgrowth from the isolated neurons occurs with co-cultured intact Helisoma brains (CC) or brain conditioned medium (CM). Growth in vitro (6-40 μm/hr at 23°C) occurs at the tips of neurites by means of growth cones which can exhibit rapid extensions and retractions of membrane. Differential morphologies were observed on collagen as compared to polylysine coated surfaces.

The brain-derived conditioning factor(s) (CF) appears to be conserved in related species (Biomphalaria and Lymnaea) and acts as a noncellular, soluble substance(s) that binds to specific substrates. Its action is not mimicked by NGF or fibronectin. From our studies on CF and its effects on both electrical excitability and neurite extension we conclude that CF acts as a substrate conditioning factor and affects primarily neurite outgrowth with no apparent effect on the maintenance of electrical excitability.

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116.1 THE DISTRIBUTION OF NUTRIENTS BETWEEN FETAL BRAIN AND BODY DURING RAT DEVELOPMENT. Stephen Zamenhof and Edith van Marthens*. Mental Retardation Research Center and Brain Research Institute, UCLA School of Medicine, Los Angeles, California 90024.

During prenatal development, the nutrients are distributed between brain and body according to a schedule influenced by many factors; this distribution determines the extent of prenatal brain growth. As indices of the final outcome may serve fetal and neonatal ratios: brain weight/body weight (R_W), brain DNA/body DNA (R_{DNA}) or brain protein/body protein (R_{prot}).

We find that R_W changes from $(7.29\pm1.06)\times10^{-2}$ (16 day fetus) to $(2.76\pm0.19)\times10^{-2}$ (newborns); the dependence on age disappears towards birth. Within one age, R_W shows considerable variability (std. dev. up to 14% of the mean), not only among individual litters, but also within litters. These variations are not due to variable water content: in 18 day fetuses, water contents were brain, 87.3±0.5%, and body, 89.5±0.2%. Thus, R_W ratios of wet weights and dry weights are similar, and the variations in water contents are negligible. For 18 day fetuses R_W = (4.43±0.63)$\times10^{-2}$ (wet wts.) or $(5.31\pm0.76)\times10^{-2}$ (dry wts.), R_{DNA} (dry wts.) $=(7.52\pm1.28)\times10^{-2}$ and R_{prot} (dry wts.) $=(4.76\pm1.10)\times10^{-2}$; thus, R_{DNA} and R_{prot} show even greater variability than R_W. As expected, R_{prot} was similar to R_W.

An extensive study of 2089 normal newborns (from 231 mothers) revealed that 157 animals, or 7.5% (from 63 mothers), had R_W values significantly higher than the mean for the population (more than 2 std. dev. above the mean). Of these, 35.0% had normal body weight (101.3% of the mean) but significantly higher brain weight, as well as higher brain DNA (cell number) and brain protein. Thus, they do not represent previously studied cases of general (brain and body) overdevelopment (Zamenhof et al., Life Sci. 18, 1391 [1976]); rather, they represent cases of favorable brain versus body growth, i.e., distribution of nutrients between brain and body that is more favorable for the brain. In 3% of cases, such favorable distribution affected the entire litters; the causes probably involved maternal factors. In 49% of cases, only one or a few individuals within a litter had such favorable distribution; in such cases individual fetal factors were more likely to be the cause. For such animals, this favorable distribution occurred during prenatal neuron proliferation; depending when it occurs, it would favor different individual cortical layers whose "neuron birth" happens to occur at that time, and would result in differences in neuron distribution among individual animals. (Supported by NIH grants HD-05615 and AG-00162).

116.2 EFFECTS OF EARLY TRYPTOPHAN OR HISTIDINE EXPOSURE ON OFFSPRING SENSORIMOTOR BEHAVIOR IN RATS, P.K. Lundberg*, V.F. Nease*, H. Zenick, and D. Price*, (SPON: A. Michaelson), 1st. Author-Dept. of Psychology, Washington Univ., St. Louis, MO 63130, Others - Dept. of Environmental Health, Univ. of Cincinnati, Cincinnati, Ohio 45220.

Although it is recognized that prenatal exposure to certain drugs can result in behavioral teratogenesis (Vorhees, Brunner & Butcher, Science, 1979, 205:1220-1224), the effects of pre - and post-natal exposure to slight excesses of dietary amino acids are not well-documented. Of particular interest are the behavioral effects of tryptophan (TRY) and histidine (HIS). Since the enzymes tryptophan hydroxylase and histidine decarboxylase are not saturated in vivo (Wurtman & Fernstrom, Am. J. of Clin. Nutr., 1975, 28:638; Schwartz, Lampart & Rose, J. Neurochem., 1972, 19:801-810), exposure of dams to dietary excesses of TRY or HIS may also expose the fetuses to increased serotonin (5-HT) or histamine. Thus the aim of this study was to compare the effects of a maternal control (CON) diet and diets supplemented with l-tryptophan (0.5% & 1.0%) or l-histidine (0.6% & 1.3%) on offspring sensorimotor behavior.

Dams were randomly assigned to diets 2 weeks prior to mating. On post-natal day 3 (PN3) 4 pups per litter were randomly chosen for behavioral testing. The test battery included surface righting response (PN3-7), negative geotaxis (NG;PN6-12), pivoting activity (PA;PN7,9,11), swimming development (SD;PN6-20), startle response (PN10-13), day of eye opening, and open field activity (PN30,60). Other measures included body weights (PN 1, 7, 14, 21), brain weights and brain TRY, 5-HT and 5-hydroxyindoleacetic (5-HIAA) levels (PN 1, 21).

Offspring of the TRY dams showed significantly reduced PA and significant impairment on the NG and SD tasks. TYR offspring were less emotional than controls during PN30 open field testing and significantly more active after amphetamine (1.5 mg/kg) administration during PN60 testing. TRY brain weights were smaller than CON on PN1 but not on PN21. TRY offspring weighed less on PN7, 14 and 21 and their 5-HIAA levels were significantly elevated (PN21). HIS offspring showed superior NG performance and delayed SD. They also exhibited less emotionality during PN30 open field testing. HIS brain weights were smaller than CON on PN21 only while body weights were smaller on PN 7, 14, and 21.

116.3 BRAIN NEUROTRANSMITTER ALTERATION AFTER INTRAUTERINE EXPOSURE TO ETHANOL. W.J. Shoemaker, G. Baetge*, R. Azad*, V. Sapin*, and F.E. Bloom. A.V. Davis Center for Behavioral Neurobiology, The Salk Institute, La Jolla, CA

Neuropeptide and catecholamine neurotransmitter levels were determined in newborn rat pups that had been exposed during gestation to ethanol, protein undernutrition or both. Ethanol-containing liquid diets formulated to produce normal growth rates and birth weight of offspring when fed to pregnant rats are compared to liquid diets that are nutritionally inadequate in order to determine the roles of ethanol (ETOH) and undernutrition on brain neurotransmitters and development. By varying the protein concentration of the diets, we have produced newborn rat pups that have been exposed to one of the following conditions: alcohol plus undernutrition; alcohol with adequate nutrition; undernutrition alone; and control diet alone. All rats received such diets on gestational days 7-21. Newborn brains are dissected into hindbrain, midbrain and telencephalon for radioimmunoassay of β-endorphin and enkephalins. Catecholamines are assayed by HPLC using electrochemical detection. β-endorphin levels (pg/mg protein) in hindbrain and midbrain are consistently and significantly increased in the offspring of alcohol exposed mothers receiving an inadequate diet that contains 5% ethanol (approximate dose = 12 g/kg/day) (midbrain: ETOH-poor diet = 693; ETOH-good diet = 400; pair-fed control = 209; adequate-diet control = 315; $p < .001$, ANOVA). Similar results were obtained for hindbrain: the magnitude of the increase in β-endorphin levels over controls in both brain regions is proportional to the peak value in blood ETOH of the mothers. There were no differences in telencephalic β-endorphin in any group tested. Brain enkephalins were not statistically different in any brain region. Brain norepinephrine, on the other hand, was increased in the telencephalon of ETOH exposed newborns. Telencephalic dopamine was unchanged. These results will be discussed with reference to the anatomical alterations caused by in utero ethanol. (NIAAA 03504).

116.4 MICE IN UTERO WHILE MOTHER IS LACTATING SUFFER HIGHER FREQUENCY OF DEFECTIVE CORPUS CALLOSUM. D. Wahlsten. Dept. of Psychology, Univ. of Waterloo, Waterloo, Ontario, Canada N2L 3G1

Many mice of the BALB/c inbred strain suffer defects of the corpus callosum(CC) ranging from reduced size of the structure to total absence of trans-cortical fibres. However, the frequency of CC defect is much less among mice bred at Waterloo than those bred commercially. Commercial breeders usually leave the parents together so that the female gets pregnant during post partum estrus and delivers another litter soon after the first is weaned, but at Waterloo parents are separated shortly before parturition.

A controlled study was done of this phenomenon with BALB/cCF mice from Carworth Farms maintained as eight separate lines at Waterloo. In the ISOLATE condition males were separated from females before parturition, whereas for their littermates in the STAY condition males remained with females and sired litters in rapid succession. After breeding was completed over a period of three generations, each of 49 STAY litters which survived to maturity was matched with an ISOLATE litter of the same line and generation, and, where possible, the same birth order, season of birth and litter size. Cross-sectional area and length of CC at the mid-sagittal section were measured on brains coded to conceal their group and line identities. A defective CC was defined on the basis of a previous study as one with area less than .85mm^2 and length less than 3.0mm.

The table shows numbers of mice(n), numbers with defective CC(#D) and percent defective CC(%D) in the various groups along with one-tailed tests of significance. Frequencies in the total sample were weighted to give equal n's for the two groups in each generation.

Generation:	1		2		3		Total	
	#D/n	%D	#D/n	%D	#D/n	%D	#D/n	%D
STAY	18/76	23.7%	21/59	35.6%	16/99	16.2%	54/230	23.5%
ISOL.	8/72	11.1%	6/59	10.2%	10/142	7.0%	21/230	9.1%
z	1.769		3.068		2.025		4.039	
p	.0384		.0011		.0214		.00003	

Not only was the frequency higher but the degree of defect was also more severe in the STAY condition, where 9 of 54 mice with CC defect had total absence of transcortical fibres compared to 2 of 24 in the ISOLATE condition. Because CC fibres usually traverse from one hemisphere to the other beginning about 17 days after conception in BALB/cCF mice, the elevated frequency of severe defects of CC in the STAY condition must result from processes acting prenatally during lactation.

116.5 ACTIVITY OF THE ENZYMES DOPAMINE-beta-HYDROXYLASE AND PHENETHYLAMINE-N-METHYLTRANSFERASE IN DISCRETE BRAIN REGIONS OF THE RAT FOLLOWING ALUMINUM INGESTION. G.L. Wenk and K.L. Stemmer*. Institute of Environmental Health, University of Cincinnati, Cincinnati, OH 45267.

We have recently reported changes in Aluminum (Al) distribution within the brain following Al ingestion (Wenk and Stemmer, J. Env. Path. Tox., In Press) and alterations in catecholamine (CA) levels in various brain regions of Al-fed rats (Wenk and Stemmer, J. Neurotoxicol., Vol 2(2), 1981). These changes relate very highly to the dietary intake of the essential trace metals zinc (Zn) and copper (Cu). Al has also been shown to inhibit various enzyme systems (Trapp, J. Neurotoxicol., 1:89, 1980). We report an investigation of whether Al may be influencing endogenous CA levels by altering enzyme activities in the brain. The enzymes DBH and PNMT were investigated in the brains of rats fed diets suboptimal in Cu or Zn with and without Al added (0.1% wt/wt. diet). Thirty rats were maintained in metal-free conditions for 120 days. To adequately control for various metals in the diet, the rats were fed a purified synthetic diet (Teklad Test Diets, Madison, WI). The rats were sacrificed and the frontal cortex, hippocampus, and cerebellum were insolated. DBH was assayed according to the method of Davis and Kissinger (J. Chromatogr.,2:663, 1979); PNMT was assayed according to the method of Borchardt et al.(Anal. Biochem. 82:149, 1977).

An HPLC unit with an electrochemical detector was used to quantitate the CA products. The actitivities of both enzymes in the cortex and hippocampus were decreased in rats fed diets suboptimal in Cu with or without Al and suboptimal Zn without Al. The addition of Al to the diet of suboptimal Zn-fed rats elevated the activity of both DBH and PNMT in the cortex and hippocampus.

116.6 PROTEIN MALNUTRITION IN RATS - EFFECTS ON THE NUCLEUS RAPHE DORSALIS: A MORPHOMETRIC GOLGI STUDY. T. Kemper*, L. Cintra, S. Diaz-Cintra* and P. J. Morgane. Worcester Foundation for Experimental Biology, Shrewsbury, Mass. 01545.

Using morphometric Golgi techniques, we previously characterized three cell types in the nucleus raphe dorsalis, i.e., multipolar, ovoid and fusiform (Brain Res. 207: 1-16, 1981). In the present experiments we quantitatively examined the effects of a low (8%) protein diet, initiated prenatally, on various cell parameters in these three cell types in rats at 30, 90 and 220 days of age. The oval, fusiform and multipolar cells of normal diet (25% protein) animals all showed significant increases in synaptic spines on primary and secondary dendrites between 30 and 90 days of age. Between 90 and 220 days of age there was a significant decrease in dendritic spines on primary dendrites in the fusiform and multipolar cells and a significant decrease of these spines on secondary dendrites of ovoid cells. Thus, all three cell types showed a decrease in total dendritic spines with normal aging to 220 days. By measuring number of dendrites, length of dendrites and spine density we calculated an index of synaptic input for each cell type in order to establish degrees of synaptic contact for each cell and determine how this is altered by low protein diets. In the protein deprived animals there were minimal changes in synaptic spine densities on primary and secondary dendrites between 30 and 90 days and 90 and 220 days in the fusiform and ovoid cells. The one exception was in the primary dendrites on the ovoid cells which showed a significant increase in spine density between 90 and 220 days of age. The multipolar cells, however, showed a significant increase in dendritic spines between 30 and 90 and between 90 and 220 days of age. When the three cell types in each diet group were compared at each age the multipolar cells in the 8% protein diet rats were found to be the only cells showing increased synaptic input on primary dendrites at 30 days of age and on secondary dendrites at all three ages. With the single exception of the secondary dendrites on fusiform cells at 90 days of age all other comparisons showed either no change or a decreased synaptic spine input in the 8% diet rats as compared to controls. These data indicate that the multipolar cells, which are thought to be the serotonin-containing cells of the raphe, show a response to protein deficiencies different from that of non-serotonin cells (ovoid and fusiform cells), i.e., show increased synaptic input with age in the face of malnutrition and do not lose spines as do the ovoid and fusiform cells. This appears to be in conformity with our studies in which we showed highly significant increases in serotonin in the brain of protein malnourished rats dating from birth. (Supported by grant #06364, NICHHD).

117.1 TWO MUTATIONS IN DROSOPHILA DIFFERENTIALLY AFFECT THE SYNTHESIS OF OCTOPAMINE, DOPAMINE AND SEROTONIN BY ALTERING THE ACTIVITIES OF TWO DIFFERENT AMINO ACID DECARBOXYLASES. Margaret S. Livingstone* (Spon: J.A. Paton) Dept. Biology, Princeton Univ., Princeton, NJ08540

The monoamines octopamine, dopamine and serotonin have been detected in nervous tissue from many insects. Intact Drosophila melanogaster brains, with cuticle removed, can be incubated in radioactive neurotransmitter precursors and analyzed by electrophoresis. When incubated in tritiated tyrosine the brains synthesized and accumulated labeled dopamine, octopamine and tyramine. When incubated in tritiated tryptophan they accumulated labeled serotonin.

Mutations in the Ddc locus, identified by Wright et al. (1976) as the structural gene for the enzyme dopa decarboxylase, decreased the synthesis by brains of both dopamine (see also Wright, 1977) and serotonin but did not affect the synthesis of octopamine or tyramine. Brains from per° flies, on the other hand, accumulated 25% as much octopamine and tyramine as wild type flies, but their synthesis of dopamine and serotonin was normal. The per° mutant, isolated by Konopka & Benzer (1971), has abnormal (arrhythmic) circadian eclosion and activity rhythms, abnormal fluctuations in the male's courtship song (Kyriacou & Hall, 1980) and an abnormally irregular heartbeat (Aceves-Piña, unpublished).

The biochemical results suggest that there are two different aromatic amino acid decarboxylases in drosophila brains: one that decarboxylates ℓ-dopa and 5-hydroxytryptophan and another that decarboxylates tyrosine. Measurements of tyrosine, ℓ-dopa and 5-hydroxytryptophan decarboxylating activity in brain homogenates from the different strains confirmed this suggestion and indicated that the decreased octopamine synthesis in per° brains is due to a reduction in tyrosine decarboxylase activity. Tyramine ß-hydroxylase activity was normal in per° brains.

The per locus is probably not the structural gene for the enzyme tyrosine decarboxylase because flies with deletions of the per locus had some enzyme activity and because per°/+ heterozygotes had normal activity. The per° mutation may affect some property of octopaminergic neurosecretory cells. Konopka (1980) found that the mutation affects the morphology of a group of neurosecretory cells in the brain. The pericardial cells along the heart were also morphologically abnormal in per° flies (and may be responsible for the irregularity of the heartbeat). These cells stained with neutral red and fluoresced yellow with glyoxylic acid, suggesting that they may be octopaminergic. Isolated hearts with the pericardial cells attached accumulated tritiated octopamine when incubated with labeled tyrosine, and octopamine synthesis was reduced in per° hearts. Supported by NIH grants GM25578 (to W.G. Quinn) and F32NS06393.

117.2 A MUTATION IN DROSOPHILA THAT REDUCES DOPAMINE AND SEROTONIN SYNTHESIS ABOLISHES ASSOCIATIVE LEARNING. Bruce L. Tempel* and Margaret S. Livingstone* (SPON: R. Cholewiak). Biology Dept., Princeton University, Princeton NJ 08544.

Monoamine neurotransmitters may be involved in various aspects of vertebrate learning, attentiveness, and reinforcement. (For example, see R.A. Wise.) Here we report that a mutation blocking synthesis of dopamine (DA) and serotonin (5-HT) in Drosophila blocks associative learning but does not affect other forms of behavior.

Using a deletion, Df130, and a temperature sensitive allele of the dopa-decarboxylase gene, Ddc^{tsl} (T.R.F. Wright), known to decrease the levels of DA and 5-HT synthesis (M.S.L., previous abstract), we have bred a number of fly stocks with different genotypes at the Ddc locus. Each of these mutant stocks is raised at a permissive temperature, 21°. We obtain the severely affected $Ddc^{tsl}/Df130$ (29°) mutants by raising them at 21° until the adults have completed cuticle hardening, then shifting them to a restrictive temperature, 29°, at which they survive and appear behaviorally normal for several weeks.

To assay dopa-decarboxylase activity, we dissect out brains of 3 day old flies and incubate them for 30 min with ^{3}H-L-dopa, the precursor of DA. The product, ^{3}H-DA, is isolated by high-voltage paper electrophoresis and measured by liquid scintillation counting. DA synthetic activity of the mutants ranges in the following order from 50% to < 1% of the activity in co-shifted, wild-type C-S control flies:

$$\text{wild-type (30 fmoles/min/brain)} > \frac{Df130}{C\text{-}S} > \frac{Ddc^{tsl}}{C\text{-}S} > \frac{Ddc^{tsl}}{Df130}\ (21^\circ) > \frac{Ddc^{tsl}}{Ddc^{tsl}} > \frac{Ddc^{tsl}}{Df130}\ (29^\circ)\ (< 1 \text{ fmole/min/brain}).$$

Three to five day old, 29° Ddc/Df mutants do not demonstrate associative learning in either a negatively reinforced shock avoidance paradigm (learning index, $\bar{\Lambda}$=0.00±0.02) or in a positively reinforced sucrose reward paradigm ($\bar{\Lambda}$= -0.02±0.04). In contrast, co-shifted, wild-type controls learn normally ($\bar{\Lambda}\ominus$ = 0.32±0.05; $\bar{\Lambda}\oplus$ = 0.22±0.06). The learning deficit in the Ddc/Df flies cannot be explained by general behavioral debility because the phototactic, geotactic and odor discrimination abilities of the mutant are similar to controls. In our other Ddc fly stocks, learning ability closely parallels dopa-decarboxylase enzyme activity levels.

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117.3 VISUAL ORIENTATION OF FLIES AFTER LASER-BEAM ELIMINATION OF SPECIFIC INTERNEURONS, G. Geiger* and D.R. Nässel* (SPON: H. Anderson). European Molecular Biology Lab., 6900 Heidelberg, F.R.G.

To study the role of a specific configuration of nerve cells in information processing in the fly, we selectively eliminated neurons in the larva and then studied the adult animal's visual orientation behaviour. In flies the innermost neuropile region of the optic lobe, the lobula complex, is divided into two parts: an anterior lobula and a posterior lobula plate. In the lobula plate 12 different classes of large neurons have been identified anatomically and electrophysiologically that are directionally motion sensitive. Of these, 3 cells (H-cells) respond to horizontal and 8-11 cells (V-cells) to vertical motion (K. Hausen, z. Naturforsch. 31c:629 1976; H. Eckert and L. Bishop, J. Comp. Physiol. 126:57 1978). The H-cells are believed to be involved in the optomotor turning response and visual orientation towards objects, whereas the V-cells are considered to be important in thrust lift and landing responses. We were able to eliminate reproducibly the large H- and V-cells in one brain hemisphere by making micro laser-beam ablations of their precursors in the 2nd instar larva of Musca domestica. To achieve this, the ablation parameters were progressively tuned by correlating them with the resulting anatomical defects in the optic lobe observed in serial sections stained with reduced silver. The visual orientation of the experimental flies were tested in the following way: a. a flying fly was tethered to a torque-thrust transducer and its torque and thrust response to a vertical stripe moving horizontally clockwise and counterclockwise around the fly were measured. b. the fly's ability to visually fixate a vertical stripe was recorded. The torque and thrust responses were not significantly different between the ablated and normal sides nor between experimental and control flies. However, the response in the ablated side was slightly more noisy. The flies' visual fixation was also normal but more noisy. Following the behaviour tests the lack of H- and V-cells was determined histologically. From our results we conclude that the H- and V-cells are not crucial in information processing of visual orientation towards single objects but are mainly concerned with background motion detection whereas single object motion information is processed elsewhere.

117.4 ROLE OF IDENTIFIABLE PROPRIOCEPTORS IN THE FEEDING MOTOR PROGRAM OF APLYSIA. B. Jahan-Parwar, Worcester Foundation for Experimental Biology, Shrewsbury, MA 01545

Each buccal ganglion of Aplysia contains two identifiable coupled neurons, B4 and B5 (Gardner, J. Neurophysiol., 1977). Other workers have suggested that B4 and B5 are motor neurons or interneurons involved in mediating feeding behavior. Several lines of evidence from my laboratory suggest, however, that B4 and B5 are centrally located somata of the proprioceptors for two ipsilateral buccal nuscles: Both B4 and B5 have their axons in the ventral buccal nerve which innervates these muscles. This was demonstrated by both electrophysiological and morphological (dye filling) criteria; moderate stretch (<4g) applied to the target muscles resulted in a burst of action potential in B4 and B5 with their extracellularly recorded axonal spikes preceding their intracellularly recorded somatic spikes. After blocking synaptic transmission in the CNS the responses of B4, B5 to muscle stretch persisted, but the responses of simultaneously monitored motor neurons for the target muscle did not. In the same preparation, driving a motor neuron by intracellular current injection caused contraction of the target muscle, but driving B4 and B5 failed to do so. These data clearly establish the B4 and B5 as the proprioceptors for these buccal muscles. We have begun to investigate the role of B4 and B5 in the feeding motor program. Recordings were made simultaneously from all buccal nerves in both intact and more simplified preparations. In intact preparations, stimulation of the chemoreceptors with food often produced a highly consistent cyclic pattern of burst activity in the buccal nerves. These patterns of bursting unit discharges were highly stereotyped both within a given nerve and across all buccal nerves. For example, 11 discrete bursts could be distinguished across four nerves which innervate the buccal mass proper. The durations and phase relations of these bursts appeared to remain constant from cycle to cycle and from preparation to preparation. This cyclic across-nerve pattern of activity was always correlated with rhythmic muscle contractions in the buccal mass and thus is likely to represent a part of the feeding motor program. Removal of the buccal mass had no significant effect on most parameters of the motor program, suggesting that it was generated centrally. Using the individual components (bursts) in the central motor program (CMP) as a guide, we have identified a number of neurons in the buccal ganglia which contribute to the CMP. Among these were the previously identified B4 and B5. These were always active during the CMP and contributed to a particular burst in the program. Removal of the buccal mass did not have any significant effect on the B4,B5 discharges during the CMP. Both B4 and B5 receive rhythmic synaptic input from the oscillator system (unpublished observation) and are known to have extensive synaptic interactions with putative sensory and motor neurons. These data and the fact that they can contribute to the CMP suggest that they play an important role in the generation and maintenance of the feeding motor program. This would raise a serious question regarding the present "dogma" on the role of the proprioceptors in the CMP generation in general. (Supported by grant NS 14388)

117.5 PRE-SYNAPTIC INHIBITORY PATHWAYS TO PARACEREBRAL FEEDING COMMAND NEURONS IN PLEUROBRANCHAEA CALIFORNICA, J. A. London and R. Gillette. Dept. of Physiol. & Biophys. Univ. of IL, Urbana, IL 61801.

Food avoidance conditioning in Pleurobranchaea is paralleled by synaptic inhibition of feeding command neurons, the paracerebral neurons, PCNs, in response to food stimuli (Davis and Gillette, Science, 199: 801, 1978). Similar inhibition of the PCNs in response to food has also been observed in naive but satiated animals. Preliminary to investigating the role of these neurons in the mechanisms of food avoidance learning, we are characterizing the circuitry and behavioral context of these neurons in the isolated CNS, the semi-intact (Head, buccal mass, esophagus and CNS) and whole-animal preparations of naive specimens. We have identified neurons which polysynaptically drive IPSPs on the PCNs and have found inhibitory neurons directly presynaptic to the PCNs.

PCN inhibitors, PIs, are a group of cells of unknown number, located on the dorsal surface of the brain, which mediate apparent monosynaptic, short duration IPSPs on the PCNs (n=2). The PI excitor cells, PIEs, also located on the dorsal surface of the brain are found in two bilateral groups, each group composed of at least two electrically coupled neurons. Stimulation of a PIE results in the occurrence of long latency, heterogeneous amplitude IPSPs on the PCNs (n=15). In one experiment, stimulation of a PIE resulted in a slow depolarization on the PI; negative feedback from the PI to the PIE was manifested as apparently monosynaptic IPSPs. The PIEs also appear to make a potent excitatory monosynaptic connection with the MCG previously implicated (Gillette and Davis, J. Comp. Physiol., 116: 129, 1977) in the feeding rhythm (n=5). During cyclic motor activity the PIEs are co-active with bursts of retractor motoneuron activity, in antiphase with PCN activity and in phase with MCG activity.

In 3 whole-animal preparations and 10 semi-intact preparations the PIEs were excited by compound EPSPs in response to food stimuli applied to the oral veil. This application also caused an increase in IPSP activity on the PCNs, consistent with excitation of the PIEs. The MCG was excited by the application of food. An aversive stimulus (10% EtOH) caused prolonged hyperpolarization of the PIEs (n=5 animals). These results are consistent with the role of the PIEs as cyclically active elements of the feeding network and suggests their potential role in the tonic inhibition of the PCNs in food avoidance conditioned animals. Supported by NSF-BNS-79-18329 to R.G.

117.6 CYCLIC ADENOSINE 3',5'-MONOPHOSPHATE PHOSPHODIESTERASE (cAMP-PDE) AND ITS ROLE IN LEARNING IN DROSOPHILA. S. L. Shotwell* (SPON: M. Kennedy). Div. of Biology, California Institute of Technology, Pasadena, CA 91125.

Drosophila can learn in several associative conditioning paradigms. Flies carrying the mutation dunce (dnc) perform poorly in one such task (Dudai et al., PNAS 73:1684, 1976). dnc mutants are deficient in activity for one of the two cAMP-PDEs present in normal flies, PDE II, and have normal activity for the other, PDE I. dnc mutants show elevated levels of cAMP (Byers et al., Nature, 289:79, 1981). The experiments described below indicate that the normal dnc gene (dnc^+) codes for PDE II itself, rather than a regulator that affects PDE II and possibly other activities.

In Drosophila, the amount of gene product, as a rule, is proportional to the number of copies of the gene present. Flies with carefully controlled genetic backgrounds were assayed using a micro-assay technique developed to allow separate measurement of PDE I and PDE II when both are present in a mixture. Homogenates of flies with gene arrangements providing the equivalent of 0, 0.5, 1.0 and 1.5 times the normal dose of dnc^+ had, respectively, 0.0, 0.6, 1.0 and 1.6 times the PDE II specific activity of normal flies. In contrast, PDE I activity was constant. This close correlation between PDE II activity and amount of the dnc^+ gene suggests strongly that this gene codes for PDE II.

To test directly for a modulator of PDE II activity, mixtures were made of homogenates of normal flies with those of flies carrying a small deficiency for the dnc region ($Df(1)N^{77\,1}/w^+y$). Mixtures having 25, 50 and 75% normal homogenate contained 26, 52 and 75% of the normal PDE II activity. Thus the normal homogenate does not activate the deficiency homogenate, nor does the latter inhibit normal PDE II activity.

Two other experiments support the suggested role for PDE II in learning. 1) PDE I and PDE II activities were determined in homogenates of various normal fly tissues. All contained both enzymes, but in different ratios. PDE II specific activity was highest in the nervous system. 2) Larvae carrying a mutant allele dnc^1 fail the larval learning paradigm of Aceves-Piña and Quinn (Science, 206:93, 1979). dnc^1 larvae were assayed, and had similarly reduced PDE II activity as dnc^1 adults.

These data do not directly prove that dnc^+ is the structural gene for PDE II. It could encode a stoichiometrically-required activator subunit. Alternatively, dnc^+ may regulate transcription or translation affecting the final level of PDE II. However, such regulation would seem unlikely to yield the linear relationship demonstrated above between PDE II activity and dose of dnc^+. The data strongly suggest that the normal dnc gene encodes PDE II, indicating that this enzyme plays a role in learning, presumably by controlling levels of cAMP. (Supported by NIH grant 07616, and NSF grant PCM-7911771 to S. Benzer.)

117.7 NEUROPHYSIOLOGICAL CORRELATES OF CONDITIONING IN IDENTIFIED PUTATIVE MOTOR NEURONS IN HERMISSENDA. T. Crow, Dept. of Physiology, Univ. of Pittsburgh Sch. of Med., Pittsburgh, PA 15261.

The temporal association of light and a gravitational stimulus, rotation, produces a long-term modification of photopositive behavior of the Pacific nudibranch Hermissenda crassicornis (Crow and Alkon, 1978). The behavioral modification consists of a significant increase in the animals latency to locomote in response to light (Crow and Offenbach, 1979). Cellular neurophysiological correlates of this conditioning have been identified in type B photoreceptors following training (Crow and Alkon, 1980). In this report neuronal correlates of the conditioning procedure are examined in identified presumptive pedal motor neurons.

As an initial step in the cellular analysis of conditioning in the motor system of Hermissenda, intracellular recordings were made from newly identified pedal cells P5 and P6 in the isolated circumesophageal nervous system following training. Intracellular iontophoresis of HRP into P5 and P6 indicated that the presumptive motor neurons had peripheral axons in pedal nerves. Impulse frequency and cyclic burst activity of cells P5 and P6 is altered by illumination of the eyes. The two identified cells in the pedal ganglion probably receive indirect synaptic input from the photoreceptors. The change in neuronal activity is mediated by the visual system and not by extraocular sources. Intracellular recordings from P5 and P6 in preparations where the optic nerves were cut before entry into the cerebropleural ganglion did not result in significant changes in pedal cell activity during illumination. Simultaneous intracellular recordings from P5 and P6 showed no synaptic interactions, but they do receive synaptic input from at least one common source (putative interneuron I).

Animals (N=10) receiving paired presentations of light and rotation showed significant increases in the latency to initiate locomotion in response to illumination as compared with controls (N=10) that received independent random presentations of light and rotation ($p<.025$). Paired animals (N=9) showed a significant decrease in spike frequency and bursting activity in cells P5 and P6 in response to light ($p<.01$) as compared with the random controls (N=7), which actually responded to illumination with an increase in spike frequency and cyclic bursting activity. The neural correlates may reflect changes intrinsic to the motoneurons or alternatively, modifications in cells presynaptic to P5 and P6 such as the photoreceptors. The pre- or postsynaptic nature of the light evoked changes in motoneuron activity following behavioral training is currently being investigated.

117.8 ASSOCIATIVE NEURAL AND BEHAVIORAL CHANGE IN HERMISSENDA: CONSEQUENCES OF NERVOUS SYSTEM ORIENTATION FOR LIGHT- AND PAIRING-SPECIFICITY. Joseph Farley*, Dept. Psychology, Princeton Univ., Princeton, NJ 08544 and Daniel L. Alkon, NINCDS, NIH, MBL, Woods Hole, MA 02543.

Locomotor behavior in the nudibranch Hermissenda, following three training sessions of exposure to paired light (30 sec duration, intensity 4.6×10^3 ergs. cm^{-2}. sec^{-1}) and rotation (94 rpm; 2.24 g) presentations, was characterized by light- and pairing-specific changes whose magnitude depended upon the gravitational orientation of the animals during testing. This associative diminished phototaxic behavior reflected both an increase in the latency for initiation of movement, as well as a decrease in the velocity of locomotion.

Tests of animals' locomotion while oriented vertically rather than horizontally, revealed: 1) a general decrease in locomotor latencies both before and after training, 2) greater light-specificity of the associative behavioral change, 3) greater pairing-specificity.

These associative behavioral changes in intact animals and the relation to test orientation were closely paralleled by the results of intracellular recordings from photoreceptors in the eye of Hermissenda. Type B photoreceptors from animals which had been exposed to paired light-rotation presentations exhibited greater impulse frequencies to a broad range of light intensities, 24 and 48 hours after the conclusion of training, than did cells from animals exposed to random light-rotation presentations. These impulse frequency differences were accompanied by facilitation of paired cells' long-lasting depolarization following offsets of light, increased input resistances of B cells during the dark, but no differences in spontaneous activity during the dark nor resting potential.

These associative changes observed for single type B photoreceptors were also manifest in ther interaction of pairs of type B photoreceptors, as well as pairs of type B and A photoreceptors, 24 hours after training.

The greater light-specificity of changes in locomotor behavior for the vertical testing orientation was accompanied by greater light/dark impulse activity ratios for paired B photoreceptors in the vertical orientation. This greater neural light-specificity is consistent with the tonic synaptic inhibition of B photoreceptors by caudal hair cells of the animals statocyst, which occurs in the vertical orientation.

The data provide evidence that type B photoreceptors define primary locii of neural change, which furthermore are causally related to the observed associative behavioral change.

117.9 ASSOCIATIVE LEARNING BY THE ISOLATED CNS OF A TERRESTRIAL MOLLUSK, LIMAX MAXIMUS, OCCURS CENTRALLY. A. Gelperin, N. Culligan* and S. Wieland. Dept. of Biology, Princeton University, Princeton, N.J. 08544.

We are pursuing the analysis of synaptic events underlying associative learning by developing an isolated CNS preparation which can be conditioned in vitro (J.J. Chang and A. Gelperin, Proc. Natl. Acad. Sci. USA 77: 6204, 1980). The sensory input pathway is via chemoreceptors on the two lips. The output is feeding motor program (FMP) recorded extracellularly from nerve roots of the buccal ganglia. By paired application of a normally attractive food-derived chemostimulus (conditioned stimulus, CS) and an aversive chemostimulus (unconditioned stimulus, US) to the lip chemoreceptors, the in vitro lip-brain-buccal ganglia (LBBG) preparation can be trained to stop emitting FMP in response to the CS, while continuing to emit FMP in response to a second food-derived chemostimulus (S_2) not paired with the aversive taste.

We have extended this basic finding by using a new stimulus delivery system which allows us to apply chemostimuli to either the right lip alone or the left lip alone. Using unilateral chemostimulus application, we applied the CS (e.g., standard potato extract) to the right lip and 5 min. later the US (saturated quinidine sulfate) to the left lip. Subsequent tests with the CS and a new chemostimulus S_2 (e.g., standard carrot extract) applied to the left lip showed a significant reduction or elimination of the FMP response elicited by the CS but not S_2 (4 of 6 preparations). The selective suppression to the CS was expressed only on the side which received the US. These data provide further evidence for associative learning by the isolated LBBG preparation of Limax and support the conclusion that the associative event occurs in the CNS, not at the periphery.

A further test of the central nature of the synaptic change was made by applying the CS and the US sequentially to the same lip, and testing for FMP responses to the CS and S_2 using the opposite lip. In 4 of 10 cases, the preparation showed suppression of the FMP response to the CS but no suppression to S_2 when tested at the "naive" lip. In these 4 preparations the lip where the training occurred also expressed selective suppression to the CS. Only 1 of the 6 preparations which did not express learning at the naive lip showed any signs of learning at the trained lip. These data are further indication of the central nature of the associative event.

We are now mapping the cells and cell clusters of the Limax cerebral ganglion and surgically reducing the LBBG preparation to the smallest remanent that will learn reliably in order to further localize the associative event. Supported by NSF Grant BNS 8005822.

117.10 DIETARY CHOLINE INCREASES RETENTION OF AN ASSOCIATIVE LEARNING TASK IN THE TERRESTRIAL MOLLUSC, LIMAX MAXIMUS. C. L. Sahley, S. R. Feinstein* and A. Gelperin. Dept. of Biology, Princeton University, Princeton, New Jersey 08544.

Recent experiments in our laboratory have been directed toward determining the effect of dietary neurotransmitter precursors on learning and memory. Adult slugs were maintained on choline enriched (11.4g/kg) and choline-deficient (.3g/kg) diets (BioServ). After two weeks on the diet, slugs were tested for acquisition and retention of an associative odor-preference modification task which we have used previously (C. Sahley, A. Gelperin & J.W.Rudy, Proc. Natl. Acad. Sci. USA 78: 640, 1981). Separate groups of slugs were tested for acquisition (24 hr following training) and for long-term retention (7 days following training). Results indicated that no differences in learning were apparent between slugs on choline-deficient and choline-enriched diets on the initial test (24 hr), $p > 1$. In contrast, dramatic differences in performance between slugs on the choline-deficient and choline-enriched diets emerged on the long term retention test. That is, 7 days following training, slugs on the choline-deficient diet showed little evidence of retention of the learned task whereas slugs on the choline-enriched diet showed excellent retention of the learned odor-modification $p < .01$. In fact, performance of slugs on the choline enriched diet 7 days following training was no different from performance of slugs on either diet 24 hr following training, $p > 1$. Dietary choline enrichment then significantly facilitated memory of a learned task.

A radioenzymatic assay for free choline revealed that slugs maintained on a choline-enriched diet possessed significantly higher concentrations of choline in their blood than their counterparts maintained on the choline deficient diet ($p < .01$).

Choline's action may be mediated via increasing ACh synthesis and release at cholinergic synapses. The effect of choline on ACh release was studied at a cholinergic neuromuscular junction (NMJ) between an identified motoneuron, the salivary burster (SB), and the muscle cells of the salivary duct. When the isolated nervous system was incubated in saline enriched with choline at concentrations within the normal physiological range, synaptic transmission at this NMJ was enhanced. Further experiments demonstrated that this effect was due to increased choline uptake, ACh synthesis and release by the SB neuron. Thus, increases in exogenous choline for Limax have both dramatic behavioral and physiological consequences. That is, increasing dietary choline results in facilitated memory for an associative learning task and in vitro exogenous choline significantly augments synaptic transmission at a NMJ. We feel that choline's effect on memory may be related to this synaptic phenomenon.

117.11 ASSOCIATIVE LEARNING IN A SIMPLE REFLEX OF APLYSIA. T. J. Carew, E. T. Walters* and E. R. Kandel. Center for Neurobiol. & Behavior, Depts. Physiol. & Psychiat., P & S, Columbia Univ., and N.Y. State Psychiatric Inst., New York, N.Y. 10032.

The ability of Aplysia and other molluscs to exhibit associative learning has encouraged the search for this type of learning in a behavior controlled by a simple and well analyzed neural circuit. Towards that end we have now produced classical conditioning in the defensive siphon and gill withdrawal reflex of Aplysia.

A light tactile conditioned stimulus (CS) to the siphon was paired for 15 trials (i.t.i.=5 min) with an unconditioned stimulus (US), a 1.5 sec electric shock to the tail. Paired animals (N=10) received the CS immediately prior to the US, unpaired animals (N=10) received the CS and US separated by 2.5 min. Animals were tested (blind) with the CS alone shortly after training. Paired animals showed significantly longer siphon withdrawal in response to the CS ($\bar{x}$=64.7 sec) than unpaired animals ($\bar{x}$=16.7 sec., $p < .005$). We next examined acquisition, extinction and retention of the conditioned response in six groups: (1) paired, (2) unpaired, (3) random CS-US, (4) US alone, (5) CS alone, and (6) untrained. During acquisition siphon withdrawal in the paired group progressively increased from $\bar{x}$=8.6 secs after trial 1 to $\bar{x}$=32.5 secs after trial 31 ($F_{6,48}$=2.8, $p < .05$). Moreover the paired group exhibited significant facilitation compared to random and unpaired groups after only one trial ($p < .025$ and $< .005$ respectively). In the 24-hour retention test the paired group again showed significantly longer siphon withdrawals ($\bar{x}$=55 secs) than all other groups ($p < .005$). In addition several differences not apparent during training and extinction now emerged among the control groups: the US-alone group ($\bar{x}$=20.1 secs) was significantly greater than both unpaired ($\bar{x}$=9.1 secs) and random ($\bar{x}$=10.5 secs) groups ($p < .005$ in both cases) while CS-alone ($\bar{x}$=5.4 secs) and untrained groups ($\bar{x}$=6.8 secs) were significantly lower than all other groups.

Preliminary cellular experiments indicate that conditioning can be monitored in the identified interneurons and motor neurons of the siphon and gill withdrawal reflex. It has also recently been shown that (1) the tail is a powerful input pathway for gill and siphon withdrawal (Carew et al., 1980, 1981); (2) sensory neurons that mediate the US are located in the pleural ganglia (Walters et al., 1981); and (3) the US powerfully activates identified facilitatory interneurons in the abdominal ganglion (Hawkins, 1981). Thus neurons have been identified in pathways for the CS, the US, and the conditioned response. Consequently, it may now be possible to identify neurons causally related to the conditioned response in this simple reflex. Since this reflex exhibits nonassociative learning, it may also be possible to compare associative and nonassociative learning on a mechanistic level.

117.12 IDENTIFICATION OF SENSORY NEURONS INVOLVED IN TWO FORMS OF CLASSICAL CONDITIONING IN APLYSIA. E.T. Walters*, T.J. Carew and E.R. Kandel. Center for Neurobiology & Behavior, Depts. Physiol. & Psychiat., P & S, Columbia Univ., N.Y. State Psychiatric Inst., New York, N.Y. 10032.

Aplysia has now exhibited two forms of aversive classical conditioning and in both cases sensory input from the tail plays a prominent role. Walters et al. (1979, 1981) showed that tail-elicited escape locomotion, inking, and siphon withdrawal are facilitated markedly by a chemosensory conditioned stimulus that has been paired with noxious shock to the head during a classical fear conditioning procedure. More recently Carew et al. (1981) have shown that direct classical conditioning of siphon withdrawal can be produced by pairing weak siphon stimulation with strong shock to the tail. Thus the tail can act both as a test pathway for expressing the effects of fear conditioning, and as the reinforcement pathway for direct conditioning of siphon withdrawal.

We here report the identification of a population of sensory neurons that respond to both tactile and electrical stimulation of the tail. These cells (30-70 μ) are clustered within a large group of sensory neurons on the ventral side of each pleural ganglion. They have receptive fields on the ipsilateral half of the tail while nearby cells have receptive fields adjacent to the tail on the posterior parapodia, body wall, and foot. The properties of these neurons resemble closely those of identified mechanoreceptors in the abdominal and cerebral ganglia (Byrne et al., 1974; Rosen et al., 1980): 1) they show slowly adapting trains of action potentials in response to mechanical or electrical stimulation of the tail; 2) the number of spikes is a graded function of the intensity of the stimulus; 3) the spikes lack prepotentials; 4) spikes can be triggered antidromically by stimulation of appropriate peripheral nerves; and 5) impulses evoked by skin stimulation persist in Co^{++} solutions which block synaptic transmission in the skin and the CNS.

Intracellular stimulation of individual sensory neurons can evoke polysynaptic EPSPs in identified siphon and ink motor neurons. Monosynaptic excitatory connections (0.5-15 mV) from the sensory neurons were found to a group of newly described motor neurons which produce withdrawal of the tail. These tail motor neurons are clustered on the ventral side of each pedal ganglion. As with other sensory neurons in Aplysia, monosynaptic EPSPs from the tail sensory neurons decrement with repeated activation. Preliminary evidence indicates that in fear-conditioned animals these monosynaptic EPSPs are not facilitated by the CS even though concurrently recorded complex EPSPs from tail stimulation are facilitated in ink and siphon motor neurons. Thus the modulation of tail input by classical fear conditioning may act at a locus downstream from the primary sensory neurons.

117.13 IDENTIFIED FACILITATING NEURONS ARE EXCITED BY CUTANEOUS STIMULI USED IN SENSITIZATION AND CLASSICAL CONDITIONING OF APLYSIA. R. D. Hawkins. Center for Neurobiol. & Behavior, Columbia University, P & S, New York, N.Y. 10032.

The Aplysia gill and siphon withdrawal reflex can be sensitized by tactile or electrical stimulation of the neck, mantle shelf, or tail. Electrical stimulation of the pleuroabdominal connectives, which carry input from the neck and tail regions, produces presynaptic facilitation at synapses from siphon mechanosensory neurons to gill and siphon motor neurons (Castellucci et al., 1970; Castellucci and Kandel, 1976). This facilitation can also be produced by intracellular stimulation of single identified neurons, the L29 group, which are excited both orthodromically and antidromically by connective stimulation (Hawkins et al., 1981). As a test of the hypothesis that the L29 neurons mediate the effects of natural sensitizing stimuli, I now report the activity of those neurons in a semi-intact preparation.

Single L29 neurons are excited by cutaneous stimulation over a large area of the body surface including the tail, posterior parapodia and body wall, siphon, and mantle shelf. Stimulation of more anterior portions of the body recruits IPSPs in L29 neurons. Within the excitatory region, stationary tactile stimuli produce an adapting "on" response and also an "off" response, while moving tactile stimuli and strong electrical stimulation produce a high frequency "on" response followed by sustained firing throughout the period of stimulation (average rate = 35 spikes/second).

These results show (1) that L29 neurons are excited by cutaneous stimuli to the tail and mantle shelf which have been used to produce sensitization of the gill and siphon withdrawal reflex, and (2) that rates of L29 firing produced by these stimuli are comparable to those which have been used to produce presynaptic facilitation at sensory neuron to motor neuron synapses with intracellular L29 stimulation (Hawkins et al., 1981). Sensitization of the gill and siphon withdrawal reflex can also be produced by stimulation of anterior body regions which do not excite L29 neurons in the semi-intact preparation, suggesting that other, as yet undiscovered neurons may mediate sensitization from those regions.

Carew, Walters and Kandel (1981) have recently demonstrated classical conditioning of the siphon withdrawal reflex, using tactile or electrical stimulation of the siphon as the CS and and electrical stimulation of the tail as the US. Both the CS and the US produce firing of L29 neurons with the parameters of stimulation used in the behavioral experiments. It therefore seems possible that the L29 neurons may be critically involved in this associative form of learning as well as in nonassociative sensitization of the gill and siphon withdrawal reflex. Experiments are in progress to test this possibility.

118.1 LATERAL GENICULATE NUCLEUS ORIGIN OF THE CORTICOTECTAL PATHWAY IN THE CAT. C.L. Colby, Dept. of Psychology, MIT, Cambridge, MA 02139

The lateral geniculate nucleus (LGN) of the cat has three sets of layers: the A laminae, the C laminae and the medial interlaminar nucleus (MIN). Functionally, three cell types have been identified in cat LGN: X, Y and W cells. These three cell types do not show a clear laminar segregation, yet each of the LGN laminae is quite distinct in the manner in which it projects to visual cortex. Since Y cells appear in all of the magnocellular laminae, it is likely that the functional connectivity of these cells is determined by their laminar origin.

This hypothesis was tested by reversibly blocking synaptic transmission in the LGN by microinjection of cobalt chloride while recording single or multi-unit activity in the topographically corresponding region of the superior colliculus (SC). Inactivation of the A laminae had little or no effect on collicular cell responses to light stimulation. In contrast, inactivation of the C laminae abolished visual responsiveness at one quarter of the SC recording sites, reduced responsiveness at another quarter of the locations and left half of the recording sites unaffected.

These results show that input to corticotectal cells does not arise equally from all LGN layers. The Y cells in the C laminae project extensively onto corticotectal cells while the Y cells in the A laminae appear to have other target sites. These results suggest that one function of the lamination of the lateral geniculate nucleus is to segregate cells according to their projection sites in cortex.

118.2 EFFECTS OF BLOCKING A-LAYER GENICULATE INPUT ON CAT AREA 17. J. G. Malpeli. Dept. of Psychology, 603 E. Daniel St., Champaign, IL 61820.

Injections of 115 nanoliters of 4 millimolar cobalt chloride into layer A of the lateral geniculate nucleus selectively and reversibly block synaptic transmission in this layer without blocking axonal conduction in fibers of passage. I have assessed the effects of layer A inactivation on the activity of single cells and multiple-unit recordings in area 17 of the visual cortex. Blocking layer A virtually abolished visual activity in cortical layers 4 and 6. In contrast, such blocks had little effect on the activity, direction selectivity, or orientation selectivity of complex cells in layers 2 and 3. The effects of layer A blocks on layer 5 cells were quite variable, ranging from complete loss of visually driven activity to no effect. The layer 5 cells least affected by the cobalt block were the special complex type, which are thought to project to the superior colliculus (1,2).

From these observations I conclude:

Major receptive field properties of layer 2 and 3 complex cells and many layer 5 complex cells do not depend on the integrity of interlaminar connections within the cortical column.

Interlaminar connections do not account for the presence of a common orientation selectivity within an orientation column.

Layer 4 simple cell activity is not necessary to support the visually driven activity of complex cells in layers 1 and 2 or the activity of special complex cells in layer 5.

As in the monkey (3), the corticotectal downflow does not strongly depend upon X-cell inputs to cortex.

The division of the lateral geniculate nucleus into discrete layers segregates populations of relay cells having unique patterns of access to individual cortical outputs. This segregation may facilitate selective dynamic modulation of cortical outputs by non-retinal inputs to the visual thalamus.

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118.3 AN INTRACELLULAR STUDY OF GENICULO-CORTICAL CONNECTIVITY IN AREA 17 OF THE CAT. D. Ferster* and S. Lindström*. (SPON: T. Wiesel). Dept. of Physiology, University of Göteborg, Sweden.

It is widely held that an understanding of the function of the visual cortex requires detailed knowledge of the arrangement of its input from the lateral geniculate nucleus (LGN). We have therefore undertaken yet another study of the synaptic input to the visual cortex from the LGN. Potentials evoked electrically from the optic tract (OT), LGN, the optic radiation (OR) and the superior colliculus (SC) were recorded intracellularly from cells with identified receptive field properties. Potentials mediated by the geniculo-cortical pathway were distinguished from those due to antidromic activation of intracortical collaterals by their ability to be evoked from the OT, LGN and OR at appropriate latencies, intensities, and frequencies. Intracortical latencies, excluding afferent conduction times, were obtained by extrapolation. A histogram of intracortical latencies for all epsps and ipsps formed 3 peaks of 0.6 ms width beginning at 0.6, 1.1 and 1.7 ms. These were assumed to contain mono-, di- and trisynaptic potentials.

The laminar distribution of monosynaptically activated cells could, in part, be predicted by the distribution of geniculate afferent terminals: all cells recorded in layer 4, the bottom of layer 3 and the upper portion of layer 6 received monosynaptic epsps and disynaptic ipsps. There were also cells outside these layers that received monosynaptic epsps: in layer 5, the lower part of layer 6 and higher parts of layer 3. These latter regions, however, contained cells that were disynaptically excited and di- or tri-synaptically inhibited. As a result, every cell below layer 3 received mono- or disynaptic excitation from the LGN. The only cells whose earliest epsps were trisynaptic were found in layer 2 and possibly the upper part of layer 3.

As previously reported, simple cells were confined to layers 4 and 6 and were therefore all monosynaptically excited from the LGN, whereas complex cells could receive mono-, di- or trisynaptic excitation. Of those cells with subcortical projections, the majority of cortico-collicular cells were disynaptically activated. All cortico-geniculate cells, however, were monosynaptically excited from the LGN, forming a disynaptic excitatory feedback pathway to LGN principle cells.

Our results differ from those obtained in previous extracellular studies of spike latency. We propose several reasons for this: 1)differences in the classification of receptive field types; 2)the use of extrapolation to arrive at intracortical latencies; 3)the variable delay between the onset of an epsp and the initiation of a spike; and 4)further delay or even blocking of the spike by ipsps superimposed on an epsp.

118.4 LINEARITY OF RESPONSE DEPENDENCE ON BAR AND EDGE VELOCITY. Robert C. Emerson and Linda S. Ide. Center for Visual Science, University of Rochester, Rochester, NY 14627.

We tested the extent to which lateral geniculate (LGN) and cortical units in the cat realize linear predictions by comparing unit responses with those of a receptive field (RF) analog whose spatial distribution included one excitatory central and two inhibitory flanking regions. We constructed the analog from silicon photocells and other linear components to facilitate interpretation of its responses and the corresponding effects in actual neurons. When the temporal properties of the analog were adjusted to match LGN and cortical responses to flashing bars, the shape and amplitude of analog responses as a function of position of a moving bar or edge showed a strong dependence on stimulus speed, especially in the 2-32°/sec range. Analog responses as a function of the speed of a bar closely approximated those of most X-LGN units, those of many Y-LGN units at low speeds, and (for the preferred direction) those of simple cortical units with balanced flanking regions. Analog responses to moving edges exhibited clearly separated regions of response to bright- and dark-edge stimuli. This finding suggests a linear basis for the disparate edge discharge centers that are considered diagnostic for simple units, as contrasted with the (nonlinear) overlapping discharge regions of cortical units in the complex family. Responses of complex units to flashing bars also could not be mimicked by a linear analog. The linear analog demonstrates that spatiotemporal interactions in all moving stimuli require responses to be highly dependent on stimulus speed. Comparisons between unit and analog responses, however, indicate that this dependence can be explained roughly on a linear basis for most LGN units and many cortical units of the simple family.

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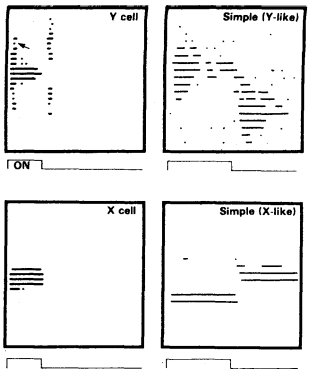
118.5 RECEPTIVE FIELDS AND LAMINAR DISTRIBUTION OF X-LIKE AND Y-LIKE SIMPLE CELLS. W. H. Mullikin*, J. P. Jones*, L. A. Palmer. Dept. Anat., School of Medicine, University of Pennsylvania, Philadelphia, Pa. 19104

There is evidence that simple cells in area 17 of cats may be differentially innervated by Y or X axons. Accordingly, we anticipated that the receptive field organization of simple cells would resemble, in part, properties of geniculate X and Y cells and that X-like and Y-like simple cells would be distributed by lamina according to the distinct patterns of arborization of X and Y axons.

Similarities between the receptive field organization of simple cells and either X or Y LGNd cells were revealed by plotting a cell's firing rate as a function of the position(y-axis) and time course(x-axis) of a small bar of light turned on and off (see figure). The receptive fields of Y-cells were distinguished from those of X-cells by the presence of transient "arms" extending to either side of the central excitatory response (arrow). In a similar manner, the excitatory responses of most simple cells were found either with or without "arms". A few cells had mixed organization (ie. on-excitation without "arms"; off-excitation with "arms"). Simple cells with "arms"(y-like) had a transient response while cells without "arms"(x-like) were sustained.

Y-like simple cells were found in layers III, IVab and VI, corresponding to the terminal distribution of Y axons. X-like simple cells were found at the III-IV border, IVab, IVc and VI, even though X axons have been found to arborise only in layers IVc and VI. X-like cells at the III-IV border and IVab could result from intracortical projections from layer IVc. Presently, no Y-like simple cells have been found in layer IVc.



These results lead us to conclude that many simple cells are dominated by either X or Y inputs from the LGNd. Few cells may receive both X and Y input. Thus, the X and Y pathways may remain largely separate even within striate cortex.

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118.6 A COMPARISON OF CYTOCHROME OXIDASE AND DEOXYGLUCOSE PATTERNS IN MACAQUE VISUAL CORTEX, Roger B. H. Tootell* and Martin S Silverman*, Dept. Psychology, Univ. Cal. Berkeley, Cal. 94720 (spon: G. Westheimer).

In order to clarify an initial 2-14C-deoxyglucose (2DG) color study (Tootell, Silverman, and De Valois,1980) which predated the demonstration of endogenous cytochrome oxidase (CO) variations in monkey striate, CO and 2DG patterns were compared in the same section in different monkeys which were shown: 1) a black/white horizontally-oriented pattern of various spatial frequencies; 2) an equiluminant red/grey oriented pattern of various spatial frequencies shown at all orientations; 3) a ganzfeldt; and 4) 5) and 6) large-field, temporally-modulated equiluminant color/grey (n=2), equiluminant multiple-color (n=1), or black/white (n=2) patterns. Sections were cut parallel with the plane of flat-mounted opercular V1 and V2 in order to view lamina-specific patterns over large expanses of cortex.

In striate, 2DG uptake following visual patterns without a constant orientation component appear to superimpose the upper-layer CO dots exactly. Equiluminant colored large-field patterns are quantitatively much more effective in producing 2DG patterns in either V1 and V2 than are black/white patterns with identical spatio-temporal parameters or a ganzfeldt. However, the 2DG patterns produced by large-field color stimuli do not appear to be hue-specific in either V1 or V2. The CO dots near layer 4A are often connected in parallel strips perpendicular to the V1-V2 border, curving to run parallel with the horizontal meridian down the middle of the operculum: a pattern highly reminiscent of the ocular dominance slabs. The major difference between the large-field color/grey 2DG patterns and the striate CO patterns is in layer VI: the 2DG pattern reveals an obvious second tier of dots directly below each upper layer dot; the CO reaction product reveals only a very faint differential stain in layer VI (which nevertheless appears to superimpose the upper layer CO dots). The one single-orientation case we have done gives a different result. When the CO dots in striate layers 3-4A are compared to 2DG (horizontal) orientation columns in the same section, these two patterns apear to be much less clearly related to each other.

In V2, columnar strips of increased CO reactivity can be seen running parallel to each other approximately 3 mm apart and loosely perpendicular to the V1-V2 border. Large-field stimuli produce 2DG patterns which superimpose these CO strips exactly. A columnar horizontal-orientation 2DG pattern appears in V2 (perhaps indicating V2 orientation columns) which is entirely different from the CO slab pattern in the same section.

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118.7 ELETROPHYSIOLOGICAL VERIFICATION OF DEOXYGLUCOSE SPATIAL FREQUENCY COLUMNS IN CAT STRIATE CORTEX. Martin S. Silverman* Roger B.H. Tootell* and Russell L. DeValois*,University of California, San Francisco and Berkeley, Calif.

Using the ^{14}C-2-deoxyglucose, (2DG) functional mapping technique a stimulus containing 1 spatial frequency presented at all orientations produces autoradiographic patterns that traverse all cortical laminae. No columnar patterns are seen if multiple spatial frequencies are presented at all orientations. Thus, a columnar organization for spatial frequency is demonstrated (Silverman, et al, ARVO abst., '80, and Tootell, et al, OSA absts., '80). When viewed from the surface these columns are aligned in strips that run in a anteroventral - to - posterodorsal direction along the medial bank of the lateral gyrus (Silverman, et al, ARVO absts., '81).

This columnar organization for spatial frequency as demonstrated by the 2DG technique was verified using single and multiple unit electrophysiological techniques.

The orientation and spatial frequency tuning characteristics of cells and cell groups were determined every 100u or less along electrode penetrations running posterior-to-anterior in the medial bank of the lateral gyrus.

We found that along such penetrations the peak spatial frequency tuning of successively encountered cells changed in a gradual oscillatory manner with a period of about 1mm. This is approximately the repeat distance seen for spatial frequency columns in our 2DG autoradiographs.

Orientation tuning for these same cells also changed in a regular manner as would be expected from the columnar organization for this parameter in cat striate cortex.

In comparing the pattern for spatial frequency and orientation tuning along these electrode penetrations it was found that the oscillations in spatial frequency peak tuning were at least as regular and periodic as those for orientation tuning. This is in agreement with our finding that the spatial frequency columnar organization shown by the 2DG technique was as regular and periodic as the organization for orientation specificity.

118.8 PROJECTION BANDS IN VISUAL CORTEX. C.D. Gilbert and T.N. Wiesel, Dept. of Neurobiology, Harvard Med. Sch., Boston, Mass. 02115.

Cells projecting from one cortical area to a given site in another cortical area are distributed in a number of discrete clusters, as shown by horseradish peroxidase (HRP) tracing experiments. We have now found that even within an individual cortical area, there exist extensive tangential connections that have a similar clustered appearance. This is seen not only with HRP used as an extracellular retrograde tracer, but with 3-dimensional reconstructions of the axons of cells injected intracellularly with HRP. We previously reported that, when reconstructed in three dimensions, the cortico-cortical projection clusters lined up into a system of branching and anastomosing bands which showed no clear relationship to the ocular dominance columns. We have begun to relate the projection bands to the pattern of labeling achieved by 14C 2-deoxyglucose (DOG) uptake after stimulation with lines of a given orientation. In one experiment in which the animal was stimulated with vertical lines we found that the cells in area 17 projecting to area 19 lay between the bands of highest DOG uptake. Whether or not this indicates a relationship between the projection bands and orientation columns requires further experiments.

From HRP experiments we noticed that the clusters projecting from area 17 to area 18 and to area 19 were similar in appearance, though they appeared to have a different sublaminar distribution within the superficial layers. To determine the precise relationship between these two sets of clusters we used two fluorescent retrograde tracer compounds, true blue and bisbenzamide. One dye was injected in area 18 and the other in area 19, with the two injections placed at retinotopically corresponding points. When area 17 was examined for labeled cells, we saw that, as with the HRP experiments, the cells labeled with either dye lay predominantly in the superficial layers, though now we saw many more labeled cells in the deep layers as well. The cells were clustered, and the cells projecting to areas 18 and 19 lay within the same clusters, with intervening unlabeled zones remaining. Numerous cells were labeled with either dye exclusively, and our preliminary impression was that there were no double-labeled cells. Furthermore, though there was overlap, within the superficial layers cells projecting to area 18 were distributed more deeply than cells projecting to area 19. It appears, therefore, that different cell populations project to different areas, though from the same system of bands. The projections of the cells that lie in the unlabeled zones remain to be determined. Our results raise the possibility, however, that cells inside and outside the projection bands may play distinct functional roles. (supported by NIH grants NS16189, EY00606 and EY01995 and by the Medical Foundation).

118.9 THE EFFECT OF COOLING AREA 18 ON CELLULAR RESPONSES IN STRIATE CORTEX OF THE SQUIRREL MONKEY. J.H. SANDELL and P.H. SCHILLER*. Dept. of Psychology, M.I.T., Rm. E10-120, Cambridge, Mass. 02139.

Reciprocal connections between primary visual cortex (Area 17) and extrastriate visual cortex (Area 18) have been anatomically documented in the squirrel monkey (Saimiri sciureus) by Tigges et.al. (JCN, 148:481, 1973). These reciprocal connections exist between retinotopically corresponding regions in the two areas. It is known that cells in Area 18 of the macaque become completely unresponsive when their input from striate cortex is removed (Schiller, P.H. and Malpeli, J.G., Brain Res., 126:366, 1977). However, the functional significance of the input from Area 18 to Area 17 is not known. This question could not be addressed in the rhesus monkey because Area 18 is largely buried under striate cortex. We chose the squirrel monkey for this study since in this species part of Area 18 is present in a strip of cortex 5-6 mm wide adjacent to Area 17 on the dorsolateral surface.

While recording the ongoing activity of cells in Area 17 of paralyzed, anesthetized squirrel monkeys, we reversibly inactivated the corresponding region of Area 18 by cooling the cortex to 10-18°C. The cells' response to optimal visual stimulation was recorded before, during, and after cooling of Area 18. Some cells in Area 17 became completely unresponsive when their input from Area 18 was temporarily removed. Other cells in Area 17 showed a significant increase in firing rate when Area 18 was cooled. Occasionally, this increased response resulted from a discrete change in the stimulus preference of the cell, such as the emergence of a response to an edge moving in the previously non-preferred direction. Not all cells in Area 17 were affected by cooling the corresponding region of Area 18; affected cells were more common in the infragranular layers of the cortex. Often only a few micra separated a cell which was affected by cooling Area 18 from cells which were completely unaffected. Cells in Area 17 which were affected by cooling returned to their pre-cooling firing rates within a few minutes of rewarming Area 18 to body temperature.

Thus the feedback from Area 18 to striate cortex plays a crucial role in defining the response properties of some cells in Area 17 of the squirrel monkey.

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118.10 BINOCULAR INTERACTIONS IN CATS WITH UNILATERAL LESIONS OF THE VISUAL CORTEX. J.C. Gardner and M.S. Cynader. Dept. of Psych., Dalhousie Univ., Halifax, N.S., Canada B3H 4J1.

Binocular interactions were examined in over 300 units from the 17/18 border of normal cats and cats with large unilateral lesions of the visual cortex. Stimuli were presented at seven retinal disparities and moved in the same and in opposite directions on the two retinae. Since binocular interactions in normal cats are extremely pronounced in superficial layers and since the corpus callosum is known to project to layer III, we wondered if unilateral visual cortex lesions would differentially effect responses in the superficial and deep cortical layers.

In normal cats, 66% of the units with very large binocular interactions were found in the superficial layers. In this region only 7% of the cells appeared insensitive to stimulus disparity, contrasting to 20% in deep layers. Average dynamic range by depth peaked about 800-900 microns below the cortical surface. To determine if a particular component of the binocular response was responsible for the larger interactions seen in superficial layers, the distributions of maximum inhibitory and excitatory interactions were analyzed seperately. Across the two cortical regions, no differences were seen in excitatory responses but a clear differenced appeared in the strength of the inhibitory component of binocular responses. The larger dynamic range of units in the superficial cortical layers was due to binocular inhibition.

Unilateral lesions of the visual cortex produced a significant reduction in binocular inhibition. No change was seen in excitatory responses. Average dynamic range showed the greatest effect around a depth of 600-1000 microns where a large increase was seen in the number of units which were insensitive to stimulus disparity. A small increase in unselective cells also appeared at the very bottom of the cortex. Differences between the two preparations were significant when the population was considered as a whole, when only superficial units were compared, but not when units in the deep layers were analyzed seperately. Effects were seen across all ocular dominance (OD) groups. Average dynamic range was largest in units of OD groups 1 and 7, followed by units in groups 2 and 6, 3 and 5, and 4. Units in all OD groups showed reduced dynamic range in the superficial layers and no change in deep layers.

In summary, unilateral lesions of the visual cortex had a significant effect on binocular interactions along the opposite 17/18 border. Lesion cats showed reduced binocular inhibition in units of all ocular dominance groups. Effects on binocular interactions appeared quite local in the cortex and corresponded well to the known terminations of callosal fibers.

118.11 POSITRON EMISSION TOMOGRAPHY STUDIES OF HUMAN VISUAL CORTEX. Eric L. Schwartz. Brain Res. Lab.,NYU Med. Ctr. New York,N.Y.

A series of experiments have been performed which make use of hemi-field stimulation,together with specific spatial stimulus structure,in order to provide a recognizable "signature" for cortical area V1 in PETT studies of human cortex. Based on a quantitative model of the topography of V1(Schwartz,Vision Res. 20:645(1980),a computer animation has been constructed on 8mm film,using a 512x521x1 bit digital display. This stimulus consists of picture elements which are scaled according to human cortical magnification, and is designed to be projected in one visual hemi-field.Visual stimulation is obtained by reversing the black and white pixels of the display, in a spatial pattern which consisted of four logarithmically scaled octants of visual field,which were alternately "on" (i.e. reversing) or "off" (i.e. static and blank). The display was restricted to either the right or left hemi-field, relative to a small (1^0 size) fixation letter. If it were possible to view an idealized and "flattened" V1, the representation of this stimulus would resemble a "check--erboard" of four equal sized patches.
This lateralized cortical "checkerboard" was intended to provide a recognizable "signature" for V1 (and possibly V2). The fixation letter was randomly alternated with a test letter(also about 1^0 in size), which was presented for 200 msec with an average interval between tests of 1 sec. The subject was required to verbally report each test letter. A high score was interpreted as evidence of compliance with the fixation task,since it is very difficult to recognize small rapidly changing letters without disciplined fixation.Subjects viewed a film loop(3 min. loop) of this computer graphic for 15 minutes; then, an i.v. injection of F^{18} labeled 2-deoxy-glucose (FDG) was adminstered,followed by fifteen minutes of stimulation. Subjects were then placed in the Brookhaven National Laboratory PET III scanner and serial scans were performed (10-12 minutes/slice). Successful observation of lateralized FDG counts in the presumed area of V1 was obtained, and local variations of FDG counts which were consistent with the topographic structure of the stimulus were observed. Application of this approach to constructing a cortical "signature" may be of increasing importance as higher resolution PETT scanners soon come on-line,since the confident identificat--ion of cortical areas is a necessary pre-condition for using PETT to study the functional architecture of human visual cortex.

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118.12 REGIONS OF POOR ORIENTATION TUNING COINCIDE WITH PATCHES OF CYTOCHROME OXIDASE STAINING IN MONKEY STRIATE CORTEX. David H. Hubel and Margaret S. Livingstone*. Dept. of Neurobiology, Harvard Medical School, Boston, MA 02115.

In a field not lacking in controversies it has been generally accepted that cells in layers 2 and 3 of the macaque monkey striate cortex show a high degree of orientation selectivity. Cells with poor orientation tuning when encountered have been difficult to interpret because local injury or deteriorating condition of the animal can lead to a loss of response specificity. In a series of 13 tangential penetrations in striate cortex of macaque monkeys, traversing a total of 54 mm in layers 2 and 3, we were surprised to find irregularly recurring sequences of cells with either poor orientation selectivity or none at all. These sequences occurred roughly every 1 to 2 mm, lasted about 200 μm, and were separated by regions in which all cells had sharp orientation tuning. At the onset and termination of a sequence tuning tended to be broad; in the middle of a sequence cells were often completely unoriented. These completely unoriented cells had center-surround receptive fields and often responded to diffuse light. As the electrode advanced there were the usual regular swings in eye dominance: the groups of cells with poor orientation tuning were centered in regions in which one eye strongly dominated.

The monocularity, size, and spacing of the regions of poor orientation tuning suggested that these regions might coincide with the patches of cytochrome oxidase staining found in layers 2, 3, and 6 of primate striate cortex (Horton & Hubel, Humphrey & Hendrickson: Neurosci. Abs. 1980). These patches or blobs are arranged in rows centered on the eye-dominance columns, are about 150-200 μm in diameter, and are spaced roughly 450 μm apart.

We therefore reconstructed the electrode tracks, using several electrolytic lesions in each track to establish reference points and staining tangential sections for cytochrome oxidase. On 22 occasions the electrode traversed a blob; each time there was an associated sequence of poor orientation tuning. And every sequence of poorly oriented cells was associated with a cytochrome oxidase blob. We conclude that the cytochrome oxidase patches are regions in which cells lack orientation tuning. This result would explain why the regions in layers 2 and 3 that stain most densely for cytochrome oxidase are also labeled by 2-deoxyglucose after stimulation with either diffuse light (Humphrey & Hendrickson) or stripes in any orientation (Horton & Hubel). The denser cytochrome oxidase staining of the blobs could reflect a higher overall rate of activity related to lack of stimulus specificity. It could also reflect differences in the organization of these regions. Supported by NIH grant EY00605.

119.1 BRAIN STEM ELECTROPHYSIOLOGICAL CORRELATES OF THE CLASSICALLY CONDITIONED NICTITATING MEMBRANE RESPONSE IN RABBIT. J. W. Moore, N. E. Berthier, and J. E. Desmond*. Dept. of Psychology, Univ. Mass., Amherst, Mass. 01003.

Electrophysiological recordings of multiple-unit activity (MUA) were obtained from unanesthetized rabbits during classical conditioning of the nictitating membrane response (NMR) to both an auditory and visual conditioned stimulus (CS). The unconditioned stimulus was electrostimulation of facial tissue marginal to one eye (paraorbital shock).

Single monopolar tungsten microelectrodes (Frederick Haer) were implanted stereotaxically into the dorsolateral pontine brain stem for chronic recording during conditioning. Animals were anesthetized during implantation and allowed at least three days of recovery before testing.

Training consisted of two daily sessions of 100 reinforced trials with a constant intertrial interval of 30 sec. Half of these trials employed a light CS and half employed a tone CS, presented in a random sequence and in a forward-delay arrangement. The CS-US interval was .5 sec. throughout. Acquisition training was followed by extinction training in which each CS was presented without the US for at least two sessions. For some animals, acquisition training was preceded by training of the eye contralateral to the electrode. Other animals received preliminary "pseudo-conditioning" to the ipsilateral eye, i.e., the CSs and US were explicitly unpaired.

Concurrent polygraphic recordings of integrated MUA and NMRs from both eyes indicated high correlation between MUA and conditioned responses of the ipsilateral NMR. The degree of coupling was greatest from electrodes located in the dorsolateral tegmentum at the level of the 5th nerve and from electrodes at the level of the 6th nerve near the motor pathway of the NMR.

Stimulation via electrodes located in these regions evoked full extension of the ipsilateral nictitating membrane, suggesting afferent synaptic linkages to relevant motoneurons. In addition, single-pulse stimulation via US electrodes (paraorbital shock) to either eye elicited short-latency (1.5-2 ms) evoked potentials from electrodes in the dorsolateral pons.

119.2 SENSORY-BEHAVIORAL CORRELATES IN INDIVIDUAL HIPPOCAMPAL NEURONS OF THE RAT ACROSS FOUR SITUATIONS. John L. Kubie and James B. Ranck, Jr., Dept. of Physiol., Downstate Med. Ctr., Brooklyn, New York 11203.

A number of studies demonstrate consistent correlations between hippocampal unit firing and behavior for a variety of behavioral situations ranging from classical conditioning to maze running. The most common descriptors used are the animal's position in space and various aspects of conditional stimuli or responses. In most cases the description implies the animal must learn something about his environment for the sensory-behavioral correlate to apply.

The aim of our study was to record from the same neurons during several behaviors performed in distinctly different environments. We hoped to see both consistencies and transformations from one environment to the next. We made chronic recordings of single hippocampal neurons in lactating female rats using either fine wires or moveable microelectrodes. Four environments were used: a large home box containing the rat's pups; a Skinner box in which the rat had learned a DRL-16; a familiar Olton 8-arm radial maze in a familiar room; and an unfamiliar Olton maze in an unfamiliar room. Recordings were made from twenty-four isolated complex-spike cells in at least two of these contexts. Rotating the apparatus within the room and manually carrying the rat through each environment were routinely performed.

We have found: 1) The most common sensory-behavioral correlate was to the rat's position in its environment. This is consistent with the findings of O'Keefe and others. 2) Spatial correlates were always defined by distal cues in the Olton maze and proximal cues in the other environments. 3) A spatial correlate in one environment could not predict a spatial correlate in a second environment, although similarities were often noticed. 4) Many cells turned off almost entirely (less than four action potentials/min) in one of the environments while firing rapidly in the others. 5) Tonic firing rate varied dramatically from one environment to the next. 6) Although most cells were spatial, conditional factors were also important. For instance, carrying a rat through its spatial field could either enhance or diminish the effectiveness of the field. 7) Cells which were recorded in the novel Olton maze (N=5) slowly established spatial correlates over a span of about five minutes.

One model consistent with these findings is that tonic unit firing in the hippocampus relates to context (situation) and phasic firing relates to space, specific to each environment. (Supported by NIH grant NS 14497 and NSF grant BNS 77-09375 to James B. Ranck, Jr. and NIH fellowship 1F32NS06152 to John L. Kubie).

119.3 SENSORY EVOKED POTENTIALS IN THE DENTATE GYRUS OF THE CHRONIC RAT DURING DIFFERENTIAL DISCRIMINATION LEARNING. M.O. West, E.P. Christian*, J.H. Robinson* and S.A. Deadwyler*. Dept. Physiol. and Pharmacol., Bowman Gray Sch. Med., Winston-Salem, NC 27103.

Rats prepared for chronic recording by means of a detachable microelectrode drive system were trained on a simple tone discrimination task and then switched to a differential discrimination paradigm in which responses to the previously conditioned tone (3.5 kHz) continued to be reinforced (positive tone), while responses to a second tone (2.4 kHz) were never reinforced (negative tone). Animals received 100 randomly sequenced trials per session and reached criterion behavior (100% responding to the positive tone and less than 10% to the negative tone) within 5-10 sessions. Averaged tone evoked potentials (AEP) recorded from the outer molecular layer (OM) of the dentate gyrus consisted of two monophasic negative components, termed N1 and N2, which were differentially controlled by inputs from entorhinal cortex and medial septum, respectively (Deadwyler et al., Science 211:1181-1183, 1981). OM AEPs recorded during criterion behavior showed that the amplitude of the N1 component, while not differing between the two tones, was significantly larger than during single tone conditioning. N2 amplitude to the positive tone was significantly larger than that to the negative tone and did not differ from that recorded during single tone conditioning. Following reversal of the reward contingency, animals responded maximally (90-100%) to both tones for 3-5 sessions and then reached criterion within 10-15 sessions. OM AEPs showed that N1 increased to both tones during the sessions following reversal and then declined to pre-reversal amplitudes when criterion performance was achieved on the reversed contingency. Conversely, N2 decreased significantly to both tones following reversal and then increased to pre-reversal amplitudes during criterion performance. OM AEPs were also analyzed according to trial sequence. Individual trials obtained during criterion behavior were sorted by a computer according to position in a sequence of like trials, e.g., first positive, second positive and so on, and then separately averaged. No differences were observed between OM AEPs corresponding to first positive vs. first negative tones; prominent N1 and N2 components were present in both. However, as trial sequence progressed, N1 amplitude decreased during positive sequences but increased during negative sequences; conversely, N2 amplitude increased during positive and decreased during negative sequences. These results provide further insight into the apparent reciprocity between the types of information conveyed to the hippocampus by entorhinal and septal inputs regarding the behavioral significance of sensory events.

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119.4 HIPPOCAMPAL, MEDIAL SEPTAL AND ENTORHINAL RESPONSES DURING AUDITORY SIGNAL DETECTION BEHAVIOR IN THE RABBIT. Ronald E. Kettner*[1], Gregory A. Clark*[1], Stephen D. Berry[2], and Richard F. Thompson[1]. Dept. of Psych.[1], Stanford Univ., Stanford, CA 94305 and Dept. of Psych.[2], Miami Univ., Oxford, OH 45056.

A model system for studing the neural basis of auditory signal detection behavior was developed using classical conditioning of the rabbit nictitating membrane (NM) response. Animals were conditioned to a white noise conditioned stimulus (CS) using a corneal airpuff unconditioned stimulus. The CS intensity was then reduced to behavioral threshold and multiple or single unit responses were compared on behavioral detect versus nondetect trials at a constant threshold level intensity. In past work, recordings from the anteroventral cochlear nucleus, the central nucleus of the inferior colliculus and the ventral division of the medial geniculate body have indicated that the behavioral variability observed at threshold cannot be accounted for by neural variability within the mainline auditory pathway. These auditory nuclei show well defined and essentially identical responses on detect and nondetect trials to a constant threshold level auditory CS.

In contrast to these auditory nuclei, these data suggest that the CA1 region of the hippocampus and its two major inputs, the medial septum and entorhinal cortex, show responses which relate significantly to signal detection performance. Hippocampal neurons showed a strong increase in firing which formed a temporal "model" of the behavioral NM response on behavioral detect trials. This increase was not present during nondetect trials. The medial septum showed a significant increase in firing to the onset of the auditory CS on detect trials which was reduced or nonexistent on nondetect trials. Entorhinal cortex displayed increases in firing on detect but not nondetect trials which tended to parallel the behavioral NM response. In addition, some entorhinal records also showed an initial inhibition to the CS, but this inhibition was present whether or not behavioral responding occurred. These data suggest that limbic areas are actively involved in determining auditory signal detection behavior.

119.5 REMEMBERING REWARDS IN THE ENVIRONMENT: ENDOGENOUS HIPPOCAMPAL OPIATES MODULATE REINFORCEMENT-MEMORY ASSOCIATIONS. T. J. Collier*, J. S. Miller*, G. Quirk*, J. Travis* and A. Routtenberg. Cresap Neuroscience Laboratory, Northwestern University, Evanston, Ill. 60201.

The granule cells of the hippocampal dentate gyrus support brain stimulation reward (BSR; Collier, T. J., et al., Neurosci.Abs. #41.5, 1980), and appear important for normal performance in spatially-cued working memory tasks (Olton, et al., Behav. Br. Sci., 2 313-365, 1979). Recent histochemical evidence (Gall, et al., in press, 1981) indicates that dentate gyrus granule cells and their mossy fiber axons exhibit enkephalinergic immunoreactivity. To investigate the functional role of these endogenous opiates, we studied the effects of systemic administration of the opiate antagonist naloxone, on dentate gyrus granule cell stimulation in two behavioral paradigms. A) BSR tests. Rats implanted with a single electrophysiologically guided monopolar stimulating electrode in the dentate granule cells were trained to lever press for brain stimulation in daily 15 min BSR tests. Electrodes directly in either limb of the granule cell layer supported low, but reliable rates of responding (mean lever presses/15 min=59.5). Following response stabilization animals received injection of a saline vehicle or naloxone (0.01-5.00 mg/kg) 20 min prior to BSR tests. Granule cell self-stimulation was suppressed in a dose-dependent manner following naloxone, while hypothalamic self-stimulation and operant behavior of controls were not affected by equivalent naloxone doses. B) Radial maze tests. Animals with similar granule cell implants were trained on a 2-trial memory task in a radial 8-arm maze. Animals were required to locate food, present in only one arm, on trial-1, and return to the single baited site, after a delay, on trial-2. Ten seconds of granule cell stimulation administered during the delay disrupted memory for the baited site. Pretreatment with naloxone (1 mg/kg) prevented the stimulation-induced performance deficit. These results suggest a role for opiates in both reward and memory functions. Release of endogenous opiates from the dentate mossy fiber system may participate in an endogenous reinforcement system involved in working memory. Supported by N.I.M.H. 25281 to A. R.

119.6 PREFRONTAL NEURON ACTIVITY RELATED TO TASK REVERSAL. K. KUBOTA AND H. KOMATSU* Dept. Neurophysiol., Primate Res. Inst., Kyoto Univ., Inuyama, Aichi, 484 JAPAN

Single neuron activities were recorded from the dorsolateral and ventral prefrontal cortex of two monkeys, in order to analyze neuronal mechanisms of a task reversal. To release the depressed lever (Go) or to continue lever depression (No-Go) for reward was combined with red or green discriminative stimulus. After fixed intertrial intervals (3 s), a yellow lamp was lighted. If the monkey depressed the lever, the lamp was turned off, 0.9 s later a green or red lamp was lit for 0.8-2.2 s and turned yellow, indicating the response period. Then the monkey performed Go or No-Go response. A correct response was rewarded with juice. The following two tasks were interchangeable. In one task, the monkey released lever to red and continued lever press to green. In the other task, the monkey released the lever to green and continued lever press to red. When the tasks were reversed, the monkey had to recognize the change of stimulus response relations through its own erroneous unrewarded responses.

Out of more than 200 neurons related to task events, 41 neurons showed a clear activity change, when the task was reversed. After the absence of reward, 7 neurons showed a transient increase of activity, 25 showed steady increase, continuing throughout intertrial intervals and 4 showed a mixed pattern. Two neurons showed a transient suppression and 4, a steady suppression. An increased activity continued often until the lamp stimulus onset or rarely until the response period. If, within tasks, the monkey responded erroneously or reward was not delivered to correct responses, these neurons showed changes similar as those in a task reversal. Increased activity was not abolished even if juice was delivered. In 3 neurons increased activity was present during 3-4 initial trials after the reversal. And correct responses were correlated to higher activity during preceeding intertrial interval periods.

It appears that switching of the response to be performed from No-Go to Go or from Go to No-Go is accomplished in the prefrontal cortex as follows: transiently increased neuron activity detects an absence of expected reward presentation and induces a tonic activity which leads to a correct response.

119.7 DRUG EFFECTS ON CLASSICAL CONDITIONING OF THE RABBIT NICTITATING MEMBRANE RESPONSE. J. A. Harvey and I. Gormezano*. Departments of Psychology and Pharmacology, The University of Iowa, Iowa City Iowa 52242.

These studies examined the effects of several drug classes on the rate of acquisition of conditioned responses (CRs) during classical conditioning of the nictitating membrane response. Conditioning was accomplished by the presentation of tone and light conditioned stimuli (CSs) for 800 msec prior to delivery of the unconditioned stimulus (UCS) consisting of a 100 msec electric shock to the skin over the paraorbital region of the head. Drug effects were determined by the frequency of CRs over the 10 daily conditioning sessions and by the number of trials required to reach a criterion of 5 consecutive CRs to the tone and light CSs. Haloperidol, a neuroleptic drug, and scopolamine hydrobromide, a muscarinic blocking agent, retarded the rate of CR acquisition to both tone and light CSs. In contrast, the two hallucinogenic agents, d-lysergic acid diethylamide (LSD) and d,l-2,5-dimethoxy-4-methylamphetamine (DOM),enhanced the rate of CR acquisition to both tone and light CSs. d-Amphetamine (AMP), a sympathomimetic, enhanced the rate of CR acquisition to the light CS but had no effect on acquisition to the tone CS. The non-hallucinogenic congener of LSD, d-2-bromolysergic acid diethylamide (BOL) had no effect on CR acquisition to either the tone or light CS. The ED 50s for these drug effects on the rate of CR acquisition were (μg/kg, as the base): haloperidol, 160; scopolamine, 38; LSD, 0.8; DOM, 52; and AMP, 280. To test for possible nonassociative effects of these drugs on CR acquisition, seperate groups of rabbits received explicitly unpaired presentations of stimuli (tone alone, light alone and shock alone). Results from the shock alone trials indicated that none of the drugs affected the amplitude of the unconditioned response elicited by the 3 ma UCS, thus changes in CR acquisition could not be attributed to an effect on the unconditioned nictitating membrane reflex. In addition, responding during the unpaired presentation of CSs was quite low for vehicle controls (1-2%) and not affected by any dose of haloperidol, scopolamine, LSD and AMP. Thus the altered rate of CR acquisition produced by these drugs appeared to be solely due to an effect on associative factors. However, DOM produced small but significant changes in nonassociative responding of approximately 10% above vehicle controls and thus its effects on CR acquisition could not be simply attributed to an effect on associative factors. Additional experiments indicated that the enhanced acquisition of CRs produced by LSD and the retarded acquisition of CRs produced by haloperidol could be attributed to an alteration in the sensory processing of the CSs. This research was supported by DA 01759 and MH 16841.

119.8 CHOLINERGIC DRUG INTERACTIONS: ENHANCEMENT AND IMPAIRMENT OF MEMORY RETENTION. James F. Flood* and Arthur Cherkin. GRECC, VA Medical Center, Sepulveda, CA 91343.

Animals injected with cholinergic agonists after training show striking improvement of memory retention (Flood, Landry and Jarvik, Brain Res., in press). Cholinergic agonists used clinically to alleviate dementia in the elderly produce only modest improvements in memory. Recent efforts to improve clinical results involve combinations of two drugs, e.g., a precursor of acetylcholine (choline or lecithin) with an anticholinesterase (physostigmine) or other agent (e.g., piracetam or L-dopa). The behavioral pharmacology of drug combinations which affect memory processing has received little attention. We have therefore undertaken studies to determine if low doses of combinations of cholinergic drugs can improve retention without undesirable potentiation of toxicity.

Mice received one of four cholinergic agonists, or saline, within 3-min after T-maze footshock avoidance training. The injections were intraventricular, to establish a central mechanism of action. Memory retention was tested 1 wk later. Exp. 1 was a dose response study of the effect of arecoline, deanol, edrophonium chloride or oxotremorine on retention. The results for each drug indicated that relative to controls, very low doses did not affect retention, low doses improved retention ($p<0.001$, t-test) and higher doses impaired retention ($p<0.005$). In Exp. 2, drug interaction effects on retention were studied by administering the 6 possible pairs of the 4 drugs. Each drug was administered at the dose per mouse which was found optimal for improving retention (arecoline, 0.1 μg; deanol, 1 μg; edrophonium chloride, 0.1 μg; oxotremorine, 0.01 μg). If the interaction were only additive (equivalent to giving twice the dose of a single drug) no amnesia would occur, based on the results of the dose-response study (Exp. 1). However, potentiation of either drug in a pair, by a factor of 5 or more, would result in a significant amnestic effect. The results showed significant amnestic effects by all 6 drug combinations; 4 pairs showed potentiation by a factor of at least 10 and 2 pairs by factors of 5-7. Studies in progress indicate that drug pairs also enhance retention, when the dose of each drug is reduced to one-fifth or less of its enhancing dose. Toxicity studies in chicks, using paired combinations of arecoline, deanol and physostigmine, have revealed no potentiation of toxic effects. Appropriate drug combinations may offer a clinically useful means of treating memory disorders associated with senile dementia.

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119.9 6-HYDROXYDOPA PRESERVES ILLNESS-INDUCED AVERSIONS TO ODORS IN RAT PUPS. Catherine A. Cornwell-Jones and Leila M. Azar*, Dept. of Psychology, Princeton University, Princeton, NJ 08544.

A treatment which depletes central norepinephrine selectively, prolonged retention of a conditioned odor aversion in rat pups. On the day of birth (Day 0), male Sprague-Dawley rats were injected systemically with either 6-hydroxydopamine HBro (6-OHDA) 100 µg/g, or with 6-OHDOPA, 60 µg/g. On the morning of postnatal Day 2, animals in the 6-OHDOPA groups received a second 60 µg/g injection. Control littermates received saline-ascorbic vehicle. On the evening of Day 2, pups were placed on a screen over lemon-scented pine shavings. They were injected, 5 min later, with either distilled water or with .02 cc/g of 0.15M lithium cloride (LiCl) and placed over the lemon-scented shavings for an additional 30 min.

Fifteen days later, on Day 17, males were given two odor preference tests on an apparatus having a screen floor placed on a two-compartment odor tray. One compartment always contained cedar shavings. The other compartment held either unscented pine shavings, or lemon-scented pine shavings. Pups were tested individually for 2 min and the time spent over the lemon-scented or unscented pine shavings was monitored.

On the cedar vs. pine test, all groups averaged significantly more than half of test time over unscented pine in preference to cedar, indicating an ability to orient to odors in the testing situation. On the lemon vs. pine test, scores of water-treated animals were similar regardless of neurotoxin treatment, while scores of LiCl-treated animals differed significantly, with 6-OHDOPA-treated animals averaging significantly less time over lemon odor than either the NaCl or 6-OHDA lithium-treated groups.

Catecholamine levels in the olfactory cortex and left ventricle of the heart were assayed flourometrically following sacrifice on Day 40-45. Compared to controls, olfactory cortex NE but not DA was reduced by 40-60% in neurotoxin-treated animals, and concentrations in the two groups were statistically similar. In contrast, 6-OHDA decreased ventrical NE levels by 67%, while 6-OHDOPA had no significant effect.

Our data suggest that aversions to odors paired with illness during the first postnatal week persist for two weeks in pups treated neonatally with 6-OHDOPA, but not 6-OHDA or vehicle. The absence of an aversion in 6-OHDA treated animals may indicate that this neurotoxin, by partially denervating the peripheral sympathetic system, reduces the ability of LiCl to induce gastrointestinal illness. These findings imply that brain NE may degrade substrates underlying responses acquired during the early postnatal period.

119.10 RETROGRADE AMNESIA PRODUCED BY ELECTRICAL STIMULATION OF THE AMYGDALA: RELATIONSHIP TO EFFECTS ON CORTICAL NOREPINEPHRINE CONCENTRATION. P. Gold and K. Welsh*. Dept. of Psychology, Univ. of Virginia, Charlottesville, VA 22901.

Under a variety of training-treatment conditions, our laboratory has observed a consistent relationship between transient posttraining changes in brain norepinephrine (NE) concentrations and later retention performance. The extent of these changes is maximal when measured 10 min after training+posttraining treatments that enhance or impair memory storage. Generally, a 15-25% decrease in forebrain NE concentration predicts good retention performance; larger or smaller changes predict poor retention performance. The present experiment used posttraining subseizure electrical stimulation of the amygdala (5 sec delay; 30 µA/side, 100 Hz, 0.1msec pulses for 30 sec) as an amnestic agent and examined changes in cortical NE concentrations 10 min after training and stimulation.

Following bilateral electrode implantation, the animals were trained in a one-trial inhibitory (passive) avoidance task with a 2 mA, 2 sec footshock. Animals were divided into groups for catecholamine assay (decapitated 10 min after training) and groups for behavioral testing 24 hr later.

On the retention tests, animals which received the posttrial amygdala stimulation exhibited retrograde amnesia, confirming the results of several laboratories. NE was measured with a COMT-linked radiometric assay. As observed previously, the training footshock resulted in a significant decrease in cortical NE in unimplanted animals. The major results seen in the implanted animals were: a) Amygdala implantation resulted in decreased cortical NE concentrations. b) Implanted animals exhibited an increase in cortical NE concentrations following the training footshock, as opposed to the decrease observed in unimplanted animals. c) Posttraining amygdala stimulation attenuated the effect of footshock on cortical NE concentrations. Analyses of other brain areas indicate that amygdala stimulation has widespread effects on forebrain NE levels. Thus, well-localized electrical stimulation of the amygdala, and perhaps of other areas as well, may act on memory storage via alterations in transmitter function of disparate brain regions.

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119.11 PROPRANOLOL-INDUCED ATTENUATION OF BOTH MEMORY FACILITATION AND AMNESIA PRODUCED BY FRONTAL CORTEX STIMULATION. Debra B. Sternberg, James L. McGaugh, and Paul E. Gold. Department of Psychobiology, University of California, Irvine, CA 92717; and Department of Psychology, University of Virginia, Charlottesville, VA 22901.

When administered shortly after training, many acute treatments, such as electrical stimulation of the brain and inhibition of protein synthesis, impair later retention performance. However, under certain training conditions, many of these amnestic treatments also can enhance retention performance. Previous studies showed that many adrenergic receptor antagonists attenuate the severity of the retrograde amnesia produced by these same agents (cf. Sternberg and Gold, Behavioral and Neural Biology, 29, 289-302, 1980). If common underlying systems are involved in both enhancement and impairment of memory, then adrenergic antagonists should also attenuate the production of memory facilitation. Thus, the purpose of the present study was to obtain facilitation with the classic amnestic treatment of supraseizure frontal cortex stimulation, and then to assess the effects of an adrenergic blocking agent on both the facilitation and the amnesia produced by this treatment.

Male ARS Sprague-Dawley rats (90 days old) were trained in a one-way active avoidance task. Thirty minutes prior to training, animals received an i.p. injection of either the β-adrenergic receptor antagonist propranolol (0.5 mg/kg) or saline. Animals were then given eight training trials using either high or low footshock. Shortly after training, the rats received sham or frontal cortex stimulation (5.0 mA/1.0 sec) through previously implanted cortical screw electrodes. Twenty-four hours later the animals received eight retraining trials. Retention was measured as the number of avoidances on Day 2 minus the number of avoidances on Day 1. The results indicate that frontal cortex stimulation enhances later retention of training with a low footshock, but impairs the retention of high footshock training. Importantly, pretreatment with propranolol resulted in attenuation of both amnesia and facilitation produced by frontal cortex stimulation.

These findings add further support to the view that there may be common mechanisms, including adrenergic involvement, underlying the memory modulation produced by many treatments.

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119.12 BEYOND THE 3/2-RULE: UNEQUAL LENGTHS AND DIAMETERS AND THEIR EFFECTS ON TRANSIENT VOLTAGES IN NEURONS WITH BRANCHING DENDRITIC TREES. Barry Horwitz, Physics Department, Texas Woman's University, Denton, Texas 76204.

Much evidence has accumulated which implicates changes in dendritic morphology with learning, aging, and such mental pathologies as senile dementia. The extensive branching of most dendritic arborizations, however, imposes formidible obstacles on studies which seek to elucidate the relation between changes in morphology and associated modifications in neuronal electrical activity. The theoretical models which have been the most utilized in studying electrotonic voltages, especially analytical approaches, have generally avoided the complexities of branching. Rall's model, for example, provides a way to reduce an entire dendritic tree to a single equivalent cylinder. Although this model has been of great use, it requires that the 3/2-rule for dendritic branch diameters be obeyed, which seems not to be the case for many neuronal types (e.g., Hillman, 1979).

I previously reported on a theoretical method I developed which enables one to calculate analytical expressions for voltage transients at specific locations in branching dendritic systems in response to synaptic current inputs at other sites in the trees (Neurosci. Abstr. 1979,1980). Exact results were obtained for a number of systems which possessed certain symmetries: all branch lengths had to be integral multiples of one another, and all branch diameters had to be equal. Because the second of these conditions is unduly restrictive, the method has been generalized to treat dendritic trees whose branch diameters differ from one another. The method entails adding onto the results obtained for the symmetric cases a sum of correction terms. Each term is proportional to a factor which depends on the deviation of the diameters from equality, raised to a power n, and a factor which is evaluated by going to the symmetric limit (when all diameters are equal), and is thus the same for all deviations. The correction terms, as well as the symmetric results, are all written as combinations of two closely related families of functions. These functions provide a precise formalism for analyzing systematically the way in which the voltage transients at a given point depend on the geometrical structure of the dendritic tree. Several examples will be presented which will show how the first order (n=1) and the second order (n=2) corrections alter the transient voltage responses.

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120.1 THE ORIGIN OF MONOAMINERGIC PROJECTIONS TO THE SPINAL CORD. STUDIES USING FLUORESCENT TRACERS IN CONJUNCTION WITH THE FALCK-HILLARP METHOD IN THE NORTH AMERICAN OPOSSUM. G.F. Martin, T. Cabana and A.O. Humbertson. Dept. Anat., Sch. Med., The Ohio State Univ., Columbus, Ohio, 43210.

Monoaminergic axons are present in the opossum's spinal cord and after spinal injections of HRP neurons are labelled within brainstem nuclei known to contain monoamines. However, most of the nuclei in question also contain non-monoaminergic neurons making it difficult to know which neuronal type was labelled by the retrograde marker. The purpose of this report is to describe the results of studies which utilized the retrograde transport of fluorescent tracers in conjunction with the Falck-Hillarp method. Injections of either True-Blue or bisbenzimide were made into different levels of the spinal cord in 10 anesthetized opossums. The operated animals were allowed to survive for 1-7 days and prior to sacrifice they were treated with L-tryptophan and Pargyline (Sigma) to enhance indolamine fluorescence. The brain and injected segment of the spinal cord were removed from the deeply anesthetized animal and subjected to a modification of the Falck-Hillarp method. In all cases neurons were labelled by the fluorescent marker in those areas expected from the HRP studies. Such neurons were visualized best at 360nm using a Leitz fluorescence microscope. The fluorescence characteristic of monoamines was seen most easily at 490nm, but it could also be observed at 360nm. Neurons showing the fluorescence of the transported marker as well as catecholamines were found dorsal and medial to the lateral reticular nucleus, adjacent to the superior olivary nucleus, within the dorsolateral and rostrolateral pons as well as within the periventricular nuclei of the hypothalamus. They were most numerous within the dorsolateral pons where they were located within the fascicles of the facial nerve, dorsal and lateral to the motor trigeminal nucleus, within the nucleus coeruleus pars alpha and the adjacent reticular formation as well as within the nucleus locus coeruleus proper. Many of those within the rostrolateral pons were located medial to the ventral nucleus of the lateral lemniscus. Neurons containing the injected marker but not catecholamines were also found in most of the above regions. Neurons containing the transported marker plus indolamines were present in the nuclei raphae pallidus, obscurus and magnus as well as within adjacent areas of the reticular formation. In most areas of the raphe non-indolamine neurons were also found to contain the transported marker.

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120.2 ORIGIN OF DENDRITES EXTENDING INTO CORTICOSPINAL TERMINAL FIELDS. Patricia L. Mensah. Dept. of Anatomy, USC School of Medicine, Los Angeles, CA. 90033.

The completely crossed corticospinal tract (CST) of rat travels in the ventralmost portion of the dorsal funiculus (DF) and terminates in the dorsal horn (DH) by forming axodendritic synapses throughout Rexed laminae I-III, and in medial regions of laminae IV and V (Brown, Jr., L.T., Exp. Brain Res., 13: 432-50, 1971). This study was undertaken to test the hypothesis that CST fibers contact dendrites of neurons located throughout the central gray. Twelve adult rats were sacrificed by anesthetic overdose and perfused with a glutaraldehyde-paraformaldehyde solution. Spinal segments C_6-T_1 were removed and treated according to the Golgi-EM procedure of Fairen et al. (J. Neurocytol., 6:311-37, 1977). Laminae I-III neurons extend from one to several dendrites into CST territory. Medial, lamina IV neurons extend a dendrite into both the CST portion of the DF and into the CST terminal field. Lamina V neurons extend medially-coursing dendrites into CST territory in laminae IV-V; while laterally-coursing dendrites lie outside this area. Laminae VII and VIII neurons extend either obliquely- or vertically-ascending dendrites into the medial portion of lamina V, some of which reach the CST terminal field. The main conclusions to be drawn from this data are that: 1) the dendrites of spinal cord neurons extend into several different terminal fields; 2) dendrites from neurons outside the DH project into the CST terminal field; and 3) as in other mammals, the CST of rat may influence both sensory and motor systems. Subsequent work will focus on the hypothesis that all dendrites projecting into CST territory are synaptically contacted by CST axon terminals.

120.3 PONTOMESENCEPHALIC INDUCED HYPERPOLARIZING POTENTIALS IN LUMBAR MOTONEURONS DURING ACTIVE SLEEP. S.J. Fung, P.A. Boxer*, F.R. Morales* and M.H. Chase. Brain Research Institute and Depts. of Physiology and Anatomy, School of Medicine, Univ. of California, Los Angeles, CA 90024.

The pontomesencephalic reticular formation has been shown to exert state-selective postsynaptic inhibition of brainstem motoneurons that arises only during active sleep[1]. The present study was undertaken to determine whether similar inhibitory effects were also present in spinal cord α-motoneurons. Accordingly, 3 cats were prepared for recording intracellularly from antidromically identified motoneurons under chronic conditions during sleep and wakefulness. Electrodes were implanted to record EEG, EOG and EMG activity and to stimulate the pontomesencephalic reticular formation (A1 to P1, H-2 to -4, L2 to 3). Twenty-one motoneurons (action potentials ranging from 55 to 94 mV) were examined during wakefulness (W), quiet sleep (QS) and active sleep (AS). The response of these cells to stimulation of the reticular formation (2 to 4 pulses, 0.5 msec, 1 to 5 volts) was studied with intracellular glass micropipettes filled with 2M K-citrate. In 18 motoneurons reticular formation stimulation promoted, specifically during AS, a hyperpolarizing potential with an average amplitude of 3 mV (maximum 6.4 mV), a latency to peak of 45 msec, and a duration of 20-65 msec. This response was seen neither in QS nor in W. However, in several cases it appeared in the transition period between QS and AS. Spontaneous discharges of the motoneurons were inhibited during these hyperpolarizing potentials. This postsynaptic potential was observed even though motoneurons were hyperpolarized when the cat entered AS. Reticular stimulation also promoted early synaptic potentials that were not state-dependent.

These results indicate that the pontomesencephalic reticular formation stimulation produces, specifically during AS, inhibition of spinal cord motoneurons. Latency measurements further suggest that the pathway mediating this inhibitory action either is polysynaptic and/or contains fibers with a slow conduction velocity. We have proposed that this pattern of motor inhibition is due to the activation of inhibitory interneurons located in the medulla, which may be selectively driven during AS by pontine cells[1]. Supported by NSF Grants INT-7722299 and BNS-79012897.

[1]Chase, M.H. The motor functions of the reticular formation are multifaceted and state-determined. In: The Reticular Formation Revisited, J.A. Hobson, M.A.B. Brazier, eds. Raven Press: New York, 1980, 449-472.

120.4 IS CONTROLLED LOCOMOTION IN CATS PRODUCED BY ACTIVATION OF CEREBELLO-RETICULAR CONNECTIONS? E. Eidelberg, J. Yu and L.H. Nguyen* Res. Program, V.A. Hospital, and U. Texas Health Sci. Cent., San Antonio, TX 78284

It is known that decorticate or thalamic cats are capable of spontaneous locomotor activity. Such activity can also be driven by localized electrical stimulation of the subthalamic region (SLR), of an area ventral to the inferior colliculi (MLR) or of sites in the lateral pontine tegmentum (PLR). This phenomenon is usually referred to as "controlled locomotion". The SLR and MLR lie close to axons of the superior cerebellar peduncle, a major cerebellofugal pathway. We hypothesized that controlled locomotion might be mediated by indirect activation of the reticulospinal system, via cerebello-reticular fibers or through descending collaterals (Cajal) of the superior cerebellar peduncle. However, controlled locomotion could be elicited from SLR and MLR sites in thalamic cats who had been subjected to destruction of the fastigial nuclei, or total cerebellectomy 1-3 weeks previously. This interval was judged to be sufficient for axon degeneration. These results seem to falsify the hypothesis.

120.5 PROJECTIONS OF INDIVIDUAL MUSCLE SENSORY FIBERS TO HOMONYMOUS AND HETERONYMOUS MOTONEURONS IN THE BULLFROG. Jeff W. Lichtman and Eric Frank. Department of Neurobiology, Harvard Medical School, Boston, Mass. 02115.

The size of synaptic potentials in adult spinal cord motoneurons was measured for various individual stretch-sensitive axons using the method of spike-triggered averaging. Discharges from single sensory axons were elicited by tapping sensitive areas on each of the three heads (internal, external, and medial) of the triceps brachii muscle and observed by recording en passant in the mixed nerve to the muscle. The observed action potential was used as a trigger for averaging while we recorded intracellularly at high gain from motoneurons innervating the same muscle head as the sensory axon (homonymous) or motoneurons innervating the other triceps heads (heteronymous).

The homonymous connections were, on average, larger than the heteronymous ones irrespective of which class of sensory axons was tested. Thus sensory axons innervating the medial head of triceps evoked an average depolarization of 221±15μV (±s.e.m.) in motoneurons that innervated the medial head compared to 89±8μV in motoneurons innervating the internal and external heads. Similarly sensory axons from the internal or external heads gave rise to a 171±12 μV depolarization in homonymous motoneurons and a 63±7 μV depolarization in motoneurons innervating the medial head.

The difference between homonymous and heteronymous synaptic connections was due in part to the fact that individual sensory axons innervated nearly all of the homonymous motoneurons tested whereas they produced measurable responses in only about one half of the heteronymous motoneurons. However, a difference in projection frequency was only part of the explanation since homonymous inputs were, on average, about 80-90 μV larger than heteronymous inputs even when comparing only the measurable responses.

These results show that despite the absence of obvious stretch reflexes in the frog, the monosynaptic projections of individual muscle sensory axons onto motoneurons are strong and specific as in the cat. This specificity requires not only that axons find appropriate postsynaptic partners, but also establish synaptic connections of the appropriate strength with those partners.

120.6 LATERAL COLUMN PROJECTION TO LUMBAR MOTONEURONS IN THE FROG: FUNCTION AND SYNAPTIC PHYSIOLOGY RE-EXAMINED. A.R.Blight. Depts. Neurosurg. and Physiol. and Biophys., New York Univ. Med. Ctr., New York, N.Y. 10016.

The lateral column (LC) of the frog spinal cord has been held to terminate in excitatory synapses on somata or proximal dendrites of most ipsilateral lumbar motoneurons (LMs). On the basis of this widespread and powerful excitatory connection, it has been suggested that the LC mediates supraspinal control or initiation of hindlimb extension in the jump. This interpretation was reinvestigated in frogs (Rana temporaria): a) using selective lesions of the upper thoracic cord (segment 4/5) and b) recording intracellular potentials in LMs in response to thoracic LC stimulation. Recordings were made in situ, the frogs decerebrated and paralysed with tubocurarine or succinylcholine.

Unilateral LC destruction had little effect on locomotion as analysed by videotape, except for the presence of a noticeable delay (100 msec to several sec acutely) in bringing the ipsilateral hindlimb into the normal flexed posture after a jump. Bilateral lesions resulted in a general flexion weakness in the hindlimbs, but extension in the jump was little impaired. This suggests that the essential role of the LC in locomotion is the transmission of information related to flexing the hindlimb into the sitting posture as the frog lands. This may be specific visual, vestibular, tactile or proprioceptive forelimb information or a more general, facilitative excitation of flexors.

Electrophysiological analysis showed the upper thoracic LC projection to LMs to be complex and heterogeneous. Monosynaptic potentials (i.e. with latencies less than 2 msec) were found in both flexor and extensor motoneurons, though they were widely variable in amplitude and rise time and several were hyperpolarizing. Few LMs fired an action potential from these monosynaptic components. Polysynaptic potentials (latencies greater than 3 msec) were found in all cells and generally gave rise to action potentials. In contrast, lower thoracic or upper lumbar stimulation (segment 6/7) resulted in monosynaptic activation of most LMs, probably through stimulation of intrinsic lumbar neurons. It is concluded that the LC pathway does not provide a homogeneous direct descending input to ipsilateral LMs but rather has a selective and heterogeneous termination which remains to be defined more closely.

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120.7 WHAT IS THE ROLE OF THE GOLDFISH MAUTHNER CELL IMPULSE IN THE "TAIL FLIP" REFLEX? John T. Hackett and Donald S. Faber, Div. Neurobiology; Dept. Physiology; SUNYAB, Buffalo, NY 14214.

The teleost Mauthner cell is well suited for initiation of a startle reflex, characterized by a rapid and unilateral tail flip, because of its multimodal inputs and extensive projection to motoneurons in the medulla and spinal cord. In the present study intracellular microelectrodes were employed to analyze the organization of the neural networks underlying the reflex evoked by auditory or optic inputs.

Experiments were performed on goldfish that were maintained by perfusion of water through the mouth. Movements were restricted by supports and flaxedil injection. Fine bipolar electrodes electrically stimulated either the posterior branch of the 8th nerve, the optic tectum, the pretectum, or the spinal cord. The output of the reflex was monitored by recording electromyograms in the mandibular or trunk muscles. Following penetration of a cell, two tests were performed to characterize its function: (1) Does an impulse directly evoked by an intracellular current pulse trigger the entire reflex in an all-or-none fashion? (2) Does orthodromic activation of the cell trigger the reflex, and in turn, can the reflex be blocked when this activation is prevented by hyperpolarizing the axon? Finally, recorded neurons were injected with dyes to provide histological identification.

Whenever the Mauthner axon was activated, its impulse was followed by a fixed latency reflex EMG (about 3 ms), regardless of the mode of stimulation. Mauthner axon hyperpolarization blocked this reflex even when suprathreshold orthodromic stimuli were used which activated other bulbar neurons. Similarly, following injections of strychnine (5 μg/g, i.m.) to increase neuronal excitability, the reflex could still be blocked by hyperpolarizing inactivation of the Mauthner axon spike. In contrast, direct activation of other medullary neurons responsive to the same orthodromic inputs produced a partial reflex. Hyperpolarizing block of their action potentials removed a fraction of the Mauthner mediated reflex.

In conclusion, we provide definitive evidence that a single Mauthner cell impulse can trigger a reflex which is the same as that evoked orthodromically. One excitatory drive to this reflex is from the 8th nerve, though higher threshold afferents were found in the optic pathway. Other neurons, presumably postsynaptic to the Mauthner cell, can relay impulses to motoneurons of the reflex and can be driven independently of the Mauthner cell. These data indicate that the Mauthner cell impulse functions to coordinate cranial and spinal motoneurons for rapid reflex initiation. (Supported by NIH Grant NS15335)

120.8 SPINAL NEURONAL ACTIVITY DURING FICTIVE LOCOMOTION IN THE LAMPREY. K. A. Sigvardt and S. Grillner. Dept. of Physiology III, Karolinska institutet, Lidingövägen 1, S-114 33 Stockholm, Sweden.

Basic vertebrate locomotory patterns are known to be generated by networks of neurons in the spinal cord. An understanding of how these central pattern generators operate requires that we know what neurons in the spinal cord are essential components of the generator as well as the properties of these neurons. The in vitro lamprey spinal cord preparation has been developed to study the neuronal organization of the pattern generator for swimming, one basic type of vertebrate locomotion (Cohen and Wallén, Exp. Brain Res. 41:11, 1980; Poon, J. Comp. Physiol., 136:337, 1980). In the lamprey, fictive locomotion (rhythmic activity recorded in the ventral roots with the same intra- and intersegmental coordination as in swimming) can be initiated by adding excitatory amino acids activating NMDA receptors to the bathing solution (Grillner et al. to be publ.). We have recorded from interneurons and motor neurons in the lamprey spinal cord during fictive swimming using microelectrodes filled with the fluorescent dye Lucifer yellow (Stewart, Cell 14:741, 1978). This procedure reveals both the physiological properties and the anatomy of each neuron. Motor neurons have been recorded from, particularly to elucidate the organization of their presynaptic neurons and to determine the extent of their dendritic arborizations. Interneurons that are active during fictive locomotion have been described that have their activity or membrane oscillations either in phase, in antiphase or in an intermediate phase relationship with the burst of activity recorded in the ventral root of the neuron's hemisegment. The structure of the cells as revealed by Lucifer yellow allowed us to identify these interneurons as local interneurons, intersegmental neurons with ascending and/or descending processes, and whether or not the neuron was limited to one half of the spinal cord or had crossed projections. Tetrodotoxin (TTX, 10^{-6} M) has been added to the bathing solution while recording from the neurons. The membrane potential of most neurons recorded from returns to a flat baseline at the moment the spike activity recorded in the ventral roots is blocked. However, after TTX treatment two neurons whose activity was correlated with the ventral root burst in normal Ringer showed large amplitude (20 mV) oscillations in membrane potential 20 min after ventral root activity has disappeared. The oscillation began with an initial slow depolarization followed by a faster rise to peak. The membrane remained depolarized at about 10-15 mV above rest for approximately 2 s and then returned to baseline to begin the cycle again. Whether or not this membrane oscillation is related to pattern generation is not known.

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120.9 SENSORY ACTIVATION OF THE RHYTHMIC SWIMMING MOTOR ACTIVITY IN IN VITRO PREPARATIONS OF THE LAMPREY. A. D. McClellan and S. Grillner, Dept of Physiology III, Karolinska institutet, S-114 33 Stockholm, Sweden.

Electrical stimulation of single "command neurons" in invertebrates and specific brainstem regions in vertebrates can activate the rhythmic motor activity patterns which underlie a variety of behaviors. In some cases, the significance of these findings may be unclear. For example, it is also critical to record from these presumed "command structures" when the same motor activity is elicited by natural means. In this regard, two lamprey preparations (Ichthyomyzon unicuspis) have been developed to examine the organisation of higher order pathways which activate the swimming motor activity.

Brainstem preparation. This in vitro preparation consists of the anterior region of the head with the exposed brainstem and attached spinal cord/notocord. Mechanical pressure or pinching the skin of the head elicits rhythmic, coordinated activity in spinal ventral roots which is characteristic of a turning response followed by forward swimming. This response has been verified by EMG recordings in intact preparations. The response requires an intact trigeminal nerve and electrical stimulation of this nerve can elicit similar swimming activity as the mechanical stimulus. Electrical micro-stimulation of specific regions in the rhombencephalon, similar to the "pontine locomotor region" in the cat have been found to activate strong, well coordinated swimming motor activity in spinal ventral roots. These regions appear to traverse in pathways approximately 200-300 um lateral to the midline. The relationship between the trigeminal sensory inputs and these "rhombencephalon locomotor regions" can now be investigated.

Tail preparation. This second in vitro preparation consists of the caudal 2-3 cm of the tail with the attached spinal cord/notocord. Mechanical pressure or pinching the skin of the tail activates rhythmic activity in spinal ventral roots which can presumably be regarded as part of a forward swimming escape response. This response has been verified by EMG recordings from intact and spinalized (intact) animals. Dorsal spinal tracts localized at the borders between the medial and lateral thirds of the cord appear to be the main pathways for activating this response. It is possible to bathe the middle region of the cord for about 10-12 segments in low Ca^{++} solutions to locally block synaptic transmission. Under these conditions, pinching the tail still activates albeit weaker swimming motor activity in the ventral roots rostral to the block. Thus, at least part of the activation pathway for this response is direct.

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120.10 NORMAL AND ABNORMAL COUPLING OF CENTRAL PATTERN GENERATORS DURING FICTIVE LOCOMOTION IN THE ATLANTIC STINGRAY (DASYATIS SABINA). M.H. Droge and R.B. Leonard, Mar. Biomed. Inst. and the Dept. of Physiology and Biophysics, The Univ. of TX Med. Br., Galveston, TX 77550

Locomotor rhythms were examined in 10 decerebrated stingrays before and after immobilization with curare (i.v.). Electromyograms (EMG's) were recorded at several segmental levels before paralysis. Neurograms (NG's) were recorded with hook electrodes on elevator and depressor nerves at the same segmental levels following paralysis. Cycle times, intersegmental lag times, EMG and NG burst durations and interrelations among these were used to characterize the rhythms.

Before paralysis, all the animals swam spontaneously with timing relations previously shown to occur in both intact and decerebrated animals. After paralysis, fictive locomotion was observed in 9 of the 10 animals, either spontaneously or in response to mechanical stimulation of the tail. NG's showing rhythmic activity with normal cycle times (0.4-1.2 sec) and normal burst durations (<50% of cycle) were obtained. The relations of cycle time to intersegmental lag and burst duration were also normal in these sequences.

However, sequences with longer cycle times (up to 2 sec) were often recorded. In most of these sequences, and in some with normal times, long burst durations (>50% of cycle) were observed. In this situation, the slopes for cycle time vs. burst durations were higher while those for cycle time vs. intersegmental lag were lower than obtained before paralysis.

More aberrant patterns were also observed. In several animals phasic activity was recorded from different segments without any apparent interrelation between the segments. In all animals short and long bursts were recorded from different segments simultaneously in all possible combinations. In 2 animals the discharge pattern at one segment had higher-order periodicities superimposed on a more normal appearing rhythm. In 3 animals tonic activity was recorded from one segment simultaneously with phasic activity from a more caudal segment which showed nearly normal cycle times and burst durations. In 2 additional animals where only fictive locomotion was examined, NG's from elevator and depressor nerves exhibited rhythmic bursts that were in phase or out of phase at different times.

We conclude that the CNS is capable of generating normal locomotor rhythms in the absence of phasic afferent feedback. The data suggest that multiple spinal oscillators exist for both elevator and depressor muscles. However, the coupling among oscillators becomes labile after paralysis. Supported by grant NS 11255.

120.11 LOCOMOTOR ACTIVITY ELICITED BY SPINAL CORD STIMULATION IN THE STINGRAY, DASYATIS SABINA. C.A. Livingston, B.J. Williams, and R.B. Leonard. Marine Biomedical Institute & Dept. of Physiology & Biophysics, University of Texas Med. Branch, Galveston TX 77550

Earlier reports have indicated that locomotion can be elicited in spinalized animals by stimulation of specific areas of the spinal cord. Effective sites have typically been in the dorsolateral funiculus. However, lesion studies have suggested that portions of the ventral quadrants must be intact in order to elicit locomotion by stimulation of the mesencephalic locomotor region.

Decerebrate stingrays swim spontaneously while stingrays with high spinal transections do not. The effects of spinal cord stimulation were investigated in spinalized stingrays and the activity obtained compared to locomotor rhythms obtained in the same animal prior to spinalization. Each animal was anesthetized, decerebrated at the meso-diencephalic junction, and a series of EMG electrodes inserted in the elevator and depressor muscles of one pectoral fin. The animal was allowed to recover from anesthesia and the EMG activity was recorded during spontaneous restrained swimming. The animal was then reanesthetized and the spinal cord crushed 10-15 segments below the spino-medullary junction. After recovery from anesthesia each animal was examined to be certain it did not locomote spontaneously. The spinal cord was systematically tracked with a stainless steel stimulating microelectrode using 100 µsec. pulses, 60-90 µA in intensity, delivered in long trains between 25 and 70 Hz. Sites from which rhythmic activity was elicited were marked with the Prussian blue technique.

Rhythmic activity in fin muscle can be elicited by stimulation of the contralateral spinal cord in either the dorsolateral or ventrolateral funiculus. The most effective stimulus frequency is about 40 Hz, but the period of the rhythm is affected by the stimulus frequency. The cycle periods during the rhythmic activity evoked by stimulating either location are similar to, but typically of a more restricted range than, those observed in the same animal during decerebrate swimming. Analyses of the relation between the intersegmental lag and the cycle period show that many points during stimulation lie among the points obtained during spontaneous locomotion.

Thus, our evidence indicates that stimulation of the spinal cord at either site can produce rhythmic activity which is similar to that seen in intact or decerebrate stingrays.

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120.12 PROPERTIES OF INTRACELLULARLY RECORDED PECTORAL FIN MOTONEURONS OF THE ATLANTIC STINGRAY, DASYATIS SABINA. Benjamin J. Williams, Michael H. Droge & Robert B. Leonard. Marine Biomedical Inst. & Dept. of Physiol. & Biophys., Univ. of Texas Medical Branch, Galveston, TX 77550

Using intracellular recording techniques, we have obtained data on the cellular properties of motoneurons which innervate the pectoral fin of the stingray. Such information is a prelude to future studies of this motor system and the spinal mechanisms which underlie stingray locomotor behavior.

Decerebration, laminectomy, and exposure of peripheral nerves were performed on anesthetized stingrays. After curarization, animals were artificially respired and allowed to recover from anesthesia. Motoneurons were identified by antidromically stimulating the peripheral nerve component containing only ventral root axons (Coggeshall et al., J. Neurophysiol., 41: 97, 1978).

Stable intracellular recordings from the somata of 30 antidromically activated pectoral fin motoneurons have been made. In 22 impalements, the resting membrane potentials were greater than -60mv. Conduction velocities ranged from 15 to 60 m/s, and these values correspond well with the range of ventral root axonal diameters (Coggeshall et al., ibid). The antidromically activated, overshooting action potentials (APs) typically had an inflection on the rising phase which is comparable to the IS-SD configuration seen in other vertebrate motoneuron recordings. SD spikes can be blocked by hyperpolarization of the soma membrane. In all but 4 cells, delayed depolarizations (D-D) of up to 7 mv in amplitude occur on the repolarizing phase of the AP. Tests have shown that the D-D occurs all-or-none with the full IS-SD spike. Following D-D, a wave of after-hyperpolarization occurs, the duration of which is inversely related to the conduction velocity. By passing up to 8 nA of current through the cell membrane with the intracellular electrode, the input resistance of the soma has been determined. These values range from 0.8 to 4.0 MΩ, and are also inversely proportional to conduction velocity. These properties of stingray motoneurons are quite comparable to those of other vertebrate motoneurons.

Occassionally spontaneous bouts of oscillating membrane potential have been recorded. These oscillations have an amplitude of up to 10 mv and a cycle period of 0.6 to 1.0 s, which is similar to the cycle period of the locomotor rhythm of the intact and decerebrate animal. APs occur on the rising phase and peaks of the oscillations, and vary in number from bursts of 1-2 spikes to 3-5 spikes per cycle. In future experiments, we intend to measure the changes in membrane conductance during such bouts of rhythmic activity. Supported by grants NS 06268 and NS 11255.

121.1 ELECTROPHYSIOLOGICAL RECORDINGS FROM THE NUCLEUS AMBIGUUS OF THE RAT. S. L. Stuesse, S. E. Fish, and K. Stockton*. Neurobiology Program, N.E. Ohio College of Med., Rootstown, OH 44272.

Cardiac efferent innervation regulates myocardial contractile force, automaticity, and conduction times through the heart. Two major areas in the brainstem, the nucleus ambiguus (NA) and dorsal motor nucleus of X, are known to send projections through the vagus to the heart. Recent morphological studies from our laboratory have indicated that the majority of preganglionic parasympathetic fibers which terminate in the myocardium originate from the NA and scattered neurons ventral to it. Very little is known about the possible role these cells play in CNS regulation of cardiac function. Consequently we have made electrophysiological recordings from the medulla of anesthesized rats in an attempt to locate neurons whose activity is correlated with cardiac function.

Extracellular single unit recordings were made in the region of the NA while stimulating the ipsilateral cervical vagus. The locations of recording sites were confirmed by histological reconstruction of electrode tracts. The conduction velocity of the axons of antidromically driven NA cells was in the B fiber range. As yet, we have recorded no spontaneous activity in this portion of the NA. Electrical stimulation of these cells through the recording electrode had no noticable effect on heart rate, so the function of these cells is still to be elucidated. However, the largest concentration of cells projecting to the heart are found in the same area of the NA as the single unit antidromically activated cells. This region of the NA is surrounded by cells whose activity is correlated with respiration. There was a tendency for cells above the NA to fire with bursts of spikes during inspiration while expiratory cells were found below the NA. It has been suggested that respiratory cells are located with the NA. We have found no antidromically driven respiratory cells, however, vagal stimulation occasionally suppressed the activity of respiratory units.

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121.2 A CORRELATION BETWEEN MULTIPLE UNIT ACTIVITY IN THE HYPOTHALAMUS AND ELECTROCARDIOGRAPHIC CHANGES OBSERVED AFTER A SUBARACHNOID HEMORRHAGE. Priti S. Lacy* and A.M. Earle. Dept. of Anatomy, University of Nebraska Medical Center, Omaha, NE 68105.

A decrease in the electrical activity of the cortex (EEG) has been observed associated with an increase in intracranial pressure (Moody et al., J. Neurosurg., 30:482, 1969; Meiner et al., Brain Research, 86:439, 1975; Hubschmann and Kornhauser, J. Neurosurg., 52:456, 1980). Transient bradycardia followed by tachycardia has also been observed following a depression in cortical EEG (Meiner et al., Brain Research, 86:439, 1975) after subarachnoid hemorrhage in female albino rats. It was not known, however, if this suppression in cortical EEG extended to subcortical structures including the hypothalamus.

An experimental subarachnoid hemorrhage was simulated in urethane anesthetized male Sprague-Dawley rats under the cirle of Willis. EKG was monitored and multiple unit activity (MUA) recordings were made from a stereotaxically placed tungsten microelectrode (exposed tip = 20 μ, impedance = 4 megaohms) in the posterior lateral hypothalamus before and after subarachnoid hemorrhage. Suppression in MUA, measured as root mean square (RMS activity was observed to precede bradycardia by 5 to 10 seconds. This suppression in MUA was observed throughout the period that bradycardia persisted. Animals showing a transient suppression (5 seconds or less) in MUA and then returning to normal MUA levels did not exhibit bradycardia. Animals which showed recovery to normal heart rate after developing bradycardia also showed a recovery in amplitude of MUA. Sudden bursts in MUA appeared in some animals during the period when MUA was suppressed and bradycardia was present. These bursts in MUA were followed immediatel by premature atrial contractions. In all animals that developed arrhythmias, a decrease in heart rate preceded the appearance of arrhythmias. These results suggest that: (1) a decrease in heart rate observed in experimental subarachnoid hemorrhage may partially result from a suppression of MUA and (2) sudden bursts in MUA when heart rate is decreased may be related to the appearance of premature atrial contractions.

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121.3 HYPOTHALAMIC NEURONS WITH ACTIVITY RELATED TO SYMPATHETIC NERVE DISCHARGE (SND). Susan M. Barman and Gerard L. Gebber. Dept. of Pharmacol./Toxicol., Michigan State Univ., East Lansing, MI 48824.

Spike-triggered averaging was used to examine the relationships between the spontaneously occurring discharges of single hypothalamic neurons, inferior cardiac SND and cortical activity (EEG). In addition, post-R wave interval analysis was used to determine whether hypothalamic unit discharges were influenced by the baroreceptor reflexes. These analyses allowed us to identify 3 hypothalamic unit types with activity related to SND in 12 baroreceptor-innervated cats. The discharges of SR neurons (n= 11) were temporally related to SND and the R wave of the ECG. The discharges of SER neurons (n=10) were in addition related to EEG activity. The discharges of SE neurons (n=12) were related to SND and EEG activity but not to the R wave. SR and SE neurons could be subdivided into 2 groups depending upon whether their activity was followed by an increase or a decrease in SND. The discharges of SER neurons were always followed by a decrease in SND.

Bilateral section of the carotid sinus, aortic depressor, and vagus nerves (12 cats) was performed to eliminate the relationships in spike-triggered averages arising as a consequence of baroreceptor input shared by "non-sympathetic" hypothalamic units and central networks governing SND. Under these conditions, 10 hypothalamic units with activity related only to SND (S neurons) were found. These neurons are tentatively classified as elements of hypothalamic sympathetic networks and may be counterparts of SR neurons in the baroreceptor-innervated cat. In this regard, S and SR neurons were similarly distributed in anterior, dorsal, lateral and posterior hypothalamic regions.

SE neurons (n=31) were also located in baroreceptor-denervated cats. As was the case for SE units in baroreceptor-innervated cats, the results with spike-triggered averaging suggested that most of these cells received cortical inputs and directed their outputs to sympathetic nerves. However, other SE units (and all SER units in baroreceptor-innervated cats) appeared to affect both SND and cortical activity. Such a unit discharge pattern presumably is indicative of coupling between functionally distinct hypothalamic networks via local mechanisms.

These data support the view that the hypothalamus is involved in setting the level of SND in the anesthetized cat and that at least one (SR unit) of the neuronal types in the hypothalamic sympathetic network is under baroreceptor reflex control. (Supported by PHS Grant HL-13187 and a Michigan Heart Association Grant-in-Aid.)

121.4 CAUDAL RAPHE NEURONS INVOLVED IN GENERATING THE CARDIAC-RELATED RHYTHM IN SYMPATHETIC NERVE DISCHARGE (SND). S.F. Morrison and G.L. Gebber. Dept. of Pharmacol./Toxicol., Michigan State Univ., East Lansing, MI 48824.

The cardiac-related rhythm in SND results from the entrainment of a brain stem oscillator by the baroreceptor reflexes (Gebber, Am. J. Physiol. 239: H143-H155, 1980). Thus, it is possible to alter the phase relations between the pulse-synchronous components of baroreceptor and sympathetic nerve discharges by changing heart rate. This observation presented us with the opportunity of distinguishing neurons which comprise or receive inputs from the brain stem sympathetic oscillator from other unit types which exhibit pulse-synchronous discharges. Spike-triggered averaging and post-R wave interval analysis were used to locate 34 neurons in the caudal raphe nuclei of the cat medulla with discharges synchronized to the cardiac-related slow wave in the inferior cardiac sympathetic nerve. The temporal relations among the point of peak probability of unit discharge (as determined from spike-triggered averages), the onsets of the rising and falling phases of the cardiac-related slow wave of SND and the arterial pulse wave were compared at different heart rates (set between 1.5 and 4 beats/s with ventricular pacing) in the same experiment. The interval between the point of peak probability of discharge of 20 raphe units and the onset of the rising phase of the cardiac-related SND was not changed during increases or decreases in heart rate which led to marked alterations in the phase relations between SND and the arterial pulse wave. The interval between the discharges of 10 additional units and the onset of the falling phase of cardiac-related SND remained constant when heart rate was increased or decreased. These unit types presumbly were contained within or received inputs from those components of the brain stem oscillator responsible for the rising and falling phases of cardiac-related SND.

The point of peak probability of discharge for the remaining 4 raphe units changed with regard to the onsets of both the rising and falling phases of cardiac-related SND when heart rate was increased or decreased. In contrast, the phase relations between the discharges of these units and the arterial pulse wave remained constant during ventricular pacing. Peak probability of discharge of 2 of these neurons occurred 70 ms after the R wave of the ECG. This observation suggests that these units were interneurons in the afferent limb of the baroreceptor reflex arc. The other 2 neurons exhibited a period of reduced activity time-locked to the pulse-synchronous component of baroreceptor nerve discharge. (Supported by PHS Grants HL-13187 and NS-06693.)

121.5 SYMPATHETIC NERVE DISCHARGE (SND) IN CHRONIC SPINAL CATS. J.L. Ardell*, S.M. Barman and G.L. Gebber. Dept. of Pharmacology and Toxicology, Michigan State University, East Lansing, MI 48824.

Recordings were made from the central ends of the sectioned external carotid (ECN) and renal (RN) postganglionic sympathetic nerves one month after transection of the spinal cord at the sixth cervical segment (4 chloralose-anesthetized cats). In contrast to the asynchronous character of SND in acute spinal cats under normocapnic conditions (McCall and Gebber, Brain Res. 89: 139-143, 1975), SND in chronic spinal preparations exhibited synchronous slow wave activity in the 2-6 c/s range. This rhythmic activity pattern could be synchronized to single shocks applied at a rate of 1/s to the afferent sciatic nerve. No evidence was obtained for a baroreceptor-like reflex mechanism acting to control SND in chronic spinal cats. In this regard, there was no locking of 2-6 c/s SND to the cardiac cycle either at control or elevated levels of blood pressure (as determined with post-R wave interval analysis). Furthermore, there was no consistent change in the amplitude of 2-6 c/s SND during the pressor response produced by intravenous norepinephrine.

Crosscorrelation analysis demonstrated locking between 2-6 c/s activity recorded from different postganglionic nerves (left ECN → right ECN) whose preganglionic inputs arise from the same spinal segments. This observation reveals the existence of intraspinal pathways which function to synchronize the discharges of separate groups of preganglionic sympathetic neurons. However, crosscorrelation analysis failed to reveal a relationship between 2-6 c/s activity recorded from postganglionic nerves (ECN → RN) whose preganglionic inputs arise from different spinal segments.

The phase relations between the R wave-related component of SND and the arterial pulse wave in animals with an intact neuraxis can be shifted by changing heart rate (Gebber, Am. J. Physiol. 230: 263-270, 1976). Such shifts reflect the ability of the baroreceptor reflexes to entrain those central oscillators responsible for 2-6 c/s SND. In 3 acute spinal cats (C_1 transected), we found 8 brain stem raphe neurons whose R wave-related discharges shifted in time with respect to the arterial pulse wave when heart rate was slowed with ventricular pacing. On the basis of criteria developed by Morrison and Gebber (this volume), it can be assumed that these neurons were contained in sympathetic networks. Thus, we conclude that 2-6 c/s SND can be generated either in brain stem or spinal circuits which can act independently of each other. (Supported by PHS Grant HL-13187.)

121.6 CORRELATIONS OF SINGLE-NEURON ACTIVITIES IN THE GIGANTOCELLULAR RETICULAR NUCLEUS WITH CARDIOVASCULAR EVENTS IN THE CAT. Julie Y. Hwa and Samuel H.H. Chan. Dept. of Life Sciences, Indiana State University, Terre Haute, IN 47809.

Previous studies from our laboratory suggested that the gigantocellular reticular nucleus (GRN) in the medulla oblongata of the cat is intimately involved in the regulation of arterial blood pressure (ABP) and heart rate (HR). Electrical activation of the GRN elicits significant vasodepression and bradycardia. Furthermore, the antihypertensive agent clonidine may utilize the GRN to promote hypotension and cardioinhibition. The present study was carried out to further delineate the role of the GRN in cardiovascular controls, using electrophysiologic techniques.

Experiments were performed on adult cats that were anesthetized with α-chloralose and urethane (40 and 350 mg/kg, i.p.). Extracellular single-neuron activities were recorded from bilateral GRN (6-9 mm anterior to the obex, 1.5-2.5 mm lateral to the midline, 2-4 mm from the ventral surface) using tungsten microelectrodes and conventional electronic devices. They were monitored simultaneously with ABP and HR.

Evaluation of 611 spontaneously discharging GRN neurons revealed that they exhibited a wide spectrum of spike frequencies (0.5-100 Hz), although 60% of them had a discharge rate of 0.5-20 Hz. Furthermore, most GRN neurons recorded displayed an irregular discharge pattern, and almost all of them did not manifest a time-locked correlation with baseline ABP and HR.

Administration of phenylephrine (5 μg/kg, i.v.) elicited a transient hypertension, followed by a reflex bradycardia. Of the 60 GRN neurons studied, 40 responded to this cardiovascular perturbation with a drastic decrease in the spike frequency, while 14 displayed an increase in the discharge rate, and 6 did not show any change in their activities.

Intravenous injection of clonidine (10 μg/kg) resulted in an immediate bradycardia, and a transient hypertension followed by a prolonged hypotension. Interestingly, the GRN neurons also exhibited an initial brief decline in discharge rate and a subsequent long-lasting heightened activity that paralleled the time courses of the cardiovascular changes. However, these events were only manifested by those reticular neurons that responded to phenylephrine-induced hypertension and reflex bradycardia with a reduction in spike discharges.

These results substantiated our previous postulation that the GRN may be a modulator of the baroreceptor reflexes. Furthermore, the GRN may be an active site for clonidine to exert its antihypertensive effects.

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121.7 SINGLE UNITS IN THE AMYGDALA OF THE CAT RESPONDING TO STIMULATION OF BUFFER NERVES. D. Cechetto* and F.R. Calaresu. Dept. of Physiology, Univ. of Western Ontario, London, Canada. N6A 5C1.

Stimulation of the amygdala is known to elicit cardiovascular changes and to influence the baroreceptor reflex. Anatomical evidence showing a projection from the nucleus tractus solitarius (a known site of termination of cardiovascular afferents) to the amygdala suggests that buffer nerves may alter the activity in neurons in the amygdala. This possibility was tested in 11 cats anesthetized with chloralose. Electrical activity of spontaneously active units in histologically verified sites in the amygdala was monitored for changes in firing frequency during electrical stimulation of the carotid sinus (CSN) and aortic depressor (ADN) nerves. Stimulation of the ipsilateral CSN altered the firing frequency of 35% (49/139) of the units in the amygdala. Of these units 59% (29/49) were inhibited with a mean latency of 65±4.3 ms and the remaining units were excited with a delay of 38±5.2. Units excited were found mainly in the lateral amygdala and the medial area of the basal amygdala. Units inhibited were found mainly in the lateral amygdala with a few scattered throughout the basal and central amygdala. Stimulation of the ipsilateral ADN elicited a change in firing frequency of 18% (29/157) of the units. Of these 72% (21/29) were excited with a mean latency of 19±2.2 ms while 8 units were inhibited with a delay of 49±2.2 ms. Responsive units which showed an excitatory response were located in the lateral and central amygdala and the lateral portions of the basal amygdala. The few units which were inhibited were not localized to specific regions within the amygdala. To study the possibility of convergence of inputs from the CSN and the ADN on the same unit 104 units were tested for a response to both CSN and ADN stimulation. Of the 44 responsive units 32 responded only to stimulation of the CSN, 5 responded only to stimulation of the ADN and the remaining 7 responded to stimulation of both nerves. These results show that the CSN inhibits a slightly larger proportion of units than it excites whereas stimulation of the ADN predominantly excites units in the amygdala. In addition, it has been shown that a small proportion (7%) of units tested for responsiveness to ADN and CSN stimulation responded to both buffer nerves. These demonstrated differences in responses of single units in the amygdala to stimulation of the two buffer nerves may have functional implications in accounting for the role of this limbic structure in the control of the cardiovascular system.

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121.8 CENTRAL SEROTONERGIC NEURONS FACILITATE SYMPATHETIC NERVOUS DISCHARGE (SND). Robert B. McCall and Stephen J. Humphrey.* The Upjohn Company, Kalamazoo, Michigan 49001.

The presence of serotonergic neurons in vasoactive areas of the brain stem and spinal cord suggests that serotonin (5-HT) has a role in the central regulation of blood pressure. The nature of the interaction between serotonergic neurons and central sympathetic pathways has not been determined. For example, centrally administered 5-HT has been shown to increase or decrease mean arterial blood pressure (MAP) and SND. The present investigation was designed to determine the effects of 5-HT on central sympathetic outflow by studying the actions of 5-HT agonists and antagonists on MAP, heart rate (HR) and SND recorded from the external carotid and splanchnic nerves of anesthetized, baroreceptor denervated cats. Intravenous administration of the 5-HT antagonists methysergide (UML, .05-1.6 mg/kg), metergoline (Met, .01-.32 mg/kg), cyproheptadine (Cyp, .05-2.4 mg/kg), and cinanserin (Cin, .20-6.4 mg/kg) was associated with a prolonged, dose-related inhibition of SND. SND was significantly reduced within 5 minutes of the administration of these agents and was maximally inhibited by the largest doses of UML (-83%), Met (-82%), Cyp (-77%), and Cin (-65%). MAP and HR were also decreased in a dose-related manner by UML (-41 mmHg/-74 bpm), Cyp (-33 mmHg/-38 bpm) and Cin (-37 mmHg/-40 bpm). Met failed to alter MAP and HR. Administration of vehicle failed to alter MAP, HR or SND. The effects of UML, Met, and Cyp were also tested in cats depleted of 5-HT by prior administration of the 5-HT synthesis inhibitor p-chlorophenylalanine (PCPA). The 5-HT antagonists failed to reduce SND in PCPA pretreated cats. Clonidine significantly inhibited SND (-73%) in these cats. These data suggest that 5-HT antagonists reduce SND via their ability to block 5-HT receptors. Finally, the effects of the selective presynaptic 5-HT agonists lisuride (Lis) and 5-methoxydimethyltryptamine (5-MeODMT) on MAP, SND, and HR were tested. At low doses, these compounds have been shown to inhibit 5-HT cell firing while having little effect on postsynaptic 5-HT receptors. 5-MeODMT (10-50 μg/kg, i.v.) and Lis (5-10 μg/kg, i.v.) markedly reduced MAP, HR, and SND. The onset of the depressor effects were rapid, while the duration of the effect was transient (3-5 minutes) with 5-MeODMT and prolonged (>1 hour) with Lis. Thus, the time course of the depressor effects of 5-MeODMT and Lis correlate well with their ability to inhibit 5-HT cell firing. This study indicates that agents which act presynaptically to inhibit 5-HT cell firing and agents which act postsynaptically to antagonize the effect of synaptically released 5-HT both mediate a central reduction in SND. Thus, these data suggest that central 5-HT neurons normally facilitate transmission in central sympathetic pathways.

121.9 RENAL AND ADRENAL NERVE ACTIVITY IN SPONTANEOUSLY HYPERTENSIVE RATS AFTER SPINAL TRANSECTION. L.P. Schramm, E.S. Chornoboy*, O.R. Simon* and K.E. McKenna* The Johns Hopkins University School of Medicine, Dept. Biomed. Engr., Baltimore, MD 21205

Spontaneously hypertensive rats (SHR's) exhibit both elevated spontaneous sympathetic activity and sympathetic hyperexcitability. The role of spinal and/or ganglionic systems in generating this hyperactivity has not been determined directly. SHR's and normotensive Wistar-Kyoto rats (WKY's), 12 to 14 weeks old, were anesthetized with alpha-chloralose, paralyzed and artificially respired. Rectified and integrated multiunit activity was measured on renal and adrenal nerves. Adrenal nerve activity was monitored after ganglionic blockade with hexamethonium and atropine. We transected spinal cords at C1 and electrically activated descending sympathoexcitatory and sympathoinhibitory pathways lying in the dorsolateral and lateral funiculi. Stimulus-response curves for both sympathoexcitation and sympathoinhibition were generated using stimulus frequencies of 5,10, 20,50, and 100 Hz. Spontaneous renal nerve activity was elevated in SHR's, and this difference persisted during both sympathoexcitatory and sympathoinhibitory stimulation. Spontaneous adrenal nerve activity was not significantly elevated in SHR's. However, sympathoexcitatory stimulation elicited larger responses in adrenal nerves of SHR's. Sympathoinhibitory stimulation was only minimally effective in reducing adrenal nerve activity in both SHR's and WKY's. To test the hypothesis that inhibition of adrenal activity was marginal because spontaneous levels of adrenal activity were low, we increased baseline adrenal nerve activity by spinal sympathoexcitatory stimulation. Superimposed sympathoinhibitory stimulation still elicited only small decreases in adrenal nerve activity.

We conclude that a component of the elevated renal nerve activity exhibited by SHR's may be generated by spinal and/or ganglionic systems. The hyperreactivity of preganglionic adrenal nerve responses in SHR's was of spinal origin. The mechanism responsible for the differential sensitivity to sympathoinhibitory stimulation of renal and adrenal activity remains to be determined. However, the relative weakness of sympathoinhibition in adrenal nerves was not simply a function of lower baseline activity.

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121.10 Differential patterns of sympathetic outflow initiated by stimulation of visceral afferent neurones. R. Meckler#, H. Fry# and L. Weaver. Dept. of Physiol., Mich. State Univ., E. Lansing, MI

Various inputs to the central nervous system cause fractionated, differential response patterns in different components of sympathetic outflow. The patterns of sympathetic responses to stimulation of cardiac vagal and thoracic and abdominal visceral spinal afferent neurones are not well known. Therefore, this study was undertaken to compare sympathetic reflex patterns caused by stimulation of receptors within the heart, upper abdominal viscera and arterial components of the vasculature. Experiments were performed in chloralose-anesthetized cats in which arterial baroreceptors had been denervated and upper thoracic (T1-T6) sympathetic chains had been removed. Activity of splenic, renal and cardiac sympathetic efferent nerves and systemic arterial pressure were recorded. Discharge of at least two nerves was recorded simultaneously. Occlusion of the celiac or superior mesenteric artery stimulated lower thoracic spinal afferent neurones to elicit greater excitation of splenic than renal or cardiac nerves. These reflexes were easily demonstrated after C1 spinal transection. Stimulation of cardiac vagal afferent neurones by intra-atrial administration of veratridine caused greater inhibition of renal than splenic sympathetic outflow. Occlusion of the descending thoracic aorta causes mechanical activation of cardiac receptors and ischemia-induced excitation of receptors in the abdominal viscera. Such occlusion caused biphasic (inhibition-excitation) responses in renal nerves and excitatory responses in splenic nerves. After cervical vagotomy, aortic occlusion caused only greater excitation in both nerves. Stimulation of cardiac vagal and peri-arterial receptors by intra-atrial injections of bradykinin also caused biphasic (inhibition-excitation) renal nerve responses and only excitatory splenic nerve responses. Again, inhibition was eliminated and excitation was enhanced by vagotomy. Stimulating peri-arterial receptors with intra-arterial injections of bradykinin caused greater excitation of splenic than renal nerves. These findings illustrate that: 1) abdominal visceral ischemia or chemical stimulation of peri-arterial receptors causes excitation of sympathetic outflow which is more pronounced in splenic than renal or cardiac nerves, 2) inhibitory influences of the cardiac vagal afferent neurons are more pronounced in renal than splenic nerves, 3) summation of these excitatory and inhibitory inputs generally leads to greater excitation of splenic nerve activity or greater inhibition of renal nerve activity. It is concluded that renal and splenic components of splanchnic sympathetic outflow receive different patterns of synaptic inputs from visceral afferent neurones. Support: National HLBI grant HL21436.

121.11 Contribution of spinal pathways to reflex influences of cardiac sympathetic afferent neurones on sympathetic outflow. H. Fry#, R. Meckler#, and L. Weaver (SPON: J. Krier). Dept. of Physiol., Mich. State Univ., E. Lansing, MI. 48824.

The extent to which neural circuits confined to the spinal cord are adequate to mediate excitatory sympatho-sympathetic reflexes of cardiac origin is not well understood. Although cardio-cardiac sympathetic reflexes are well documented in spinalized cats, the autonomy of spinal networks in the excitation of lower thoracic sympathetic outflow or in the presence of an intact neuraxis remains ambiguous. Further resolution of this question may contribute to the understanding of the organization of other spinal visceral afferent influences on the sympathetic nervous system. Experiments were conducted in anesthetized, vagotomized, sino-aortic denervated cats. Cardiac sympathetic afferent neurones were excited by epicardial superfusions of bradykinin and responses of cardiac, renal, and splenic sympathetic nerves were recorded simultaneously. Response patterns of these sympathetic nerves were compared prior to and 1, 3, and 6 hours following C1 spinal cord transection. Prior to transection, cardiac sympathetic afferent stimulation caused greater excitation of cardiac than renal activity and greater excitation of splenic than cardiac or renal activity. The same patterns were observed at all times tested after transection. The magnitude of cardiac and splenic excitatory responses often was not diminished immediately following transection and generally all responses increased with time. A second group of experiments were performed to determine if medullary neurones which could be excited by electrical stimulation of cardiac sympathetic afferent nerves similarly were excited by chemical stimulation of these nerves. These experiments were considered preliminary investigations of potential brainstem components of the sympatho-sympathetic reflex. Activity of medullary neurones was recorded with multibarreled micropipettes and enhanced, when necessary, using microiontophoretic techniques. Stimulation of cardiac receptors by bradykinin excited few of the neurones which had responded to electrical afferent stimulation. Only spontaneously active neurones responded to both electrical and chemical stimulation and these were located throughout the reticular formation near the level of the obex. Neurones which could be correlated with sympathetic activity were not excited by chemical afferent stimulation. The observation that sympathetic reflexes of cardiac origin were similar in magnitude and pattern before and after spinal transection suggests that these reflexes are largely complete within the spinal cord. The failure of medullary neurones to respond to the chemical afferent stimulation which excites sympathetic outflow also is consistent with this contention. Support: HL21436, Mich Heart Assoc

122.1 STUDIES ON THE INTERACTIONS BETWEEN SEROTONIN AND SEROTONIN BINDING PROTEIN. H. Tamir and Kuo-peing Liu,* New York State Psychiatric Institute, Division of Neuroscience and Columbia University, Department of Psychiatry, New York, New York 10032.

Serotonergic neurons of both brain and gut contain a specific serotonin binding protein that is probably a component of the amine storage mechanism. We wish to report now on the nature of the interaction between the protein and serotonin. Rat brain serotonin binding protein (SBP) was found to have essential -S-S and -SH groups. Both reduction of the disulfide bond by dithiothreitol or mercaptoethanol and modification of -SH group(s) by Ellman reagent or alkylating agents, caused loss of binding capacity. In contrast, formation of mixed disulfide bond with sodium metabisulfite did not affect the binding capacity. Serotonin, in the presence of Fe^{+2} and phosphate was found to bind to either an -SH group or to a site in very close proximity. Since addition of serotonin protected -SH groups from modification by Ellman reagent and from denaturation of protein upon storage. Lipids that enhance binding of serotonin to SBP also protected -SH groups from modification. Nucleotides were found to be strong inhibitors of the binding of serotonin to SBP. The inhibitory effect of nucleotides was due to their chelating properties and not to phosphorylation of or binding to the protein. Inhibition by nucleotides and other chelators was reversible. Binding capacity being fully restored after removal of the chelator by molecular sieve chromatography and addition of Fe^{+2}. A partial reversal of the inhibition by nucleotides was also achieved when the concentration of phosphate ions, required for binding, was elevated. The ionic environment had a marked effect on the binding: intracellular ions such as K^+ were found to enhance the binding, and extracellular ions such as Na^+ and Ca^+ inhibited the binding. Consistent with our data is formation of a complex of SBP-S-Fe-S that in hydrophobic surrounding could bind up to four molecules of serotonin in coordination bond with Fe^{+2}. Extracellular ionic conditions which favor the dissociation of the complex would free the amine to interact with its receptor or the presynaptic reuptake carrier. These results and previous studies strongly indicate that SBP is an intracellular protein that acts as a storage protein. The binding of the amine to the protein may serve to reduce the osmotic pressure within the vesicles.

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122.2 SYNAPTOSOMES CONTAIN TWO NONMITOCHONDRIAL ATP-DEPENDENT CALCIUM TRANSPORT ACTIVITIES DIFFERING IN THEIR REGULATION BY CALMODULIN. Diane M. Papazian,* Hannah Rahamimoff,* and Stanley M. Goldin (SPON: Shew Y. Chan) Dept. of Pharmacology, Harvard Medical School, Boston, MA 02115.

Synaptosomes contain two nonmitochondrial ATP-dependent Ca^{++} transport activities. These activities can be separated by fractionation of lysed synaptosomes. The synaptic plasma membrane fraction contains an ATPase activity stimulated by Ca^{++} in the presence of millimolar levels of free Mg^{++}. The Ca^{++}-stimulated ATPase is activated several-fold by calmodulin after removal of endogenous calmodulin; this observation confirms earlier reports (Sobue, K., et al., FEBS Let. 99:199-202, 1979; Sorensen, R., and Mahler, H.R., Soc. Neurosci. Abstr. 5:309,1979). The Ca^{++}-stimulated ATPase catalyzes ATP-dependent Ca^{++} transport after reconstitution into artificial phospholipid vesicles.

The synaptosomal vesicle fraction, obtained by high speed centrifugation of synaptosomal lysates, catalyzes ATP-dependent Ca^{++} uptake into native and reconstituted vesicles. The synaptosomal vesicle Ca^{++} transport activity is not stimulated by calmodulin even after removal of greater than 95% of the calmodulin endogenous to the preparation. Furthermore, the ATPase activity associated with synaptosomal vesicles, measured in the presence of millimolar levels of free Mg^{++}, is not Ca^{++} or calmodulin stimulated. Therefore, the synaptosomal vesicle Ca^{++} transport activity is distinct from the Ca^{++} stimulated, calmodulin sensitive ATPase found in synaptic plasma membranes. Since these two Ca^{++}-transport activities differ in their sensitivity to calmodulin, and in their distribution in fractions of synaptosomal lysates, they may represent two Ca^{++} pumps distinct in their regulation, location, and physiological roles within nerve terminals.

122.3 NOREPINEPHRINE NEURONAL UPTAKE SITES LABELED WITH [^{3}H] DESIPRAMINE. C.M. Lee and S.H. Snyder. Johns Hopkins University, Sch. of Med., Dept. of Neuroscience, Pharmacology and Psychiatry, Baltimore, MD 21205.

Biochemical characterization of the neuronal norepinephrine (NE) uptake processes has long been based on the measurement of the accumulation of the amine into nerve terminals. However, these analyses do not permit the distinction between interactions with the ligand recognition site from that with the translocation mechanism. It is generally known that tricyclic antidepressants of the tertiary amine type like chlorimipramine and imipramine are potent inhibitors of neuronal 5-hydroxytryptamine (5HT) uptake while those of the secondary amine type like desipramine (DMI) preferentially inhibited NE uptake. Recent evidence suggested that [^{3}H] imipramine labeled neuronal 5HT uptake recognition site (Langer, S.Z. and Briley, M., Trends in Neurosciences, 4: 28-31, 1981). We now report that the high affinity [^{3}H]DMI binding in rat brain membranes may be associated with the NE neuronal uptake site. The evidence to support this notion include drug specificity, sodium dependency and selective decrease in [^{3}H]DMI binding by 6-hydroxydopamine (6OHDA) lesion.

The absolute molar potencies of a series of antidepressant drugs in inhibiting the [^{3}H]DMI binding to cerebral cortex membranes correlated quite well with their potencies in inhibiting the cortical uptake of [^{3}H]NE, with DMI more potent than imipramine and chlorimipramine which in turn are much more potent than iprindole. Similar to the situation in neuronal NE uptake, [^{3}H]DMI binding is dependent on the presence of sodium ions. A 5-fold stimulation of [^{3}H]DMI binding was observed at 150 mM and a maximal 7-fold stimulation was observed at 300 mM sodium chloride. This effect was specific for sodium as similar concentrations of potassium, lithium and choline chloride gave little or no stimulation on [^{3}H]DMI binding. Finally, in 6 OHDA lesioned animals the loss of NE nerve terminals was accompanied by a parallel decrease in the high affinity [^{3}H]DMI binding but not that of [^{3}H]imipramine binding.

122.4 DISTRIBUTION OF γ-AMINOBUTYRIC ACID (GABA) TRANSPORT SITES IN EMBRYONIC CHICK SPINAL CORD NEURONS IN CELL CULTURE. L. A. Borden*, C. M. Czajkowski* and D. H. Farb* (SPON: J. S. Jakway). Dept. of Anatomy & Cell Biology, SUNY Downstate Med. Ctr., Brooklyn, NY 11203.

GABA is thought to be an inhibitory neurotransmitter in the CNS. Evidence suggests a similar role for GABA in 7 day embryonic chick spinal cord neurons maintained in cell culture (Choi, Farb and Fischbach, Nature 269:342, 1977). ^{3}H-GABA is accumulated by $\simeq$ 20% of the neurons in culture by a specific, high-affinity (Km = 4μM) energy dependent process (Farb, Berg, and Fischbach, J. Cell Biol. 80:651, 1979). A possible function of this system is the rapid removal of endogenously released GABA from the synaptic cleft. After 10 days in culture, accumulation of ^{3}H-GABA and the number of spontaneously occurring GABA-like IPSP's both decrease similarly with age in culture (Farb, Choi, Fischbach, unpublished). This suggests that neurons that accumulate ^{3}H-GABA are those that release GABA from synaptic terminals. Labeled neurons were identified by autoradiography and were found to be completely labeled (cell body and processes). Either ^{3}H-GABA is retrogradely transported to the cell body, or transport sites are located on all parts of the cell. To block axonal transport, cells were preincubated for 45 min. with either colchicine (10μM), vinblastine (0.5μM) or cytochalasin B (5μg/ml), and ^{3}H-GABA (final conc. 30nM) was then added. After 30 min., cells were washed, fixed, and processed for autoradiography. Label was observed over both cell bodies and processes. Assuming the maximal known rate of axonal transport (400 mm/day) substances can move $\simeq$ 25μm in 5 seconds. Cultures were incubated for 5 seconds with 250nM ^{3}H-GABA, followed by a brief wash (<5sec), and rapid fixation. Neurons with processes greater than 100μ were identified and were found to be uniformly labeled. In order to directly determine of transport sites are present on cell bodies, 4.5μM ^{3}H-GABA was applied locally to individual perikarya. Focal application was accomplished by applying brief ($\simeq$1 sec) pulses of ^{3}H-GABA by pressure ejection from micropipettes (tip diameter $\simeq$ 10μ). 15 neuronal cell bodies but not their processes were found to be labeled. Thus, GABA uptake sites are located on both processes and cell bodies of spinal cord neurons in cell culture.

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122.5 EFFECT OF PROTONS AND BICARBONATE ON STIMULATED ACETYLCHOLINE UPTAKE BY TORPEDO SYNAPTIC VESICLES. D. C. Anderson‡ Steven C. King‡ and Stanley M. Parsons. Department of Chemistry, University of California, Santa Barbara, CA 93106

In order to better define the energetics of acetylcholine (ACh) uptake into synaptic vesicles isolated from Torpedo californica we have investigated the effects of a number of mitochondrial uncouplers on both MgATP stimulated [^{3}H] ACh uptake and on the activity of the vesicle-associated ATPase. Low concentrations of nigericin, FCCP, S-13 and A23187 completely inhibit stimulated [^{3}H] ACh uptake. FCCP and S-13 also stimulate the ATPase activity by approximately 50%. These effects of uncouplers suggest that a proton gradient generated by the ATPase is coupled to concentrative uptake. Valinomycin inhibits [^{3}H] ACh uptake and also stimulates the ATPase. At low concentrations, however, valinomycin slightly stimulates [^{3}H] ACh uptake, suggesting that it may have a complex mode of action.

We also have extended earlier investigations (Parsons and Koenigsberger, Proc. Natl. Acad. Sci. USA 77, 6234, 1980) of the effect of bicarbonate on ACh uptake by looking at the pH-dependence of both the bicarbonate stimulation of [^{3}H] ACh uptake in the presence of MgATP and the specificity of active uptake with respect to [^{14}C] choline. MgATP-stimulated uptake in the absence of bicarbonate increases greatly with increasing pH from 6.6 to 7.8, where it reaches a plateau. This behavior is most consistent with inward directed proton pumping by the ATPase. Bicarbonate (40 mM) increases MgATP-stimulated uptake at pH values at or below 7.6 but has no effect at pH values greater than 7.8. Similarly, bicarbonate enhances the uptake of [^{3}H] ACh relative to [^{14}C] choline at pH values at or below 7.6, but it has no effect above pH 7.8. Corresponding direct bicarbonate stimulation of vesicle-associated ATPase activity occurs (Rothlein and Parsons, Biochem. Biophys. Res. Comm. 95, 1869, 1980). Thus, bicarbonate is not obligatory to active ACh uptake but it stimulates ACh uptake at lower pH values by stimulating the ATPase activity.

122.6 EFFECTS OF XYLAMINE ON THE RELEASE OF NOREPINEPHRINE FROM ORGAN-CULTURED RAT SUPERIOR CERVICAL GANGLIA. J.B. Fischer and A.K. Cho*. Brain Research Institute and Department of Pharmacology, UCLA School of Medicine, Los Angeles, CA 90024.

Xylamine (XYL; N-2'-chloroethyl-N-ethyl-2-methylbenzylamine) is a selective irreversible inhibitor of norepinephrine (NE) uptake which has an IC_{50} of about 0.1 μM in all in vitro preparations tested. After a single IP injection, XYL causes an inhibition of NE uptake in cerebrocortical synaptosomes that shows no recovery between 4 hr and 10 days, while striatal dopamine (DA) uptake is not affected. The same in vivo treatment causes a long lasting depletion of NE levels in the cortex and hypothalamus but no depletion of DA levels in the striatum. We have used organ-cultured rat superior cervical ganglia to examine XYL's depleting actions, a preparation that has been shown to synthesize, take up, and store NE, and to release this transmitter via a Ca++-dependent mechanism to appropriate stimuli (Vogel et al., European J. Pharmacol. 20 (1972) 308).

SCG cultured for two days (Fischer & Cho, Neuroscience Abstr. 6 #22.5, 1980) were incubated with 0.2 μM (^{3}H)NE (1 Ci/mmol) for 30 min in Krebs-Ringer bicarbonate (KRBS), then incubated with or without 10 uM XYL for 30 min, followed by two 15 min wash incubations to remove unbound XYL. The SCG were then incubated for 30 min in either normal KRBS, KRBS with 10 μM tyramine (TY), KRBS with 20 μM amphetamine (AM), or modified KRBS with 50 mM K+ and reduced Na+. Radioactivity was measured in samples from each of the incubation and wash baths. During exposure to XYL, no additional (^{3}H)NE was lost compared to control SCG. XYL caused a small increase in the efflux of (^{3}H)NE from the SCG after the wash incubations, up to 27% greater than control values. TY increase the efflux of (^{3}H)NE to 222% of control values; this increase was reduced to 49% by prior XYL exposure. AM caused a 114% increase in efflux which was reduced to 61% by XYL. 50 mM K+ increased (^{3}H)NE efflux by 120%, and prior XYL exposure increased this efflux slightly to 174%.

The actions of XYL on NE efflux are consistent with its action as an irreversible NE uptake inhibitor. TY- and AM-caused efflux probably occur via the uptake carrier, and are therefore inhibited by XYL. K+-induced release is thought to occur via exocytosis, and XYL should prevent the reuptake of the released NE, thereby increasing the (^{3}H)NE in the medium. The slight increase in efflux caused by XYL after the wash incubations but not during XYL exposure suggests a slowly developing action of XYL to cause an efflux of NE. Such an action could be involved in the in vivo depletion by XYL of NE in the brain.

123.1 AGE-EFFECTS ON FACILITATORY PROCESSES AT A SYNAPSE BETWEEN CENTRAL MOTOR NEURON L_7 AND GILL MUSCULATURE IN APLYSIA. B. Peretz and G. L. Ringham. Dept. of Physiology and Biophysics and College of Pharmacy, U. of Kentucky Med. Ctr., Lexington, KY 40536.

We have described previously an age-related change in the motor neuronal function of L_7 in the parietovisceral ganglion (PVG) of Aplysia (Peretz et al, 1980). Specifically, contraction of the antiflaring gill musculature elicited by stimulation of L_7 developed less tension in old animals (minimally 200 days old) than in mature animals (ca 120 days old). We report here that the decreased tension is associated with altered synaptic efficacy between the terminals of L_7 and the antiflaring muscles of the gill pinnules. With the innervation between the PVG and the gill intact, an intracellular electrode in L_7 permitted simultaneous recording and stimulation. A suction electrode on the pinnule overlying the antiflaring musculature provided an extracellular record of the synaptic potentials (compound junctional potentials, CJP's) produced in the muscle cells by L_7 stimulation. This technique permitted recording of evoked CJP's while measuring the tension of pinnule contraction with a force transducer.

Trains of spikes in L_7 are required to elicit antiflaring. We examined age-effects on facilitatory processes at L_7's terminals using both double pulses and 3 sec spike trains in L_7. The resulting CJP's were analyzed by calculating the ratio, $f = [CJP_i/CJP_1] - 1$, where CJP_1 is the first evoked response and CJP_i is any subsequent response. No difference was found between the two age groups for double pulse facilitation with interspike intervals of 40-500 msec. The maximum f values for mature and old animals, 1.9 ± 0.5 (n=5) and 1.7 ± 0.4 (n=5) respectively, were obtained with interspike intervals of 70-100 msec. Within each group the facilitation decayed to one-half its peak value in approximately 80 msec. A similar analysis was made of augmentation, defined here as facilitation during a 3 sec spike train. For low frequency trains (those near threshold for producing muscle contraction, 8-18 spikes/3 sec) there was no difference in augmentation of CJP's from old and mature animals. At intermediate frequencies (those which always elicited contraction, 21-28 spikes/3 sec) CJP's from mature pinnules showed more augmentation than did those from old animals, although the differences were not significant ($p > 0.05$). At high frequencies (those producing maximal contraction, 39-48 spikes/3 sec) the augmentation of CJP's was significantly greater in mature than in old animals ($p < 0.05$, n=5). Also, in old animals augmentation levels were not sustained as they were in mature animals.

These results suggest that diminished motor neuronal function of L_7 results from decreased synaptic facilitation during trains between L_7 and the pinnule musculature in old Aplysia. (MH18611)

123.2 CEREBRAL BLOOD FLOW AND CEREBRAL METABOLIC RATES FOR OXYGEN, GLUCOSE AND CARBON DIOXIDE IN FISCHER-344 RATS OF DIFFERENT AGES. H. Takei*, W.R. Fredericks*, E.D. London and S.I. Rapoport. Laboratory of Neurosciences, National Institute on Aging, Gerontology Research Center, Baltimore, Maryland 21224.

Global cerebral blood flow (CBF) and the cerebral metabolic rates for O_2 ($CMRO_2$), glucose (CMRglc) and CO_2 ($CMRCO_2$) were measured in male, conscious, partially-immobilized Fischer-344 rats at the ages of 3, 12 and 24 months. CBF was determined with ^{14}C-iodoantipyrine which was infused intravenously for 45 sec. Radioactivity in timed arterial blood samples and in the brain at 45 sec was determined by scintillation counting (Sakurada et al., Am. J. Physiol. 234, H59, 1978; Ohno et al., Stroke, 10, 62, 1979). The metabolic rates for O_2, glucose and CO_2 were calculated from the concentrations of these substances in femoral artery and sagittal sinus blood, from which arteriovenous differences were obtained and multiplied by CBF. Differences and correlations noted below were significant at $P < 0.05$.

In fourteen 3-month old rats, CBF = 1.20 ± 0.08 ml/g/min (mean ± S.E.M.), $CMRO_2$ = 4.71 ± 0.32 μmol/g/min, CMRglc = 0.72 ± 0.09 μmol/g/min and $CMRCO_2$ = -4.86 ± 1.17 μmol/g/min. Whereas CBF, $CMRO_2$ and $CMRCO_2$ did not change significantly with age ($p > 0.05$) CMRglc was greater at 12 months than at 3 months. The ratio of $CMRO_2$/CMRglc was 6.97 ± 0.51 at 3 months, and did not change significantly with age. In individual rats, CBF was positively correlated with $CMRO_2$ and CMRglc, due to coupling between blood flow and oxidative metabolism, and negatively correlated with arterial hematocrit (which was reduced by blood loss). As the superior sagittal sinus drains venous blood mainly from supratentorial cortical regions, the results indicate that global CBF and indices of cerebral cortical oxidative metabolism do not decline with age in the conscious partially-immobilized rat.

The results are not in conflict with previous observations in aging rats, that decreases in CMRglc of most cortical areas are usually less than 20% (Smith et al., Brain, 103, 351, 1980; London et al., J. Neurochem., 1981, in press). Such small decrements may be obscured by experimental errors in determinations of global CBF, CMRglc and regional CMRglc.

123.3 AGING OF DIENCEPHALIC CATECHOLAMINE NEURONS (A-11, A-12, A-13, A-14) IN THE FISCHER 344 MALE RAT. L.D. Selemon* and J.R. Sladek, Jr. (SPON: W.E. O'Neill). Center for Brain Research and Dept. of Anatomy, University of Rochester School of Medicine and Dentistry, Rochester, NY 14642.

A-12 dopamine neurons have been implicated in a LHRH modulatory activity and inhibitory control of prolactin release from the anterior pituitary. In turn, the level of activity of A-12 neurons is regulated by peripheral steroid and prolactin levels. The function of and relevant inputs to incertohypothalamic catecholamine neurons (A-11, A-13, A-14) has yet to be established. Age-matched sets, which included one Fischer 344 male rat at 3, 20, and 30 months of age, were processed simultaneously for formaldehyde-induced fluorescence. Nucleated cell profiles and total cell profiles were counted in every tenth section of serial paraffin sections. These raw counts were corrected for split-cell errors and errors due to unseen small profiles using a modified Abercombie (1946) formula. A-12 soma exhibited a visible increase in fluorescence intensity as a function of age which was confirmed by microspectrofluorometric analysis. In contrast, the visible fluorescence intensity of incertohypothalamic neurons either declined (A-13) or was unchanged (A-11, A-14) in the aged versus the young rat. Similarly, cell counts of A-12 neurons revealed a 73-100% increase in visible cell population while the visible cell population of A-11 neurons decreased 10-81%; A-13 neurons decreased 24-42%. Only a small number of A-14 neurons were counted in any of the animals studied, and age-related changes in the A-14 cell population were extremely variable between sets. Since the age-related changes seen in A-12 dopamine neurons were not mirrored by A-11, A-13, or A-14 neurons, a generalized aging mechanism for diencephalic catecholamine groups is not supported by these data. However, the reduction in fluorescence intensity noted in A-13 soma parallels that seen in brain stem catecholamine groups (Sladek and Blanchard, Anat. Rec. 199:239A, 1981). The increases in transmitter content of A-12 soma are unique to tuberoinfundibular neurons and may be linked to the endocrine function of this dopaminergic system. Alternately, failure of the axon transport mechanism may occur selectively in A-12 neurons resulting in accumulation of transmitter in A-12 soma. Supported by USPHS Grants NS 15816 and AG 00847 (JRS).

123.4 SECRETION OF HYPOTHALAMIC DOPAMINE INTO HYPOPHYSIAL PORTAL BLOOD OF AGED FEMALE RATS. M. J. Reymond* and J. C. Porter. Cecil H. and Ida Green Center for Reproductive Biology Sciences, Depts. Ob-Gyn and Physiology, Univ. of Texas Southwestern Med. Sch., Dallas, TX 75235.

There is considerable indirect evidence for the view that neuronal function is impaired in the aged brain, including the hypothalamus. In order to study the effect of aging on neuronal function, we have investigated the neurosecretory activity of dopaminergic tuberoinfundibular neurons of aged female rats by determining the concentration of dopamine in hypophysial portal blood and calculating the rate of secretion of dopamine into portal blood. Although blood flow in the pituitary stalk vasculature of both old and young rats was the same, the concentration of dopamine in plasma of hypophysial portal blood of old constant estrous rats (20-24 months of age) was much less than that of young estrous rats (3-4 months of age) (1.25 ± 0.42 vs. 5.56 ± 0.78 ng/ml plasma, mean ± SE). The secretion rates of dopamine by hypothalamic neurons were 0.36 ± 0.14 ng/hr in old female rats and 1.65 ± 0.33 ng/hr in young female rats. The low concentrations of dopamine in hypophysial portal blood of aged rats were associated with high concentrations of prolactin in arterial blood. In aged female rats compared to young female rats, the concentrations of dopamine were reduced in the median eminence, a region that contains terminals of dopaminergic tuberoinfundibular neurons. The impaired hypothalamic secretion of dopamine observed in old rats was not affected by increased availability of L-tyrosine (200 mg/kg BW, IP) administered 60 min before beginning the collection of pituitary stalk blood. However, when old rats were treated with L-DOPA (200 mg/kg BW, IP), very high concentrations of dopamine were found in hypophysial stalk plasma compared to those of vehicle-treated old rats (64.1 ± 9.7 ng/ml vs. 1.25 ± 0.42 ng/ml). The high concentration of dopamine in hypophysial portal plasma of L-DOPA-treated old rats is believed to be a consequence of the conversion of L-DOPA to dopamine by hypothalamic neurons since the concentration of dopamine in the serum of arterial blood of the same animals was much less (1.56 ± 0.27 ng/ml) than that in portal plasma. On the basis of these data, it is concluded that the neurosecretory activity of the dopaminergic neurons of the hypothalamus is impaired in old constant estrous rats. This impaired neurosecretory activity can be overcome by increasing the availability of L-DOPA, the precursor of dopamine, but not of L-tyrosine.

123.5 AGE DIFFERENCES IN RECOVERY FROM INJURY TO THE NIGROSTRIATAL DOPAMINERGIC PROJECTION. J. F. Marshall, M. Drew*. Dept. of Psychobiology, University of California, Irvine, CA 92717

After unilateral injury to the mesotelencephalic dopaminergic projection rats show marked contralateral sensorimotor impairments, including an inability to orient to touch of the body surface. Rats with this injury frequently recover from this somatosensory localization deficit, and the extent of recovery is directly correlated with the dopamine (DA) content of the affected neostriatum at sacrifice.

Because aged rodents have marked changes in the neurochemistry of the nigrostriatal projection and its target site, and because they show no proliferation of striatal DA receptor sites after chronic neuroleptics, it was important to compare the extent of recovery of somatosensory localization in aged (26+ mos.) and young adult (4-6 mos.) Fischer 344 rats given 6-hydroxydopamine (6-OH-DA) injections (2, 4, or 6 µg) into the left ventral tegmentum after desmethylimipramine pretreatment (15 mg/kg, i.p., 30 min.). Somatosensory localization was studied for one month postoperatively, after which the animals were sacrificed for striatal catecholamine analysis.

Old rats given small (2 µg) 6-OH-DA injections showed substantially less recovery of somatosensory localization than did young rats given the same amount of this neurotoxin. The depletions of striatal norepinephine (20-30%) achieved by this 6-OHDA dose were similar in young and old rats. However, the neostriatal DA depletions of young rats (20%) were significantly less than were those of old rats (90%).

When the extent of recovery of each animal was plotted as a function of that animal's striatal DA depletion, significant correlations (-.71 and -.62) were obtained for the young and old groups. (Correlations between recovery and striatal NE depletions (-.64 and -.44) were somewhat smaller). However, the regression lines relating recovery to striatal DA depletions did not differ for the young and old groups.

The results suggest that young and old rats recover similarly after an equivalent degree of dopamine cell loss. Thus, the neural changes underlying this recovery are similar in both age groups. Our findings also suggest an enhanced susceptibility of the dopaminergic projection to the neurotoxic actions of 6-OHDA in aged rats.

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123.6 DIETARY CHOLINE INCREASES DENDRITIC SPINE POPULATION IN AGING MOUSE NEOCORTEX. Ronald Mervis and Raymond T. Bartus. Dept of Pathology (Neuropathology), The Ohio State University College of Medicine, Columbus, OH 43210, and Dept. of CNS Research, Medical Research Div., American Cyanamid Co., Pearl River, NY 10965.

Dietary choline enrichment (ChE) or deficiency (ChD) has been shown to alter the behavior of mice in ways that are quantitatively and qualitatively similar in comparison to those occurring naturally along the lifespan of mice on baseline control (BC) diets (Bartus et al, Science 209:301, 1980). Thus, when 8.5 month-old mice were placed on a ChE diet for 4.5 months and later behaviorally tested when 13-months-old, they performed as well as 3-month-old control mice. Similarly, ChD mice performed as poorly as senescent (23-month-old) mice. The mice were subsequently maintained on their respective dietary regimens for an additional six months and sacrificed (when 19-months-old). Hemispheres were coronally sectioned, impregnated using the Rapid Golgi method, and, in a blind study, randomly chosen layer V neocortical pyramidal cells were selected from coded slides for analysis of dendritic spine populations along selected lengths from both apical and basilar portions of their dendritic trees. This study evaluated over 120 neurons from 26 brains divided among the 3 dietary groups. In general, ChE mice had greater, and ChD mice, fewer, numbers of dendritic spines along the sampled areas in comparison to the age-matched control mice. In particular, ChE mice showed a highly statistically significant increase in spine population along the terminal tips of both apical ($p<.01$) and basilar ($p<.05$) branches in comparison to controls. This provides the first correlative evidence for structural-functional alterations which may be attributed directly to dietary choline. It further implies that dietary choline may not only manipulate cholinergic neurotransmitter mechanisms, but, by influencing phospholipid synthesis, such long-term dietary treatment may enhance the production of dendritic membrane.

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124.1 AGE-RELATED IMPAIRMENT OF HIPPOCAMPAL FREQUENCY POTENTIATION: EVIDENCE OF AN UNDERLYING DEFICIT IN TRANSMITTER RELEASE FROM STUDIES OF MAGNESIUM-BATHED HIPPOCAMPAL SLICES. P.W. Landfield, Dept. Physiol. & Pharmacol., Bowman Gray School of Medicine, Winston-Salem, NC 27103.

We have previously found that one of the most characteristic neurophysiological alterations in hippocampus of healthy, aging rats is an impairment of monosynaptic frequency potentiation of the extracellular population spike (in field CA1) during repetitive stimulation, which impairment is restricted to orthodromic stimulation. The pattern of potentiation impairment suggests that a deficit in transmitter functions may be involved (Landfield et al., 78, Brain Res.; Landfield, 80, in: Psychobiol. of Aging, Elsevier), although the data are not conclusive.

Recently, it was reported that bathing hippocampal slices from young animals in high Mg^{++}-low Ca^{++} media substantially enhanced monosynaptic frequency potentiation in comparison to potentiation in standard media, presumably by preventing transmitter depletion (Landfield, 80, Soc. Neurosci. Abstr.)

In the present studies, hippocampal slices from both aged and young Fischer rats were studied in high magnesium (2.7 mM)-low calcium (1.0 mM) media, and compared on measures of frequency potentiation of both the population spike and the "field EPSP" in the Schaffer collaterals of the CA1 apical dendrites. Both the spike and the EPSP exhibited less potentiation in slices from aged rats and, moreover, the EPSP and spike followed very similar temporal patterns of potentiation and depression during repetitive stimulation. When spike potentiation failed, so did EPSP potentiation. Changes in the dendritic fiber potential did not account for these changes. These data strongly indicate that the age-related deficit in spike potentiation is synaptic (as opposed to, for example, being due to dendritic electrotonus or to somal spike thresholds).

Additionally, it was found that high Mg^{++} media increased potentiation of the EPSP and the spike in slices from aged as well as from young rats, although the increase was larger in slices from young rats.

The primary effect of magnesium on synaptic potentials is known to be presynaptic in many systems. The ability of Mg^{++} to counteract the age deficit in hippocampal EPSP potentiation, therefore, suggests that the mechanism underlying the age deficit may be related to impaired transmitter release from presynaptic terminals.

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124.2 BRAIN NORADRENERGIC RESPONSES TO A SINGLE TRAINING FOOTSHOCK IN THE AGING RAT. K. Welsh* and P. Gold (SPON: P. J. Best). Dept. of Psychol., Univ. of Virginia, Charlottesville, VA 22901.

Recent evidence in the Fischer F-344 rat demonstrates age-related declines in retention performance at long intervals after avoidance training. These memory impairments may be explained in part by altered neurobiological systems which modulate the extent to which recent information is stored. For example, central noradrenergic systems are particularly sensitive to avoidance training and to memory modulating treatments. In the present experiment, we were interested in measuring the central noradrenergic responsiveness to the acute stress elicited by footshock in an inhibitory avoidance task.

F-344 rats (Charles River) were obtained at 60 days, 1 year, and 2 years of age and were housed individually. Three weeks following arrival each rat was placed in a rectangular shock compartment where it received a single, moderately intense footshock (2.0 mA, 2 sec duration). Ten mintues following this experience, the animals were decapitated, the brains were removed and subsequently divided into a brainstem sample (including the medulla, pons, and midbrain) and a forebrain (telencephalon-diencephalon) sample. Norepinephrine (NE), dopamine (DA), and epinephrine content were assessed with a sensitive COMT assay.

In general forebrain and brainstem catecholamine levels did not differ among the three age groups. Forebrain DA proved to be the exception, demonstrating a progressive decrease in concentration with age but reaching significance only in the oldest age group ($P < 0.05$). This result complements the deficits in DA metabolism already well characterized in the aging rodent (Finch, Brain Research, 52, 1973). Only the noradrenergic system exhibited concentration changes following the acute footshock experience. Moreover, this system appeared to be differentially sensitive to stress depending on the age group analyzed. Following footshock, both the 70 day and 1 year old rats demonstrated 20-30% decreases (Ps < 0.05) in both forebrain and brainstem NE. In the oldest animals, NE concentration was not significantly altered by the footshock experience. These findings are consistent with earlier findings from our laboratory which indicate that post-training NE responses are correlated with later retention performance under a variety of training and post-training treatment conditions. The present results, therefore, suggest that age-related declines in central noradrenergic responsiveness to training-related stress may contribute to age-related memory deficits.

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124.3 MEMORY CONSOLIDATION IN SENESCENCE: EFFECTS OF CO_2, AMPHETAMINE AND MORPHINE. R.B. Messing, H. Rigter, and V. Nickolson*. Scientific Devel. Grp., Organon International, Oss, The Netherlands.

Alterations in memory storage processes that occur in senescence were investigated by challenging young (4 month old) and old (30 month old) female "small Wistar" rats with posttraining administration of CO_2, amphetamine or morphine and measuring retention performance. Rats were trained in a one-trial inhibitory avoidance step through task, in which they were given a 0.5 mA, 3 sec footshock upon entering a dark compartment. They were then placed in a box saturated with CO_2 and returned to their home cages. The next day, the latencies of rats to enter the dark (shock) compartment were measured. Animals treated with CO_2 for 10-30 sec were amnestic, as indicated by shorter latencies than untreated controls to enter the compartment in which footshock had been administered. Neither duration of CO_2 immersion, nor the time of CO_2 treatment after training had a differential effect with age on retention performance. Challenge with drugs, however, did reveal age-related alterations in memory storage processes. Amphetamine (0.3 or 1.0 mg/kg, i.p.) administered immediately after training, and just prior to placement of rats in CO_2, attenuated the CO_2-induced amnesia in young rats, but had no effect in old rats. This could not be attributed to a general decline in response to amphetamine in old rats, because amphetamine increased open field activity of both young and old rats. In another experiment, the amnestic effects of morphine were investigated, using a hot plate escape task. Rats were placed on a hot plate (54.5 or 56.5°C) inside a glass cylinder 18-19 cm high, and the latency to escape by jumping to the top of the cylinder was recorded. Immediately after a single training trial, rats were injected with saline or morphine (3 or 10 mg/kg, i.p.) and returned to their home cages. Escape latencies were again measured two days later. Old rats treated with 3 mg/kg of morphine failed to learn, as indicated by the lack of a significant decrease in jump latencies in the retention as compared to the training trial. Old rats treated with saline did exhibit signficant learning, and also had longer retention latencies than morphine-treated rats. In contrast, young rats treated with morphine or saline exhibited a significant decrease in retention as compared to training latencies, and no effect of morphine on retention performance was observed. Thus, morphine induced an amnesia only in old rats.

These results indicate that the memory modulatory role of catecholamine and opioid systems may be altered in senescence.

124.4 REGIONAL BRAIN METABOLISM AND COGNITIVE DEFICITS IN AGING AND SENILE DEMENTIA. S.H. Ferris, M.J. de Leon*, D. Christman*, B. Reisberg*, J. Fowler*, A. George*, M. Emmerich*, C. Gentes*, T. Farkas* and A.P. Wolf*. Dept. of Psychiatry, N.Y.U. Med. Ctr., N.Y., N.Y. 10016 and Brookhaven National Labs., Upton, N.Y. 11973

Positron Emission Tomography (PET) is a new technique for studying regional brain function. ^{18}F-2-deoxy-2-fluro-D-glucose (FDG) is a positron emitting tracer for rate of glucose utilization in brain tissue. When a PET scanner is used following intravenous injection of FDG, in vivo quantitative measurements in man of regional glucose utilization can be obtained. Periodic "arteriolized" venous blood samples are obtained for determining the time course of the tracer, glucose and blood gases. The FDG is "trapped" in the brain cells as a 6-phosphorylated derivative. The trapping rate is proportional to the metabolic rate and provides a usable functional index after about 30 min. From emission data provided by the PET scanner, a computer reconstructs tomographic images representing the regional distribution of tracer uptake in the brain. Using a mathematical model for FDG and glucose metabolism, the rate of glucose utilization (mg/100g of tissue/min) may be quantified for various regions of interest in the brain.

We applied the PET-FDG technique to study regional brain metabolism in normal elderly subjects (N=6) and in senile dementia patients (N=12) with mild to moderately severe cognitive impairment. Patients with Primary Degenerative Dementia (Alzheimer's disease) were selected based upon extensive diagnostic evaluation Regional metabolic data were obtained using the PETT III scanner (spatial resolution = 1.7 cm^3).

At the level of the basal ganglia (CM plane x 40mm), the dementia patients showed marked diminution in metabolism (35-45%) as compared to age-matched controls (p's<.001). The regions sampled included frontal and temporal cortex, thalamus and caudate. The degree of diminution was highly correlated (p's<.05) with measures of cognitive impairment, particularly with deficits in memory. Less diminution (15-20%) was found at the level of the centrum semiovale (CM + 70mm). We also examined the relation between metabolic and structural changes by sampling computed tomography (CT) densities for various brain regions. The results did not indicate a simple pattern of PET-CT correlation. Interestingly, thalamic CT changes correlated with metabolism in most other brain regions.

The PET-FDG technique shows great promise for studying in vivo the brain changes related to aging and senile dementia. It also may prove helpful for studying brain pathways related to cognitive processes, and for evaluation of drug effects.

124.5 NEUROMETRIC CORRELATES OF COGNITIVE DYSFUNCTION IN THE ELDERLY. L. Prichep*, F. Gomez-Mont*[1], H. Kaye, E.R. John, S. Ferris & J. Fridman*[2]. (SPON: H. Ahn). Brain Research Lab., Dept. of Psychiatry, N.Y.U. Medical Center, N.Y., 10016. [1]Informatics Unit, Mexican Institute of Psychiatry. [2]Neurometrics Inc., N.Y.

The electroencephalogram (EEG) and averaged evoked potentials (EPs) can be used to assess the anatomical integrity, functional status and maturational development of the brain, as well as to evaluate information processing related to sensory, perceptual and cognitive functions. Using neurometrics, features of clinical diagnostic utility are extracted from a battery of EEG and EP conditions by objective quantitative procedures. These features are evaluated statistically relative to a normative data base, yielding the objective probability of abnormality for each measure. Studying the normative distributions, we found that the frequency composition of the EEG in the normative population could be described by a set of linear equations as a function of age. Deviations from the values predicted by the neurometric equations were few in large groups of culturally different normal children (6-16 yrs.), while a high incidence of significant deviations were found in children with learning disabilities and those at risk for neurological disorders.

The extension of these findings to include adults through senescence and the ability of such EEG and EP data to discriminate between normal (N=55) and cognitively impaired (N=75) elderly is the primary focus of this paper. Preliminary results indicate that based on the predicted EEG values for adults we can obtain very high discriminant accuracy in this population. Using only 10 EEG variables selected on the basis of a portion of univariate results, we were able to correctly identify 88% of the normal and 84% of the dysfunctional patients. (Split-half replication was approximately 80% for both groups). The results based on the addition of evoked potential measures and a more optimal set of EEG features to the dicriminant analysis will be presented.

In a previous study of a population of elderly patients we were able to identify homogeneous subgroups within the population using cluster analysis. The extension of such analyses to the present population and the usefulness of such differential identification of subgroups for the purpose of understanding and treating the elderly will be discussed.

124.6 "I DON'T KNOW" RESPONSES IN DEMENTIA AND DEPRESSION. R. C. Young and M. Manley*. Dept. of Psychiatry, Cornell Univ. Med. Coll. and New York Hosp.-Cornell Med. Ctr., Westchester Div., White Plains, New York 10605.

Dementia and depressive illness are sometimes difficult to distinguish in the elderly. Clinicians have suggested that depressives with "pseudodementia" give "I don't know" responses on mental status examination while demented patients do not. Documentation of the frequency of such responses was sought.

Nineteen patients aged 58 to 90 were interviewed. Nine had primary degenerative dementia (PDD), eight had primary recurrent major depression (PRMD), and two presented with both major depression and dementia. Severity of depressive symptomatology was rated with the Hamilton Scale (HRS) and cognitive performance was tested with the Minimental State scale (MMS). Responses on the MMS were categorized as correct, incorrect or irrelevant, or "I don't know"/"I can't".

As expected, subjects with PRMD had higher HRS scores (median 25; range 18-30) than those with PDD (median 8; range 4-17; $p < .002$; Mann Whitney U test, 2tailed). The two subjects with both depressive and dementia syndromes had HRS scores of 29 and 31. Again, as expected, subjects with PDD had lower MMS scores (median 12; range 3-22/30) than those with PRMD (median 28; range 20-30; $p < .002$). The MMS scores of the two subjects with depression and dementia were both 17.

The subjects with PDD gave more "I don't know" responses (median 6; range 0-10) than those with PRMD (median 0; range 0-8; $p < .02$). While 8 of 9 subjects with PDD gave "I don't know" responses, only 3 of 8 with PRMD did so ($p = .04$; Fisher's exact test). The two subjects with depression and dementia gave 2 and 3 "I don't know" responses.

These preliminary findings call into question the usefulness of "I don't know" responses in differential diagnosis since they may be elicited often in patients with PDD.

124.7 TURNOVER OF NEUROTRANSMITTERS AND METALS IN RABBIT BRAIN AFTER TREATMENT WITH ALUMINUM AND CHELATING AGENTS. K. S. Rajan, S. Mainer, Nina L. Rajan, IIT Research Institute, Chicago, IL, and B. I. Diamond, R. L. Borison and J. M. Davis, Illinois State Psych. Institute, Chicago, IL.

A possible role for Al(III) in the neuropathology of Alzheimer's disease has been considered by a number of reports. In this context, it is important to know, (1) the nature of incorporation of excess Al(III) and the associated changes in neurotransmitter levels in the brain and (2) the effect of therapeutic removal of Al(III) on the turnover of the neurotransmitters and the cerebral metals. Studies were undertaken to determine the brain levels of Al and dopamine metabolites as a result of treatment with chelating agents. On the basis of physicochemical studies directed toward exploring suitable chelating agents for *in vivo* interaction with cerebral aluminum, two compounds have been found to be suitable, i.e., ethylenediamine di-(o-hydroxyphenylacetic acid) [EDDHA], and N,N-'di-(2-hydroxybenzyl) ethylenediamine-N,N'-diacetic acid [HBED]. The stabilities of their Al(III)-chelates (log K_{ML}) were 24.48 ± 0.06 and 24.78 ± 0.23. I.V. treatments of New Zealand rabbits (2.5 Kg, male) with the two chelating agents (7.7 mg/Kg) resulted in a 14 to 24% decrease in brain levels of Al(III). The levels of dopamine (1.1 µg/g) homovanillic acid (1.7 µg/g) and dihydroxyphenyl acetic acid (0.6 µg/g) in the caudate increased to 3.8, 4.4, and 0.9 µg/g, respectively after chelate treatment. The results are discussed in terms of a possible chelation approach to dopamine turnover and the alteration of the levels of Al and other metals in the brain. (Supported by NIH Grant No. NS11556.)

124.8 EFFECT OF REPETITIVE HYPOXIA ON LOCOMOTOR ACTIVITY AND LEVELS OF CATECHOLAMINES IN BRAIN REGIONS OF AGED RATS. Isaac F. Roubein, Larry J. Embree, David W. Jackson*, and Danny Kay*. VA Med. Cen., and Dept of Neurology, LSU Med. Cen., Shreveport, LA 71130.

Previous work in our laboratory has shown that when aged rats were exposed to 10% oxygen for 2, 13, and 36h, the levels of dopamine (DA) and norepinephrine (NE) in the brain regions examined remained unchanged after 2h, but after 13h of hypoxia, NE concentration in hypothalamus and midbrain decreased significantly; and after 36h, levels of this monoamine returned to normal (In Press, Neurobiology of Aging). The current study was undertaken to investigate the effects of repetitive hypoxia on locomotor activity and the levels of catecholamines in seven regions of aged rat brain. Groups of aged male rats (25-26 months old) and young adults (10-12 weeks old) were exposed to 10% oxygen for 6h daily for five consecutive days. Thereafter the animals were left in their shelter for 48h; at the end of this period the animals were again exposed to the hypoxic atmosphere for another 5 days and then returned to their shelter for two days; (total days of exposure to 10% oxygen were 20). Young adult animals were exposed similarly to the hypoxia. Control animals of identical ages received air under similar conditions. Behavioral studies: On 5, 10, 15, and 20 day of exposure to 10% O_2 or air, the locomotor activity was measured, using OPTO-VARIMEX. The activity of the animals was recorded every 15 minutes for 6h. At the end of the experiment, the rats were decapitated, the brain excised and dissected into the following regions: cerebral cortex, hippocampus, striatum, hypothalamus, midbrain, cerebellum, pons and medulla. Levels of DA were determined using HPLC. Protein was determined by the Lowry Procedure. DA levels were determined in hippocampus, hypothalamus, midbrain and striatum. Statistical analysis indicated that the levels of DA decreased significantly in the aged hypoxic striatum and midbrain as compared with the same brain regions of the young adult hypoxic animals. There were no significant differences in the levels of DA in the same regions from both air receiving controls (25-26 months and 10-12 weeks). An analysis of variance showed there was no group, weeks, or time of day effect in locomotor activity. The results of this investigation indicate that repetitive hypoxia had differential effects on the levels of DA in striatum and midbrain of aged and young rats. These differences seem to depend on age-related changes in DA metabolism in two specific regions of the aged rat brain exposed repeatedly to hypoxic environment. NE levels in brain regions are currently under investigation and will be reported. Supported by Veterans Administration and the LSUMC, Shreveport, LA.

124.9 RELATIONSHIP OF PLASMA NOREPINEPHRINE, BLOOD PRESSURE, RESPIRATION AND HYPOXIA IN TWO OLDER INDIVIDUALS WITH RESPIRATORY DYSFUNCTION. M.V. Vitiello*, P.N. Prinz, E.C. Giblin*, R.B. Schoene* and J.B. Halter* (SPON: R. Martin). Depts. of Psychiatry, Physiological Nursing and Medicine, Univ. of Washington, and American Lake and Seattle Veterans Admin. Hospitals, Seattle, WA 98195.

Recent studies suggest that the aging process may increase respiratory dysfunctions during sleep. These respiratory dysfunctions may be either apneas, cessation of airflow for 10 or more seconds, or hypopneas, a decrease in the amount of airflow. Either of these conditions can result in a decrease in oxygen saturation (SaO_2) and usually results in an arousal from sleep. The present study evaluated breathing and SaO_2 during sleep and its relationship to sleep patterns, plasma norepinephrine (NE) and blood pressure (BP) in 2 individuals with extensive respiratory dysfunction during sleep.

LG is a 55 year old female and OM a 59 year old male, both complained of excessive daytime sleepiness and had been previously diagnosed as narcoleptic. Subjects were off major medications 2 or more weeks prior to study. Both slept in the laboratory 3 consecutive nights. On night 3 LG received 1-2 litres/hour O_2. Nothing was administered on any of the other nights. Sleep was recorded using nasal and oral thermistors and thoracic and abdominal strain gauges. SaO_2 was recorded using ear oximetry. In addition, blood samples were obtained using an indwelling venous catheter and BP using an Arteriosonde on nights 2 and 3 for LG and night 3 for OM.

Both individuals displayed marked respiratory impairment across the night. While LG's SaO_2 was improved on the O_2 night, her sleep pattern remained severely disturbed. For both subjects there were 2 or more marked periods of oxygen desaturation per night. These desaturations always preceeded REM onset and continued throughout REM. This relationship continued during LG's O_2 night. This relationship has been observed in the general population with apneic episodes associated with REM. Hypoxemia was usually associated with a surge in NE and a labile BP. High pulse pressure occurred frequently throughout the study.

OM, who showed primarily mixed apnea, displayed smaller NE and BP responses than did LG, who had both apneic and hypopneic episodes. This association between hypopnea and apnea and NE and BP changes may be a contributing factor to the increased incidence of hypertension seen with aging.

124.10 SLEEP/WAKING PATTERNS AND PLASMA NOREPINEPHRINE IN YOUNG AND AGED NORMAL MEN. P.N. Prinz, M.V. Vitiello* and J.B. Halter*. American Lake and Seattle Veterans Admin. Hospitals, and Depts. of Psychiatry and Medicine, Univ. of Washington, Seattle, WA 98195.

It is well documented that sleep patterns change with age. Older individuals awaken more during the night and spend less time in rapid eye movement (REM) sleep and slow wave sleep (SWS) than do younger individuals. Recent studies have shown that an increase in daytime plasma norepinephrine (NE) also accompanies the aging process. This study examines plasma NE in young and aged normals during the day and at night in relation to sleep/waking patterns. All subjects were paid volunteers; normotensive nonsmokers in good physical health (based on physical examination)(16 young normals, aged 21-28 years, and 10 aged normals, aged 55-82 years). All subjects had one or more adaptation nights at the Clinical Research Center, University Hospital. Following adaptation hourly blood samples were obtained via indwelling venous catheters, and sleep stages were recorded using conventional techniques. A significantly greater plasma NE level was observed in the older subjects both at night and during the day. Additionally, a diurnal variation of plasma NE was observed in both groups with NE values lowest during sleep and highest during the late morning (approx. 11:00). It was observed that sleep quality was correlated with mean plasma NE levels during sleep ($R=.624$, $p<.01$).

These finding raise the possibility that the well known age effect on sleep may be related to increased sympathetic nervous system activity at night.

	Daytime NE c. 11:00	Nighttime NE c.23:00-6:00	% SWS of Time in Bed	% REM of Time in Bed	% Waking of Time in Bed
AN	327.00± 34.76*	225.30± 19.94	8.34± 2.21	13.79± 2.04	26.09± 5.18
YN	212.50± 26.87*	151.61± 9.35	20.91± 2.01	21.37± 1.54	9.78± 1.62

*Mean ± standard error of the mean, all variables significantly different, $p<.05$ across both groups.

124.11 EFFECT OF AGING ON FLUID HOMEOSTASIS: RESPONSE TO ACUTE OSMOTIC CHALLENGE. C.D. Sladek, T.H. McNeill, C.M. Gregg‡ M.L. Blair‡ Depts. of Neurology, Anatomy, Physiology, University of Rochester, Rochester, N.Y., 14642; and Dept. of Biology, Pennsylvania State University, University Park, Pennsylvania.

Abnormalities in vasopressin (VP) secretion may contribute to the alteration in body fluid homeostasis associated with aging. In aged rats, the serum VP response to chronic dehydration is attenuated (Sladek et al, Anat. Record 199:239A, 1980). The current study evaluates the ability of aged rats to respond to acute osmotic stimulation. Three mos. (246±5 gms) and 30 mos. (364±9 gms) male Fischer 344 rats received intraperitoneal injections of hypertonic (1,000 mosm/kg) or isotonic (285 mosm/kg) saline and were decapitated 15, 30, or 60 min. later. Control animals received no injections. Serum VP and renin concentrations (SRC) and VP content of the supraoptic nuclei (SON), paraventricular nuclei (PVN), and posterior pituitary (PP) were determined by radioimmunoassay. Injection of isotonic saline did not alter any of these parameters. Kidney function was adequate in the old rats as indicated by normal BUN, plasma albumin concentration, and protein excretion rate. Serum VP and VP content of the SON, PVN, and PP were not significantly different between the 3 and 30 mos. rats. However, relative to body and PP weight, PP concentration of VP was reduced in the old rats ($p < .025$). The hypertonic saline injection resulted in an increase in plasma osmolality (pOsm) by 15 min in both young ($p < .05$) and old ($p < .005$) animals. The increase in pOsm was greater in the old animals than in the young ($p < .005$). Serum VP was elevated ($p < .025$) and SRC was suppressed in both groups. PP and PVN content of VP were not significantly altered, but VP content of the SON was decreased 15 min post injection in the old rats ($p < .025$). Thus, the VP and renin response to hypertonic saline are maintained in aged rats. Evidence was not obtained for increased sensitivity to osmotic stimulation as observed in humans (Robertson and Rowe, Peptides 1:Suppl. 1: 159, 1980). In fact, the converse is suggested by the comparable serum VP concentrations in aged rats in spite of greater osmotic stimulation.

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124.12 INCREASE IN IMMUNOREACTIVE β-ENDORPHIN IN THE PLASMA OF OLD MALE RATS: PRESENCE OF A HIGH MOLECULAR WEIGHT FORM, L.J. Forman*, W.E. Sonntag*, N. Miki*, D.A. Van Vugt* and J. Meites* (SPON: R. Bernard). Dept. of Physiol., Neuroendocrine Res. Lab., Mich. State Univ., East Lansing, MI 48824.

In young rats, β-endorphin decreases hypothalamic dopamine and increases serotonin metabolism; decreases release of gonadotropins and increases pituitary release of PRL. A similar condition is found in old male rats since the metabolism of hypothalamic dopamine is reduced, whereas the metabolism of serotonin is increased; and pituitary PRL release is increased, whereas gonadotropin release is reduced. In the present study, plasma levels of immunoreactive β-endorphin (IR-β-ENDO) were measured in young (3-4 mo) and old (19-21 mo) male rats using a highly specific radioimmunoassay. A four-fold increase in plasma IR-β-ENDO was observed in old as compared to young male rats, and pituitary content and concentration of IR-β-ENDO was increased approximately 60% in the old rats. Chromatographic analysis of the plasma of the old and the young rats revealed that the elevated plasma level of IR-β-ENDO in the old male rats was due solely to the present of a high molecular weight form of IR-β-ENDO which was not detected in the plasma of the young male rats. The biological significance of this high molecular weight form of IR-β-ENDO in the old male rats remains to be determined. The greater release of IR-β-ENDO in the old male rats may be related to the changes mentioned above in hypothalamic and pituitary function. (Aided in part by NIH research grant AG00416 to J. Meites, and NIH post-doctoral fellowships to L. Forman, AG05208, and to W. Sonntag, AG05147 from the National Inst. on Aging).

124.13 ALTERATIONS IN ADRENERGIC CONTROL OF VASCULAR SMOOTH MUSCLE DURING AGING. S.P. Duckles. Dept. of Pharmacology, College of Medicine, University of Arizona, Tucson, AZ 85724.

A number of significant alterations in circulatory hemodynamics are known to occur in both animals and man with advancing age. However, little is known about possible changes in adrenergic control of blood vessels that may contribute to hemodynamic alterations. Therefore, the adrenergic neuroeffector mechanism of vascular smooth muscle was characterized using in vitro techniques. Blood vessels from two groups of New Zealand white rabbits were compared: young rabbits (90 days old, 2-3 kg body weight) and aged rabbits (at least 4 years old, 4-5 kg). The life span of domestic rabbits is 7 to 8 years. The content of norepinephrine (NE) measured with a phenylethanolamine-N-methyl transferase radioenzymatic assay was determined in a series of vessels. The ear artery, basilar artery, middle, anterior and posterior cerebral arteries, circle of Willis, thoracic aorta, and mesenteric artery and vein were studied. No significant differences were found in NE content in any of these vessels. Release of endogenous NE evoked by transmural electrical stimulation of superfused vessels in vitro was measured using a radioenzymatic assay. Cocaine (10^{-5} M) and desoxycorticosterone (4×10^{-5} M) were present throughout to prevent neuronal and extraneuronal metabolism. At a frequency of 8 Hz, the fractional release of NE from the ear artery was decreased in vessels from the aged rabbits. Fractional release of NE/pulse ($\times 10^{-5}$) averaged 0.73 ± 0.23 in the aged ear artery compared to 2.0 ± 0.3 in the young ear artery ($p < 0.05$). Responses of vascular smooth muscle to adrenergic nerve stimulation were compared in the two groups of animals by measuring contraction to transmural electrical stimulation in vitro. The optimum resting tension was selected in preliminary experiments. This was not different for 5 mm ring segments from young and old rabbits, being 1 gram in both cases. No significant differences in contractile responses to transmural nerve stimulation were seen. Contractile responses of the ear artery to 200 pulses at 2 Hz averaged 30% of the maximum response in young animals compared to 29% in the aged animals. Maximum contractile responses to adrenergic nerve stimulation averaged 2.27 ± 0.09 grams in the young rabbit ear artery compared to 2.15 ± 0.35 grams in the aged. Further investigation will be necessary to determine the mechanism by which contractile responses of blood vessels to adrenergic nerve stimulation are unaltered even though it appears that a smaller amount of norepinephrine is released.

124.14 AGING-LIKE BRAIN MORPHOLOGICAL CHANGE IS INCREASED IN TWO RODENT MODELS OF PROLONGED HYPERTENSION. T.A. Pitler*, R.I. Baskin*, J.A. Wren* and P.W. Landfield (SPON: J.G. McCormick). Dept. of Physiol. & Pharmacol., Bowman Gray Sch. Med., Winston-Salem, NC 27103.

The nature of the interaction of hypertension and brain aging, if any, has not yet been clarified. Although humans with senile dementia exhibit lower than average blood pressures (Busse and Wang, 74, In: Normal Aging, Duke Univ. Press.), elevated blood pressure is also correlated with cognitive impairment in aging humans (Wilkie and Eisdorfer, 71, Science).

In order to determine whether hypertension can retard or accelerate aging-like brain morphological changes, two models of prolonged hypertension were examined for brain morphological patterns, with particular emphasis on glial changes that are known to occur in brains of aging rats.

In the first experiments, 3 mo.-old male Sprague-Dawley rats were unilaterally nephrectomized, and were given a saline solution to drink. Injections of deoxycorticosterone acetate (DOCA) were given for a period of 2.5 mo. (3-6 mg/rat, 2 x week). Blood pressures (BP) were monitored with a tail cuff method. By 2 mo. BP in the DOCA treated rats ranged from 180 to greater than 220 mm Hg, and some hypertensive animals began to exhibit pathological signs of malignant hypertension. All hypertensive and control rats were sacrificed at about 2.5 mo. and their brains were stained with Cajal's gold chloride stain for astrocytes. Hypertensive rats showed increased astrocyte reactivity, and a few of the animals exhibited massive astroglial reactivity, and signs of brain edema or stroke. However, some animals with BPs below 200 mm Hg showed milder glial reactions, which appeared to be very similar in topography to the patterns of astroglial reactivity seen in the hippocampus of normally aging rats (Landfield et al., 77, J. Gerontol.; Lindsey et al., 78, ibid).

In the second experiments, male SHR and WKY controls were maintained from age 4-5 mo. until age 21-24 mo., and semithin sections were then prepared from the hippocampus of each animal. Data analyses indicate that age-related glial reactivity is more pronounced in hippocampus of aged SHRs (BP ~175 mm Hg) than of aged WKYs (BP ~115 mm Hg). Female SHRs are currently under study.

Since the patterns of glial changes that are increased in hypertensive rats are very similar to those seen in normally aging rats, these data suggest that non-lethal levels of hypertension may accelerate some aspects of aging-like changes in the brains of rats. Studies are continuing to determine whether these effects are due to direct or indirect actions on the brain.

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125.1 CORTICAL CONTROL OF SELECTIVE ATTENTION IN MAN. A REGIONAL CEREBRAL BLOOD FLOW STUDY. P.E.Roland. Dept. Clin.Physiol. Bispebjerg Hospital DK 2400 NV, Denmark.

How does the cerebral cortex select the sensory cha -nnel from which it wants to extract information, and what happens to irrelevant information from other cha -nnels? Ten subjects were simultaneously stimulated with pairs of objects pressed against their palm, pairs of ellipses projected on a screen and pairs of tones. They were asked to discriminate the input from one channel and ignore inputs from the two other channels. During the two-alternative forced-choice discrimination (2AFCD) the regional cerebral blood flow (rCBF) was measured in 254 cortical regions with the 133-Xe-intracarotid injection technique. Under normal physiological conditions the rCBF is an indicator of the regional cerebral metabolism. Previous measurements of rCBF during 2AFCD with the described stimuli and selective stimulation of either the somatosensory channel (som-ch), the visual channel (vis-ch) or the auditory channel (aud-ch) provoked rCBF increases in these areas: the visual association cortex (vis-ch), posterior superior parietal cortex (vis-ch), parieto-temporo-occipital cortex (vis-ch,aud-ch), right inferior parietal cortex (aud-ch, vis-ch), intraparietal cortex (aud-ch,vis-ch), somatosensory cortex (som-ch), auditory association (aud-ch), inferior posterior fron -tal cortex (aud-ch), frontal eye fields (aud-ch,vis-ch), superior dysgranular cortex (aud-ch,som-ch,vis-ch), anterior part of lateral prefrontal cortex (aud-ch,vis-ch), posterior part of lateral prefrontal cortex (som-ch,aud-ch,vis-ch), and the superior part of the prefrontal cortex (som-ch,aud-ch,vis-ch). Present results: An identical pattern of rCBF increases appeared, no matter what channel the subjects focussed their attention upon. This pattern was composed of increases of rCBF in all the above areas plus an increase in the left inferior parietal cortex. The rCBF increase in the superior dysgranular cortex was particularly high suggesting that this area controls the direction of attention. These results indicate that sensory information, even if it is irrelevant, is processed by the cortex as long as it does not interfere with the processing of the information actually being discriminated. Turning the attention towards either the visual or the auditory channel enhanced the rCBF further in the respective association cortex.

125.2 THE REGIONAL DISTRIBUTION OF MANGANESE IN THE NORMAL HUMAN BRAIN. E. Bonilla, E. Salazar,* J.J. Villasmil* and R. Villalobos*. Instituto de Investigaciones Clínicas. Fac. de Medicina, Universidad del Zulia, Apartado 1151, Maracaibo, Venezuela.

The cause of the higher vulnerability of the nervous tissue and the reasons for the unequal regional distribution of the brain lesions in chronic manganese intoxication are unknown. It is therefore important for the understanding of the pathogenesis of manganese poisoning to gain insight into the topographical distribution of this metal in the brain. The present study includes results from 39 areas of 8 normal human brains. The persons studied comprised 8 men with ages ranging from 11 to 75 years. The manganese content was determined by flameless atomic absorption spectrophotometry. All samples were analyzed by the method of standard additions. The general lineal model from Statistical Analysis Systems was used for the analysis of variance and the comparison between the means.

The highest content of manganese was found in the pineal gland (4.2±2.5 ug/g dry weight). It was observed that the gray matter yielded higher concentrations of manganese than the white matter (1.54 ug/g for gray matter, 0.93 ug/g for white matter). The difference was statistically significant ($p < 0.01$).

No difference was demonstrated for the concentrations of manganese in the frontal (1.60 ug/g), occipital (1.57 ug/g), and parietal (1.54 ug/g) lobes, but when comparing any of these with the content of manganese in the temporal lobe (1.22 ug/g) and with the mean obtained in the white matter of the lobes (0.78 ug/g) the differences were significant at the 5% level.

No difference was detected between the content of manganese in the head (1.29 ug/g) and the body (1.41 ug/g) of caudate nucleus. However, when comparing each of these means with the manganese concentration of the tail of caudate (1.89 ug/g) the differences were significant at the 5% level.

A regression analysis of the amount of manganese in brain and age showed no significant correlation between them. However, the significance started to be detected when alpha = 0.10. Therefore, it is convenient to repeat this analysis with a larger number of brains of different ages. (Supported by CONDES-LUZ).

125.3 AMINO ACID CHANGES FOLLOWING CONDITIONED EMOTIONAL RESPONSE. J.D. Altazan*, M.P. Sands*, D.R. Cherek, J.E. Smith and J.D. Lane (SPON: J.W. Dailey). Psychiatry Research Unit, Department of Psychiatry, Louisiana State University Medical Center, Shreveport, LA 71130

Conditioned emotional response (CER) is thought to be an animal model for anxiety. CER has frequently been used to evaluate behavioral-drug effects, with anxiolytics clearly attenuating the emotional response. To evaluate the neurochemical changes associated with this paradigm, groups of three littermate Fisher F-344 rats were food deprived and shaped to lever press in a standard operant chamber. Lever pressing was maintained by a variable interval (VI) 1 min schedule of food presentation. Following stabilization of responding, one hour classical conditioning sessions were initiated. Classical conditioning consisted of presenting conditioned stimulus (tone) of varying lengths (2 min-10 min) and unconditioned stimulus (foot shock, 1mA, 500 msec) approximately 8 times during each session. UCS was presented at the end of CS. One rat of each group received this classical conditioning for 5 sessions. To isolate the conditioning component from the shock history and animal activity, the other two littermates received yoked CS (tone) or USC (shock) only. On test day, the CS was presented to the triads of 15 minutes while lever pressing was maintained by the food presentation schedule, after which the animals were totally frozen and stored at -70°C until analysis. The pre-tone and post-tone responding on the VII schedule were compared. The CER animal responded very little (<<1 res/min) after tone presentation, while both controls continued at pre-stimulus rates (circa 15 res/min). Brains were dissected into discrete cortical and sub-cortical areas and the content and utilization of biogenic amines and amino acid neurotransmitters determined. There were very few changes in content of neurotransmitters or their metabolites, suggesting that small functional pools were being utilized. Comparison of shock history (shock only versus tone only) revealed a general decrease in the turnover of Asp, Glu and GABA in multiple areas. Comparison of the conditioning/emotional component (CER versus shock only) revealed a general increase in turnover of Asp and Glu in multiple areas and mixed changes in turnover of GABA in limbic versus motor areas. These data are consistent with CER being a model for studying emotional behavior in the presence of an aversive stimulus. Since amino acids are the neurotransmitters at a majority of CNS synapses, both general and specific roles for them in conditioning and emotion are predictable. (supported by USPHS Grant MH-31835).

125.4 RECEPTOR CHANGES FOLLOWING THE REVERSAL OF CONDITIONED EMOTIONAL RESPONSE. G.F. Guerin*, C.M. Crenshaw*, J.E. Smith, D.R. Cherek and J.D. Lane (SPON: C.D. Wood). Psychiatry Research Unit, Department of Psychiatry, Louisiana State University Medical Center, Shreveport, LA 71130.

Conditioned emotional response (CER) is thought to be an animal model for anxiety. CER has frequently been used to evaluate behavioral-drug effects with anxiolytics clearly attenuating the emotional response. To evaluate the neurochemical changes associated with this paradigm, groups of three littermate Fisher F-344 rats were food deprived and shaped to lever press in a standard operant chamber. Lever pressing was maintained by a variable interval (VI) 1 min schedule of food presentation. Following stabilization of responding, one hour classical conditioning sessions were initiated. Classical conditioning consisted of presenting conditioned stimulus (tone) of varying lengths (2 min-10 min) and unconditioned stimulus (foot shock, 1 mA, 500 msec) approximately 8 times during each session. UCS was presented at the end of CS. One rat of each group received this classical conditioning for 5 sessions. To isolate the conditioning component from the shock history and animal activity, the other two littermates received yoked CS (tone) or USC (shock) only. On test day, the CS was presented to the triads for 15 minutes while lever pressing was maintained by the food presentation schedule, after which the animals were decapitated and stored at -70°C until analysis. The pre-tone and post-tone responding on the VII schedule were compared. The CER animal responded very little (<<1 res/min) after tone presentation, while both controls continued at pre-stimulus rates (circa 15 res/min). If the CER conditioned animals were given an acute i.p. dose of diazepam, the suppression and emotional behavior were attenuated, and pre-CS VII responding returned. Brains were dissected into discrete cortical and sub-cortical areas and total particulate membrane fractions were prepared. Membrane suspensions were evaluated for binding of tritiated QNB, spiperone, muscimol, WB4101, DHA, 5-HT, imipramine and diazepam in the presence or absence of displacing agent. When comparing the CER animals with their shock-only controls, frontal and associatiative cortical areas had decreased benzodiazepine and increased muscarinic acetylcholine receptors. Diazepam administration effected QNB, spiperone, DHA, but not diazepam binding. This data is consistent with CER being a model for studying emotional behavior in the presence of an aversive stimulus and indicates that CER and its reversal by benzodiazepines is mediated by classic neurotransmitters. (Supported by USPHS Grant MH-31835).

125.5 ALTERED METABOLIC ACTIVITY IN RAT BRAIN PRODUCED BY PHENCYCLIDINE ANALOGUES: A 2-DEOXY-D-GLUCOSE STUDY. R. P. Hammer, Jr. and M. Herkenham., Lab. of Neurophysiology, NIMH, Bethesda, MD 20205

Phencyclidine-induced changes in brain metabolism have been reported to predominate in limbic structures (Meibach et al., Nature, 1979, 282:625). We have examined patterns of metabolic activity in brains of rats exposed to phencyclidine and phencyclidine analogues using ^{14}C- and ^{3}H-2-deoxy-D-glucose. Brain sections were exposed either to X-ray film, ^{3}H-sensitive LKB Ultrofilm, or Kodak NTB-2 emulsion for autoradiography. Our preliminary autoradiograms show striking patterns of columns and laminae selectively marked in various cortical regions. Comparatively greater glucose utilization occurs in the molecular layers of hippocampus, dentate gyrus, subiculum and parahippocampal cortex. The densest labeling in the entire brain is found in layer I of posterior entorhinal cortex. A striking variation of neocortical glucose utilization is noted in the form of columns which appear in register with granule cell-poor zones in layer IV of somatic sensory-motor cortex. The patterned labeling of large portions of neocortex, including at least all of SI cortex, suggests that metabolic columns are not caused by phencyclidine-induced "whisking" which would limit their appearance to vibrissal barrel fields. Rather, columns occur in cortical regions spared by inputs from thalamic relay for somatic sensation. Increased metabolic activity in the molecular layer of the hippocampus and dentate gyrus may be correlated with terminations of pathways from regions rich in phencyclidine receptors, including dentate gyrus and entorhinal and cingulate cortices (Quirion et al., PNAS, 1981, in press). Descriptions of phencyclidine "anesthesia" as a functional dissociation of cortical areas could be explained by the differential activation of limbic and neocortical zones by phencyclidine-stimulated systems. These results further elucidate the influence of phencyclidine (PCP) on brain metabolism by affecting the activity of neo- and archicortical systems.

125.6 HISTOPATHOLOGICAL CORRELATES OF REGIONAL UPTAKE OF ^{14}C-DEOXYGLUCOSE (DG) IN CAT BRAIN AFTER CONCUSSIVE INJURY. C. M. Pechura,* J. Povlishock, D. P. Becker and R. L. Hayes* (SPON: K. Corley). Div. of Neurosurgery, Med. Coll. VA, Richmond, VA 23298.

The ^{14}C-deoxyglucose (DG) technique (Sokoloff et al., J. Neurochem. 28:897, 1977) has been used to define patterns of local glucose utilization (LGU) in the CNS associated with various pathophysiological conditions. The present study examined whether or not specific patterns of DG uptake could be associated with the various histopathologies seen following mechanical brain injury in cats. The DG technique was combined with horseradish peroxidase (HRP) and hematoxylin and eosin (H&E) histological methods.

Surgical preparations were performed under Brevital. Subsequently, cats (2.5-3.0 kg) were paralyzed and ventilated under nitrous oxide (70% N_2O, 30% O_2) anesthesia. Graded head injury (n=4) was produced via a fluid pressure pulse measured in atmospheres of pressure (Sullivan et al., J. Neurosurg. 45:520, 1976). Four cats were surgically prepared but not injured. Nitrous oxide anesthesia was withdrawn following injury in the experimental group. Five minutes prior to injury HRP was injected (i.v., 50-75 mg/kg) to assess blood-brain barrier (BBB) status and the presence of contusions or hemorrhage (Povlishock et al., Brain Res. 153:223, 1978). DG (i.v., 75-100 µCi/kg) was injected one hour following insult or completion of sham surgery. Forty-five minutes following the DG injection, animals were killed by an overdose of barbiturate, perfused, the brains frozen and prepared for DG autoradiography in the standard manner. Alternate serial sections were collected for HRP visualization and H&E staining.

DG uptake decreased in contused brain areas and showed a spatial relationship to areas of tissue destruction indicated by exudation of HRP. Subarachnoid hemorrhages, seen as extracerebral fringes of HRP reaction product or H&E staining were also visible in the autoradiographs. Small intraparenchymal hemorrhages, as identified by HRP reaction and H&E staining, appeared as corresponding spots of dense DG labelling in the autoradiographs. DG uptake did not exhibit a spatial relationship to HRP extravasation associated with BBB opening produced by injury. Regions normally excluded from the BBB showed corresponding patterns of HRP reaction product and uptake of DG. These data suggest that patterns of DG uptake can be reliably associated with certain pathophysiologic events following concussive injury. These methodologies allow evaluation of the possible contributions of histopathology to changes in functional activity, inferred by the DG method, following head injury. Supported by NIH Grant # NS 12587.

125.7 MEASUREMENT OF LOCAL BLOOD FLOW AND GLUCOSE METABOLISM WITHIN THE SAME TISSUE SAMPLES IN THE FELINE CNS. D. S. DeWitt,* M. J. Rosner,* D. P. Becker and R. L. Hayes* (SPON: W. I. Rosenblum). Div. of Neurosurgery, Med. Coll. VA, Richmond, VA 23298.

Previous data have shown that, under normal circumstances, cerebral blood flow (CBF) and metabolism are believed to be positively correlated. This hypothesis was further tested in nitrous anesthetized cats using the radioactive microspheres (RMS) to calculate CBF (Rudolph & Heymann, Circ. Res. 21:163, 1967) and the [^{14}C] 2-deoxyglucose (DG) techniques (Sokoloff et al., J. Neurochem. 28:897, 1977) or to calculate local rates of glucose utilization (LGU).

Male cats (2.5-3.0 kg) were initially anesthetized with sodium methohexital. Following tracheostomy, they were paralyzed and ventilated with 70% N_2O and 30% O_2. Further surgical procedures were carried out using local anesthesia. Four CBF measurements were done over a period of two hours using 15 µm RMS labelled with ^{125}I, ^{141}Ce, ^{85}Sr, or ^{46}Sc (3M). Approximately one million RMS were injected into the left atrium for each determination and simultaneous reference samples were withdrawn from the brachial and femoral arteries for 90 seconds after the start of the injection. Flows were done prior to and during the period following the injection of DG. The animals were sacrificed 45 minutes after the DG injection by an overdose of sodium pentobarbital. The brains were removed immediately and dissected into 14 regions bilaterally. Following gamma counting, tissue samples were dissolved in a tissue solubilizer. Once the tissue was dissolved, the samples were diluted with scintillation cocktail and filtered through 10 µm teflon Millipore filters. Filtering removed over 95% of the RMS but less than 3% of the DG. This procedure prevented the overlap of the energy spectra of the gamma-labelled RMS with that of the ^{14}C-labelled DG. Samples could then be counted on a liquid scintillation counter and the amounts of LGU thus determined.

Measurements of CBF showed that the flows did not differ significantly over the two hour period of the study. Resting blood flows for the whole brain were 65.7 ± 22.7 (n=7). Preliminary studies of glucose metabolism and CBF indicate that flow and metabolism are related with the following linear regression equation: CBF = .218 + .719 (LGU) $p<.01$. This study further demonstrates the normal coupling between CBF and metabolism, a phenomenon previously undescribed in nitrous oxide anesthetized cats. Supported by NIH Grant # NS 12587.

125.8 LOCAL RATES OF GLUCOSE UTILIZATION (LGU) IN THE BRAIN STEM OF THE CAT AFTER CONCUSSIVE HEAD INJURY. R. L. Hayes,* C. M. Pechura* and D. P. Becker (SPON: A. Snow). Div. of Neurosurg., Med. Coll. of VA, Richmond, VA 23298.

Concussive injury to the brain is associated with certain disturbances in systemic and cerebral functions which may be related to disruption of brain stem activity. The present study used the method of Sokoloff et al. (J. Neurochem. 28:897, 1977) to examine changes in the uptake of [^{14}C] deoxyglucose (DG) and LGU in brain stem loci after concussive injury. Ten cats (2.5-3.0 kg) were anesthetized with sodium methohexital, paralyzed, artificially ventilated with 70% N_2O and 30% O_2 and prepared for induction of mechanical brain injury (Sullivan et al., J. Neurosurg. 45:520, 1976). The technique produces levels of injury correlated with the magnitudes of pressure transients (in atmospheres, atms) which produce deformation of neural tissue. Five cats were surgically prepared, maintained on N_2O anesthesia and not injured. Five cats were injured and anesthesia discontinued 10 minutes later. One hour after injury or completion of surgery, DG was injected (75 µCi/kg, i.v.). In 6 cats (3 uninjured, 3 injured), timed plasma samples of DG and glucose were taken to calculate LGU. The injury level was 1.9 atms, a magnitude for which morbidity and mortality (30%) were defined in a separate group of animals (n=13). Forty-five minutes after injection of DG, animals were killed by a barbiturate overdose, the brain perfused, frozen and sectioned for autoradiographic analyses by standard DG techniques.

Preliminary analyses of LGU in 15 brain stem regions indicate that, with 2 exceptions, the functional activity of brain stem structures of injured cats is depressed. For the regions showing decreased activity, mean LGU in uninjured cats was 37.3 (± 2.5 µmoles/100g/min) and 28.7 (± 3.2) in injured cats. Injury resulted in the greatest relative decreases in LGU in the vicinity of the locus coeruleus (>40%) and regions surrounding the ventral tegmental nucleus (VTN) (>50%). Smaller depressions in LGU were seen in the inferior colliculi (<10%). Increases in LGU were seen in some regions of the pyramidal tract and the VTN. Qualitative analyses of the relative amounts of glucose sequestered in neural tissue (µCi/g) yielded results consistent with the data on LGU. These data suggest that: (1) concussive brain injury results in heterogeneous regional changes in glucose utilization in brain stem structures of the cat; (2) increases in the functional activity of certain brain stem structures may mediate some of the acute responses to concussive injury. Supported by NIH Grant # NS 12587.

125.9 ELECTRODE PLACEMENT AND METABOLIC EFFECTS OF ELECTROSHOCK. A. L. Miller, D. J. Jones, W. B. Stavinoha and A. T. Modak. Departments of Psychiatry, Anesthesiology and Pharmacology, The University of Texas Health Science Center at San Antonio, San Antonio, TX 78284.

Clinical research on electroconvulsive therapy over the past 15 years has shown that electrode placement is an important parameter influencing the behavioral effects of treatment. Bilateral stimulation, with the electrodes placed over the temporal regions, produces more confusion and memory impairment than does unilateral stimulation, with both electrodes over the non-dominant hemisphere. Unilateral treatment may have slightly less anti-depressant efficacy than bilateral, but this difference is much less pronounced than are the different cognitive effects of the two placements.

To investigate possible biochemical correlates of these behavioral effects, male guinea pigs were given right-sided unilateral or bilateral electroconvulsive shock and sacrificed 7-10 s later in a 915 MHz microwave enzyme inactivator with 1.0 s of irradiation to the head. Hemispheres were processed separately and analyzed for their levels of acetylcholine, cyclic AMP, and a number of intermediates of glucose metabolism. Preliminary results indicate several trends, which require confirmation by further experiments. (1) Levels of cyclic AMP were higher in all shocked animals, as compared to controls, and were highest in the right hemispheres of right unilaterally stimulated animals. (2) Acetylcholine levels were higher in the right hemisphere of right unilaterally stimulated animals. (3) Levels of intermediary metabolites indicated a stimulation of glycolysis in all shocked animals, with no differences between hemispheres.

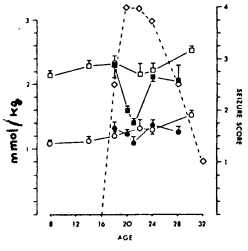
As stated, these results are preliminary and further studies are in progress. To this point, however, it appears that electrode placement does influence some of the biochemical consequences of electroshock.

125.10 CHANGES IN GLUCOSE AND GLYCOGEN CONCENTRATIONS IN BRAINS OF C57BL/6J MICE AFTER AUDIOGENIC PRIMING: POSSIBLE EVIDENCE FOR ENERGY RESERVES DEFICITS UNDERLYING SUSCEPTIBILITY TO AUDIOGENIC SEIZURES. Robert A. Schreiber, Dept. of Biochemistry, Univ. of TN. Center for the Health Sciences, Memphis, TN. 38163, USA.

The onset of susceptibility to audiogenic seizures (AGS) in genetic-developmentally AGS-prone DBA/2J mice coincides with the transition from suckling to dietary self-sufficiency. During suckling the brain is primarily dependent on ketone bodies derived from β-oxidation of fatty acids from the dam's high-fat milk as a source of acetyl-CoA for brain growth and for energy. By the end of the suckling period at 14 to 15 days of age, the brain has reached near-adult size, and begins to shift to carbohydrate-derived sources of glucose as a source of acetyl-CoA for brain.

DBA/2J mice appear to have difficulty in this transition, and as a result, the brain immediate energy reserve, glycogen, is temporarily reduced, with a time course similar to the time course (days) of susceptibility to AGS (Schreiber: J. Neurochem., 1981, in press). An external-stimulus induced large energy expenditure, such as that induced by an appropriate acoustic stimulus to newly opened acoustic pathways, may result in a functional depletion of the already reduced energy reserve during the few seconds until energy repletion processes begin; as a result, CNS activity may then briefly become disorganized, resulting in the onset of an AGS (Med. Hypot., 5, 487, 1979).

Should this heuristic model hold, then audiogenic priming should lead to similar changes in brain glycogen levels during the transient (days) period of susceptibility to AGS. C57BL/6J mice were subjected to 60 sec. of 127 ± 2 dB at 16 days of age. Mice were sacrificed in liquid N_2; 10 mg samples of brain tissue were taken, extracted, and assayed for glucose, glycogen, ATP, and phosphocreatine as previously described (Soc. Neurosci. Abstr., 6, 720, 1980). The developmental course of AGS is shown (diamonds). Pooled preliminary data ± SEM for glucose (circles) and glycogen (squares) are shown for primed (solid) and nonprimed (open) C57BL/6J mice. (N = 8 to 20 parts of brain per point; no significant differences among areas). At 20 and 21 days of age, $p < .05$ for glycogen. No differences have been found between littermate primed vs nonprimed mice in ATP and phosphocreatine.



Supported in part by MH 32583.

125.11 NOREPINEPHRINE DEPLETION ALTERS THE RESPONSE OF CEREBRAL CORTEX TO ISCHEMIA. S.I. Harik, R. Busto* and E. Martinez*. Department of Neurology, University of Miami School of Medicine, Miami, FL 33101.

Locus ceruleus (LC) lesion and the resultant depletion of norepinephrine (NE) in the ipsilateral cerebral cortex results in definite abnormalities of cerebral mitochondrial oxidative metabolism that become manifest under conditions of increased metabolic activity (Harik, et al., Science 206:69, 1979; La Manna, et al., Brain Res. 204:87, 1981). We have recently collected evidence suggesting that this imbalance in oxidative metabolic homeostasis is probably due to impaired substrate availability most likely due to the inability of the NE-depleted cortex to use glycogen during conditions of heightened energy demand, such as status epilepticus. In this study we sought to confirm this possibility under conditions of total cerebral ischemia induced by decapitation.

Unilateral LC lesions were performed in adult male Wistar rats by the stereotaxic infusion of 6-hydroxydopamine into the LC. Two weeks after LC lesion, rats were decapitated and the heads maintained at 37°C for 1 and 2.5 min before immersion in liquid N_2. Bilaterally symmetrical regions of the frozen cerebral cortex were punched out in a cold box at -20°C and tissue samples were assayed for NE, creatine phosphate, ATP, ADP, AMP, cAMP, glycogen, glucose, pyruvate and lactate. Results from the NE-depleted cerebral cortex ipsilateral to LC lesion were compared to these from the contralateral cortex by the paired t test (2-tailed). Average NE levels in the ipsilateral cortex were decreased to about 10% of the contralateral side. In both time periods studied, glycogen was significantly elevated by about 20% ($p < 0.005$) and cAMP decreased by about 25% ($p < 0.001$) in the NE-depleted cortex. These results indicate that cerebral glycogen breakdown during total cerebral ischemia is modulated by central NE. The exact mechanism through which such modulation is effected and whether it is mediated by cAMP remains unknown. (Supported by PHS Grants NS 16617 and NS 05820).

125.12 FOCAL CEREBRAL ISCHEMIA: A NOVEL AND REPRODUCIBLE EXPERIMENTAL MODEL IN THE RAT. R. Busto*, M. Ginsberg*, I. Cendan*, E. Martinez*, and P. Scheinberg (SPON: E. Ramsay). Cerebral Vascular Disease Research Center, University of Miami School of Medicine, Miami, FL 33101

Previous studies of stroke in experimental animals have been hindered by lack of an easily reversible means for producing high-grade focal ischemia resulting in consistent hemodynamic and metabolic alterations. In the present study, we report a novel and reproducible focal cerebral ischemia in the anesthetized rat. Furthermore, the severity of ischemia could be graded. Wistar rats anesthetized with N_2O received unilateral (right) common carotid artery ligation followed by elevation of intracranial pressure to 30-45 mm Hg by the intracisternal infusion of mock CSF. Mean arterial blood pressure was reduced to 110-120 mm Hg by hemorrhage. Cerebral perfusion pressure to the left hemisphere remained at 50-70 mm Hg, whereas right hemispheral perfusion was reduced to the ischemic range. After 15 or 30 min. of ischemia, animals were infused with ^{14}C-iodoantipyrine to measure relative cerebral perfusion, and the brains were frozen transcalvarially for metabolite levels. NADH fluorescence examination of coronal brain sections revealed a consistent right-sided metabolic lesion involving the hippocampus, a wedge of lateral thalamus, and the portion of the neocortex nourished by the middle cerebral artery (MCA). The territory of the anterior cerebral artery was spared. Sharp transition of phosphocreatine and ATP were observed, from values of 4.04 and 2.77 mmol/Kg in the medial neocortex to 0.74 and 0.65 mmol/Kg in the involved lateral cortex, and 0.89 and 0.47 mmol/Kg in the hippocampus. A sharp PhCr and ATP transition was also observed in the thalamic region from values of 2.56 and 1.51 mmol/Kg in the medial thalamus to 0.76 and 0.39 mmol/Kg in the lateral thalamus. ^{14}C-activity in the right MCA territory (neocortex, hippocampus, and thalamus) were 19.8, 18.2, and 30.7% of their respective left hemisphere values. Addition of prior bilateral vertebral artery occlusions to this model accentuated the metabolic gradient observed in the ischemic hemisphere and produced a milder metabolic lesion of the contralateral hemisphere. (Supported by PHS Grant NS 05820).

125.13 MULTIPARAMETER MONITORING OF THE AWAKE CEREBRAL CORTEX EXPOSED TO DECAPITATION.
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In order to study the metabolic, ionic and electrical responses of the awake cerebral cortex to complete ischemia the decapitation model was used. By decapitation the brain is transferred into a closed system in which oxygen input is zero but energy consuming processes are going on until the complete depletion of available energy. The aim of our study was to identify the exact pattern and timing of the various changes taken place in the awake brain under complete ischemia insult. We monitored pO_2, NADH oxidation reduction state, K_e^+, DC potential, Electro corticogram, Temperature and pH from the surface of awake rats (200 gr) and gerbils (60-70 gr). The technical details of the multiprobe assembly are described elsewhere (Friedli, Sclarsky and Mayevsky, in preparation, 1981). Due to the limitation of the cannula size, pO_2 electrode was used alternatively with the pH electrode but the other parameters were monitored in all experiments.

The animal was anesthetized and operated on as described previously (Mayevsky et al. Neurological Res. 1, 213, 1980). After decapitation (done in the awake animal) the first event to appear was an increase in NADH simultaneously with the decrease in pO_2 values. Electro corticogram was decreased gradually and reached its minimal level within 10-20 sec from decapitation, and it was correlated to the increase of NADH as well as a decrease in pO_2. Brain temperature decreased only slightly after decapitation (0.4-0.8°C within the first 2 min). The extracellular K^+ level showed two phases in its increase after decapitiation. The first step was a slow leakage of K^+ (0.5-1 min) followed by a fast efflux of K^+ which occurred at the time when DC potential showed a large negative shift (indicating a complete depolarization). At the same time, when the large negative shift occurred, the 366 nm reflectance trace showed a large increase although the NADH level was at its maximum and pO_2 was zero. The pH response to decapitation was a gradual acidification of the tissue starting a few sec after the decapitation. The DC response to decapitation appeared in the entire area monitored and it was identified by 3 different DC electrodes located near the NADH, pH, and K^+ probes. The same sequence of events in response to decaptitation was recorded also when complete cerebral ischemia was induced by bilateral carotid arteries ligation in the gerbil.

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126.1 INCREASED PAIN SENSITIVITY IN DEPRESSED PATIENTS WITH HYPERSECRETION OF CORTISOL. M. S. Joseph* and V. I. Reus* (SPON: E. Callaway). Langley Porter Neuropsychiatric Institute, San Francisco, CA 94143.

50% of endogenously depressed patients exhibit a failure to suppress plasma cortisol levels after the administration of dexamethasone. Corticotropin (ACTH) has been associated with alterations in experimental pain sensitivity. Depressed patients often demonstrate an increase in somatic pains. To explore the possibility of neuroendocrine basis for this observation we examined experimental pain sensitivity in depressed patients undergoing a dexamethasone suppression test.

Patients admitted over a two month period with the diagnosis of primary depression were tested during their first week of hospitalization in a medication free period. After obtaining informed consent patients received dexamethasone (dex) 1 mg orally at 11:30 p.m. The following day (post-dex) plasma was collected at 8:00 a.m., 4:00 p.m. and 11:00 p.m. and assayed for cortisol, ACTH, beta-endorphin and arginine vasopressin (AVP). At 9:00 a.m. on the post-dex day the patients underwent measurement of experimental pain sensitivity using the submaximal effort tourniquet technique. Each patient also underwent a second baseline pain test either two days before or two days after the post-dex session in a randomized counterbalanced design.

A total of 15 patients were tested. There was no difference between the baseline and post-dex tourniquet times; however, the mean time (in minutes) of the second session, 7.9 ± 6.6, was significantly shorter than the first, 11.0 ± 8.1 ($p < .025$). Because of this order effect only first session times were considered. Examination of cortisol levels showed 9 patients suppressed and 6 were nonsuppressors (cortisol > 5 ng/ml at either 4:00 p.m. or 11:00 p.m.). Each group's respective mean tourniquet time was 14.4 ± 8.2 and 5.9 ± 4.6. This demonstrates a significantly shorter time in the nonsuppressors compared to the suppressors ($p < .001$). The 11:00 p.m. cortisol values were negatively correlated with tourniquet times in nonsuppressors ($r = .819$, $p < .05$).

These data suggest an association between a neuroendocrine marker for depression and pain sensitivity. It is likely that the neuropeptides which are responsible for the cortisol hypersecretion are also involved in pain sensitivity. The above results will be related to concurrent plasma levels of ACTH, beta-endorphin and AVP. (Supported in part by NIMH grant 5 T32 MH14609-05.)

126.2 ELECTROSHOCK (ECS) INDUCED ALTERATIONS OF THYROTROPIN-RELEASING HORMONE (TRH) IN THE RAT. M.J. Kubek, D. Etchison*, and A. Sattin. Dept. Anatomy and Inst. Psychiat. Res., Indiana Univ. Sch. of Med. and V.A. Hosp., Indianapolis, IN. 46223

TRH, the hypothalamic tripeptide responsible for thyroid regulation, has been localized throughout the CNS of man and several animal species. This peptide may play a role as a Neurotransmitter or neuromodulator by interacting with brain monoamine systems. Clinical studies have shown that changes in the TSH response to TRH during ECT can predict the post-treatment course of depression. In view of these findings we examined the effect of ECS and a dopamine agonist, apomorphine (APO) on TRH content in several CNS loci in the rat.

Male S-D rats (150-180g) were given ECS or sham ECS (without current) via ear clip electrodes for 1s. The induction of a grand mal seizure required 22mA (constant current). After 5 treatments given on alternate days at 4PM, all rats were decapitated 45-51h after the last ECS and brains were immediately dissected and frozen on solid CO_2. ECS and sham controls were compared in 3 experiments of 8-14 rats each. One experiment included injection of APO(2mg/kgi.p.) 24h after the last ECS or sham treatment. TRH was assayed by a specific RIA following HAc extraction and results were expressed as pg/mg tissue (mean $\pm$ SEM). Tissue weights were not significantly different between treatment groups. Student's t-tests were performed on log-transformed data for simple and interactional effects in 2X2 tables of the dependent variable.

ECS alone increased TRH content in Ant. pituitary (AP)(5.25 ± 0.56vs9.99 ± 1.08, $P<.001$), post. pituitary (41.27 ± 8.03vs192.65 ± 38.88, $P<.001$), Brain stem (BS)(27.82 ± 2.7vs40.51 ± 5.49, $P<.02$), temporal lobe (TL)(7.06 ± 1.25vs29.02 ± 4.02, $P<.001$), and striatum (8.35 ± 1.36vs15.74 ± 3.12, $P<.001$) but had no effect on hypothalamic (H) or cortical TRH. Similar results were seen in the APO treated group ($R<.001$ in all cases) except that in H TRH decreased two-fold following ECS. APO alone produced a two-fold decrease in TL TRH and was found to potentiate the ECS effects on TRH in AP($P<.01$), BS($P<.001$), and TL($P<.001$).

These results indicate: (1)that ECS, given in a temporal paradigm used clinically, can induce significant elevations in TRH content in the pituitary and several brain loci for up to 50h, (2)APO may have a direct effect on TRH in TL, and (3)APO, given as a single dose 24h after the last ECS, can enhance the ECS effects on TRH in specific loci. Thus, elevations in brain TRH may be a favorable consequence of ECT treatment for depression since it has been shown that TRH has an overall excitatory effect on CNS activities. Supported by M.H.R.& E. Indpls. Biomed. R. and MH 29126.

126.3 VASOPRESSINERGIC MODULATION OF SHORT-TERM BEHAVIOURAL HABITUATION: DOSE, AGE, STRAIN AND SEX DEPENDENT ACTIVATING AND DE-ACTIVATING EFFECTS. A.G.Sadile, A.Cerbone* and L.A.Cioffi*. Inst. Human Physiology, 1st Med. Sch., Univ. Naples, 80138-Italy.

A diffuse network of vasopressinergic terminals has been shown to synapse in the hippocampal formation, among other extra-hypothalamic target areas (Buijis, R.M.,Cell Tissue Res., 192:423, 1978). In the present research, the physiological significance of these peptidergic pathways in the control of behavioural output has been investigated throughout postnatal development and adulthood in two strains of genetically selected rats, the Naples Sprague-Dawley High and Low Excitable (NSD-HE and -LE) of both sexes.

Dose-response curves for acute effects of a single lysin-8-vasopressin dose (LVP, 4.4, 44 and 440ng.100g b.w.$^{-1}$, s.c.) or saline, given immediately before a 10min-exposure to a novel environment, upon short-term habituation (ST-HAB) of behavioural arousal, were obtained by trend analysis of horizontal and vertical activity by a PDP11/34 computer (Sadile, A.G. et al., Abh. Akad. Wiss. DDR, 5:205, 1979). In NSD-HE strain LVP is de-activating and as early as the 16PND with 440ng only in females and this effect persists at 18PND, when it matures also in males and when 44ng become de-activating in females. In adult age (60PND) the de-activating effect of the highest dose is still present in both sexes and is accompanied by hypotonia, ataxya and sedation. In NSD-LE rats, pre-trial LVP has biphasic effects: it is activating at 16PND and only in females with 4.4 and 44ng, whereas at 18PND remains activating only in females and 44 and 440ng become de-activating, the latter only in females and the former in both sexes. At 60PND the 440ng dose has a significant de-activating effect only in females, with no behavioural abnormalities, as in the NSD-HEs.

The differential, multifactorially dependent, activating and/or de-activating acute effects of pre-trial lysin-vasopressin are suggested to be due to activation of different VPergic receptor and circuitries, which seem to mature at different developmental periods, at various organizational levels, in a genotype- and sex-dependent way.

126.4 THE EFFECT OF REM SLEEP DEPRIVATION ON SUBSTANCE P-LEVELS AND SOMATOSTATIN IN DISCRETE AREAS OF THE RAT BRAIN. L.A.Mattiace,J.Farber,A.Negro-Vilar,Departments of Psychiatry and Physiology, University of Texas Health Science Center at Dallas, Dallas, Texas 75235. (SPON: H.P. ROFFWARG).

Although peptide substance P has been extensively mapped in the periphery, it has recently been demonstrated to be located in discrete cell bodies and terminals of the CNS.Similarly, somatostatin is widely distributed in the brain and has been localized in primary sensory neurons.(Hokfelt,1976).However, the functional role of these peptides in the CNS is as yet unknown.It has been suggested that, as in the periphery, substance P may be involved in the sensory processing of pain:(Henry,1977).One method of altering sensory processing is through REM deprivation, which appears to increase neural excitation (Cohen &Dement,1965) and also waking motivational behaviors (Dement,1965;Morden et al;1968; Steiner & Ellman,1972).Using the pedestal-water technique of REM sleep deprivation (Jouvet,1964),Sprague-Dawley male rats were kept for 3 days either on a small platform (n=5)or on a large stree-control platform (n=5).The rats were sacrificed by decapitation and the brains quickly removed and frozen.Several brain areas were punched according to the Palkovits(1973)procedure. Substance P and somatostatin were extracted in 1N acetic acid and measured by radioimmunioassay.Levels of peptides were determined in a number of areas throughout the brain including the forebrain areas of the caudate,preoptic and medial hypothalamus;the midbrain areas of the medial habenula(MH), substantia nigra pars compacta (NRC) and reticulata (SNR),ventral tegmental area (A10); and the hindbrain areas of the dorsal (DR) and medial raphe (MR), central grey (CG) and the locus coeruleus (LC).Decreases in substance P levels were seen in most neuroanatomical areas examined in REM deprived animals, with greater differences observed in the SNR,RD,LC and MH.Measurements of another neural peptide,somatostin, in the same areas revealed an increased in peptide levels with REM deprivation in the more rostral regions examined (preoptic,medialhypothalamus and MH).However, in the more caudal regions, a clear decrease in somatostatin levels was observed, particularly in the CG,LC,MR and DR.The decreases in levels of substance P following REM deprivation may indicate a change in centrally-mediated pain sensitivity.Support for this notion is suggested by changes in pain sensitivity thresholds following both REM deprivation(Hicks,et al,1973;Hicks et al,1978;Hicks et al, 1979)and hindbrain lesions(Bodnar et al,1978).REM deprivation, in addition to changing catecholamine levels also appears to affect peptide levels such as substance P and somatostatin.Accordingly, the biochemical and neurochemical changes that accompany REMD do not appear to be confined to the monoaminergic neurotransmitters in the CNS.

126.5 BOMBESIN WORKS AT BOTH ENDS. J. Gibbs and D.J. Fauser*. Dept. of Psychiatry, Cornell University Medical College and E.W. Bourne Laboratory, The New York Hospital, White Plains, NY 10605.

Bombesin (BBS)-like immunoreactivity has been identified in neural and/or endocrine tissue throughout the gastrointestinal tract of several species, including rat and human. The concentration of BBS-like immunoreactivity is high in colon. While carrying out experiments to determine the satiating effect of BBS and cholecystokinin on feeding behavior, we noted a markedly increased rate of defecation following administration of exogenous BBS.

Eleven male Sprague Dawley rats were injected intraperitoneally with synthetic BBS (courtesy of R. de Castiglione, Farmitalia Carlo Erba, Milan), the synthetic C-terminal octapeptide of cholecystokinin (CCK-8, courtesy of M. Ondetti, Squibb Institute, Princeton) or equivolumetric 0.15 M NaCl control 5 min before presentation of a balanced liquid food (25% EC 116, GIBCO) during the light phase following a 3-hour food deprivation; drinking water was always available. In additional tests, the same rats were similarly injected 5 min before presentation of drinking water following a 12.5-hour water deprivation; food was removed 30 min before water presentation. During all tests, defecation was measured by counting boluses at 5, 15, 30, and 45 min following food or water presentation.

When food was present and being consumed, BBS produced a marked, dose-related increase in number of formed boluses excreted. The threshold dose of 2 $\mu g\text{-}kg^{-1}$ produced a five-fold increase during the entire 45 min period ($P < .001$). The maximally effective dose of 8 $\mu g\text{-}kg^{-1}$ produced a ten-fold increase ($P < .001$). The latency for a significant increase was 15 min. When water was consumed instead of food, the shape of the dose-response curve was identical, but the potency of BBS was reduced at each point by approximately 50%. At no time did BBS produce diarrhea. CCK-8 (1.5 $\mu g\text{-}kg^{-1}$ and 3.0 $\mu g\text{-}kg^{-1}$) also significantly increased bolus excretion, but CCK-8 was less potent than BBS and failed to produce a dose-response relationship.

The strikingly increased rate of defecation produced by exogenous BBS, particularly when food is being consumed, together with radioimmunoassay data demonstrating high concentrations of BBS-like immunoreactivity in colon (Walsh & Dockray, Gastroenterol. 74:1108, 1978), raises the possibility that one function of endogenous BBS-like peptides may be to play a role in the colonic response to eating ("gastrocolic reflex").

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126.6 NEUROPEPTIDES IN ANIMAL MODELS OF AGING. R.L. Dean, C. Loullis, D.L. Watkins*, and R.T. Bartus. Department of CNS Research, Medical Research Division of American Cyanamid, Pearl River, NY.

In recent years, evidence has accumulated that neuropeptides of hypothalamic and pituitary origins may influence behavior, independent of their neuroendocrine effects. It has been reported that, in several mammalian species, several of these neuropeptides affect many of the same behaviors which are significantly altered in the elderly. During the last few years, we have developed a number of animal models of aging based on a multidisciplinary set of criteria involving behavioral, biochemical and pharmacological factors. Several different neuropeptides (such as $ACTH_{4\text{-}10}$, vasopressin, oxytocin, etc.) were tested in these procedures over a wide range of doses to help characterize their behavioral and biochemical effects.

The results of these studies demonstrated that these neuropeptides indeed possess many interesting and differential qualities which may be relevant to correcting or compensating for certain age-related, neurobehavioral dysfunctions. These include: (1) protection of neural function under various conditions of energy deficiency, (2) alterations in certain neurotransmitter functions in rats, and (3) reduction in age-related deficits on a memory task in monkeys. These findings will be discussed as they relate to possible direct and indirect influences on neurotransmitter function in the young and aged brain.

126.7 TRIGGERING AND MODULATION OF THE CENTRAL PROGRAM FOR LOCOMOTION BY SEROTONIN AND BAG CELL EXTRACT (EGG LAYING HORMONE). S. Mackey*, T.J. Carew, and E.R. Kandel. (SPON: I. Kupfermann). Center for Neurobiol. & Behav., Dept. Physiol. & Psychiat., P & S, Columbia Univ., and N.Y. State Psychiatric Inst., New York, N.Y. 10032

Locomotion is a centrally programmed behavior in Aplysia which is triggered by appetitive and aversive stimuli and is modulated by hormonal states (Strumwasser et al., 1969) and learning (Walters et al., 1979). Thus locomotion is a useful system to explore the cellular mechanisms involved in the triggering and modulation of a complex behavior. As a first step in this direction we have examined the triggering effects of several biogenic amines and the modulating effects of bag cell extract (BCE) which contains the neuropeptide egg laying hormone.

We recorded locomotion in a modified split foot preparation (Henning et al., 1979) with strain gauges attached to the body wall and foot. After a 5 minute baseline period, we injected 1 ml. of a solution containing 5HT into the anterior aorta. A 1 ml. injection of sea water was used as a control. Threshold for locomotion was 10^{-7} 5HT which produced $\bar{x}$=1.5 steps (N=4) within 90 seconds of application compared to 0 steps (N=5) after a 1 ml. injection of sea water ($p < .05$). Increasing the concentration of 5HT produced (1) a monotonic increase in the number of steps, reaching $\bar{x}$=25.0 at 10^{-3} 5HT (N=5), and (2) a progressive decrease in the latency for locomotion. 5HT injected into the anterior aorta of freely moving, intact animals also elicited walking. Three other biogenic amines found in Aplysia (dopamine, octopamine, histamine) failed to elicit locomotion in concentrations up to 10^{-3} in preparations in which 10^{-3} 5HT produced vigorous locomotion.

We next explored the actions of BCE and found that it suppressed 5HT-elicited locomotion. We first triggered locomotion with an injection of 1 ml. 10^{-3} 5HT. Twenty minutes later a second injection of 5HT was delivered. In the experimental group (N=5) the second injection was preceded (by 2 min) by a 1 ml. injection of BCE. In the control group (N=7) a 1 ml. sea water injection replaced BCE. Experimental animals showed a 52% decrease in the number of steps following BCE compared to a 6% reduction in controls ($p < .002$). The suppressive effects were reversible. The number of steps produced by 5HT in experimental animals usually approached initial (pre-BCE) levels within 40 minutes.

Our results suggest that 5HT might be an endogenous trigger for locomotion in Aplysia. Since 5HT elicited locomotion is significantly inhibited by prior application of BCE, this should enable the study of the triggering and modulation of locomotion on a cellular level. It will also be of interest to examine the effects of BCE on withdrawal reflexes and inking in Aplysia in an attempt to characterize the modulatory effects of a known neuropeptide on a variety of defensive behaviors.

126.8 THE DISCOVERY OF A NEW ENDOGENOUS LIGAND FOR BENZODIAZEPINE RECEPTORS. D. Jackson*, B. Beer, L.R. Meyerson and A. Lippa. Dept. CNS Research, Medical Research Div. Lederle Labs, Pearl River, NY 10965.

The discovery of a pharmacologically relevant, high affinity binding site for benzodiazepines (BDZ) has raised the possibility that these sites may act as receptors for some as yet unidentified neurotransmitter systems. Several endogenous substances (i.e., purines, nicotinamide and an unidentified peptide) have been isolated from mammalian brains and are able to competitively inhibit ^{3}H-BDZ binding. Since various hormonal systems are able to modulate the anxiolytic effects of BDZ, we have searched for possible endogenous ligands in the blood of rats and humans. While crude serum is able to displace ^{3}H-BDZ binding, the presence of albumin may contribute to this effect. Albumin was removed from serum by either boiling (100°C for 30 minutes), acid precipitation (5% TCA) or ultrafiltration (with molecular weight exclusion of 20,000 daltons). In each case, approximately 30-40% inhibition of ^{3}H-flunitrazepam binding (^{3}H-FLU) was observed. Scatchard analysis demonstrated that extracts competitively inhibited ^{3}H-FLU binding. Molecular weight determinations using HPLC revealed the presence of two active fractions which co-eluted with globular proteins of 3,000 and 18,000 daltons. The hydrophilic nature of these fractions was revealed by exposure to a hydrophobic medium. Furthermore, inhibitory activity was reduced by >50% after exposure to 100°C for 10 minutes. These results demonstrate the presence in mammalian blood of hydrophilic, thermo-labile proteins which competitively inhibit ^{3}H-BDZ binding. The larger component may act as a precursor for the smaller.

127.1 THE DORSAL SUPRAOPTIC DECUSSATION AND INTEROCULAR TRANSFER IN THE PIGEON. M. A. Goodale and D. Stewart*. Psychology Department, University of Western Ontario, London, Ontario, N6A 5C2.

Pigeons trained to discriminate between visual stimuli in a key-pecking task normally show excellent interocular transfer (IOT) when trained with only one eye and then tested with the "naive" eye. However, when the dorsal supraoptic decussation (DSO) was sectioned and pigeons were then trained to peck at illuminated keys in an interocular transfer experiment, they failed to show normal IOT of a simultaneous horizontal-vertical discrimination. In contrast, another group of pigeons, in which the DSO was sectioned after they had already learned the discrimination with one eye covered, showed excellent IOT post-operatively when the blindfold was moved to the other eye. Both groups later failed to show IOT on a new visual discrimination. The results of this experiment confirm and extend earlier observations in the domestic chick (O'Connell, N. unpublished Ph.D. thesis, University of Rochester, 1979).

In a second experiment high-speed films were made of pigeons pecking at illuminated keys in a discrimination task. A subsequent frame-by-frame analysis of these films revealed that the discriminative stimulus fell within the binocular portion of the retina during the final two head fixations immediately prior to pecking. Even when the pigeons were pecking at the keys while wearing a monocular blindfold, they regarded the stimulus key with the portion of their uncovered eye that was normally binocular.

Since the DSO carries input from the binocular portion of the retina, the results of the two experiments taken together strongly suggest that IOT in the pigeon is the simple consequence of information reaching both hemispheres from a single eye via converging binocular pathways. Thus, cutting the DSO before training with the first eye eliminates IOT whereas cutting it after training with the first eye does not -- since information has already reached both hemispheres from the (normally) binocular portion of that eye. These findings complement the work of Goodale and Graves (Physiol. Behav., 25:39-43, 1980) showing that IOT is absent in normal pigeons in those situations in which they scan the discriminative stimuli with the monocular portion of their visual fields.

This investigation was supported by Natural Sciences and Engineering Research Council (Canada) A6313.

127.2 A TACHYSTOSCOPIC STUDY OF INTRA- AND INTERHEMISPHERIC PROCESSING OF VISUAL INFORMATION IN CALLOSAL AGENESIS. Hannelore Sauerwein*, Maryse Lassonde and Guy Geoffroy.* Laboratoire de Neuropsychologie expérimentale, Université du Québec à Trois-Rivières, C.P. 500, Trois-Rivières, Qué., Canada and Ste-Justine Hospital, Montréal.

Numerous studies using tachystoscopically presented stimulus material in normal individuals have established the presence of a right hemifield/left hemisphere superiority for verbal and a left hemifield/right hemisphere advantage for non-verbal stimulus processing. They have further shown that information simultaneously presented to the two visual half fields is accessible to both hemispheres due to the integrative function of the cerebral commissures, especially the corpus callosum. In contrast, tachystoscopic studies of patients suffering from agenesis of the corpus callosum have generated conflicting results concerning cerebral dominance and interhemispheric integration of visual information.

The purpose of the present study was to further elucidate the problem of cerebral asymmetry and interhemispheric communication in callosal agenesis patients. The agenic group was composed of 5 subjects, aged 12 to 22, with total agenesis of the corpus callosum as revealed by CT scan. The agenic patients were compared with two matched control groups: one group of individuals with comparable IQs and a second group of subjects with normal intelligence. The experimental task consisted of verbal identification or intra- and interocular comparisons (same-different judgements) of letters, numbers, shapes and colors presented tachystoscopically to either or both peripheral visual hemifields. Error scores and reaction times were monitored.

In the intra-hemispheric conditions both control groups displayed typically a right hemifield advantage for the verbal and a left hemifield effect for the non-verbal material. This differential response was not found in the agenic sample. The agenic patients made also significantly more errors and were markedly slower in their responses. On the interocular comparison task all groups performed above chance level. However the performance of the agenic patients was once again significantly poorer than that of the controls with respect to correctness and response latencies.

The absence of unilateral effects in the agenic sample seems to suggest a bilateral representation of function in these patients. Furthermore, the fact that the agenic subjects were able of correct interocular comparisons would point to the existence of an alternative pathway of interhemispheric transmission. This pathway, however, may be less efficient and less expedient that the callosal route.

127.3 DICHOTIC LISTENING IN CALLOSAL AGENESIS. Jean Lortie,* Maryse Lassonde and Maurice Ptito. Laboratoire de Neuropsychologie expérimentale, Université du Québec à Trois-Rivières, C.P. 500, Trois-Rivières, Qué. Canada, G9A 5H7

A previous study (Lassonde et al, 1981) has shown that two patients suffering from a congenital absence of the corpus callosum demonstrated a left ear advantage in a dichotic paradigm regardless of the nature of the stimuli. However, other experiments conducted in our laboratories (Sauerwein et al, 1981) revealed a bilateral organization of speech in the visual and somesthetic modes when callosal agenesis patients were tested. Therefore, in order to assess if the right hemisphere superiority effect could be generalised to all acallosal subjects, another auditory study was conducted using a larger sample of subjects and stimuli.

Five subjects, 3 boys and 2 girls were included in the study. All were right-handed except M.G. who showed a left-hand preference. The subjects' age varied between 11 and 23 years. All patients tested had a complete callosal agenesis as revealed by P.E.G. or CT scan. The subjects' IQ varied between 45 and 97.

Four types of stimuli were presented dichotically. They consisted of verbal material (familiar words and nonsense syllabes), and non-verbal stimuli (pure tones varying between 400 Hz - 17KHz and melodies). Each type of stimulus was presented 125 times, the first five trials being used as examples. After hearing the sample stimuli, S was presented with a binaural test stimulus. The subject's task was to decide whether or not the test stimulus was previously heard. The first administration required a verbal response. In a subsequent session the subject answered using the preferred hand. The technical set-up used in the experiment was automatized in such a way that the subjects could initiate their own trials.

An analysis of variance performed on the reaction times revealed that they varied according to the stimulus type (words, syllables, tones and melodies). The subjects were slower to respond to the melodies than to any other stimulus. Interestingly, the mode of response (manual or oral) had no significant effect nor did the stimulation site (left or right ear). Indeed no differences were found between the left or the right ear.

In opposition to previous findings (Ettlinger et al, 1972; Bryden and Zuriff, 1970; and even Lassonde et al, 1981), it therefore seems that auditory hemispheric organization in callosal agenesis patients is bilateral. The hemispheric "symmetry" could explain the characteristic slowness of response usually observed in callosal agenesis (e.g. Lassonde et al, 1981); Indeed, data processing might be delayed in these patients because of a crowding of verbal and non-verbal functions in both hemispheres.

127.4 DEPTH PERCEPTION DEFICITS IN CALLOSAL AGENESIS. Maryse Lassonde, Sylvain Bernier*, Jean Ouellet* and Michel Volle*. Laboratoire de Neuropsychologie expérimentale, Université du Québec à Trois-Rivières, Trois-Rivières, Qué., Canada, G9A 5H7.

Midline stereopsis has sometimes been reported to be deficient in patients suffering from a congenital absence of the corpus callosum (Jeeves, 1980). Similarly, a loss of binocular depth perception in the vertical meridian has been observed in a commissurotomized patient. Recently, however, the possibility was raised that at least in animals, the influence of the corpus callosum in binocular integration extends well over the vertical meridian (Payne, 1980). In order to test this assumption, we studied intra- and interhemispheric analysis of depth perception in 6 patients suffering from a total callosal agenesis. They were matched on age, sex and IQ with 6 control SS. Two solid objects differing only by their color and occasionnally their size were presented a) at 3.5° of visual angle, one to the right and the other to the left of a central fixation point (diode, 3.5 volts); b) one at 3.0 and the other at 4°, to the left or the right of the fixation point; c) one at 0.5° and the other at 3.5° to the left or the right of the fixation point; d) one to the left, the other to the right of the fixation point, the distance between both objects being less than 1°. The objects were placed on a table whose heigth corresponded to the S's eye level when seated. In the vertical plane, the objects were kept 10 cm apart. The room was completely dark and a photostimulator (Grass model PS2) located 3m above the table allowed the illumination of the objects during 150 msec. Sixty four trials were used for each condition and S's task consisted in indicating which stimulus was closer. The results showed that when the objects were presented in the peripheral field, (conditions a) and b)) the mean number of correct responses was significantly lower in the experimental group than that of the control group. Furthermore, a chi-square analysis performed on the data obtained by the agenic patients revealed that the acallosal subjects performed at chance level in both conditions. On the other hand, when at least one object was presented centrally (conditions c) and d)) the performance of the agenic subjects was above the chance level. However, their number of correct responses was once again significantly poorer than that of the control group. Noteworthy, the agenic patients were markedly better (70% of success) when both objects were presented centrally. These results confirm the findings that the involvement of corpus callosum in depth perception extends over the whole retina and further indicates that its role in binocular integration is more important in the periphery of the visual field. Finally, our results suggest that callosal disruption prevents the development of normal depth perception.

127.5 ANATOMICAL AND QUANTITATIVE ASPECTS OF THE HUMAN CORPUS CALLOSUM
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While there is an ever-increasing interest in the neuropsychology of interhemispheric relations, there are few anatomical data on the major cerebral commissure -- the corpus callosum. Our study was undertaken to investigate the topographical organization and laminar termination of the fibers of this commissure. In addition, we obtained quantitative measurements of male and female corpora callosa. Methods included computer-assisted planimetric measurements of total and partial callosal surface areas as well as a modified trichrome stain, general histological stains, and the Albrecht Fernstrom silver stain to respectively delineate extent of lesions, isolate callosal degeneration and identify terminating degenerated fibers.

The study of degeneration in the corpus callosum subsequent to focal cerebral insult, occuring generally 1-2 years antemortem, evidenced that while orbital, inferior frontal and pre- and post-central fibers course rostral to the superior and middle frontal fibers,the inferior, middle and superior temporal fibers course caudal to the frontal fibers but rostral to the occipital and parietal fibers. Selective silver impregnation of degenerated fibers in contralateral homotopic cortex demonstrated that callosal fibers terminate mainly in layers IV and V.

Planimetric measurements of corpora callosa (N=28;M=15,F=13) yielded an average callosal surface area of 662.2 sq.mm (min. 444; max. 962; std. dev. 120.9; 10-15% shrinkage was observed). A quantitative analysis of the relationship between callosal surface area and brain weight evidenced a sexual dimorphism in that the female but not the male callosal area correlated significantly with brain size (Pearson R=.47(F),.05(M)). Furthermore, a computation of maximum dorsoventral splenial width demonstrated a nearly bimodal distribution for males and females (range .9-1.41cm(M);1.4-1.8cm(F); p=.000). Planimetric measurements of the partial surface area of the posterior fifth of the corpus callosum (determined to be representative of splenial area) substantiated our observation that females have a larger splenium than males(p=.05). This sex difference is actually visually obvious: the female splenium is more bulbous than the male counterpart. We believe that our callosal topography may be clinically significant both in the evaluation of neurological syndromes and in recovery of function. Our finding of sexual dimorphism in the callosum may have neuropsychological implications for postulated gender differences in degree of cerebral lateralization.

127.6 HEMISPHERIC ASYMMETRY IN VISUO-SPATIAL PROCESSING: DIFFERENTIAL EFFECT ON REACTION TIME, ACCURACY, AND CRITERION. B. Koss. Department of Psychology, Queens College, City University of New York, New York, N.Y. 11367.

An investigation was carried out on six normal subjects to study the relative influence of perceptual difficulty and decisional strategies on cerebral asymmetry in recognition of line orientation. The dependent variables included reaction time (RT) for correct response, percentage of accurate response and two signal detection measures based on rating scales. Stimuli were black rectangles flashed tachistoscopically in the left or right visual fields (LVF, RVF) under two stimulus conditions. In condition vertical-oblique (V-O), the rectangles were oriented 90 or 95 degrees from the horizontal, whereas in condition oblique-oblique (O-O), rectangles were oriented 95 or 100 degrees from horizontal. In both conditions, subjects responded to the 95 degree stimulus by depressing a telegraph key.

With accuracy, the results support the often hypothesized right hemisphere superiority for visuo-spatial processing. The positive results obtained with accuracy, reputedly rather insensitive to laterality differences, were attributed to a combination of enhancing factors: use of male subjects, control of eye dominance, perceptual difficulty of the task and memory factors. In contrast, no difference was observed between the performance of the two hemispheres with RT. However, a highly significant interaction between VF and stimulus condition indicated that the easier condition V-O was processed faster when presented in the LVF whereas the more difficult condition O-O was processed faster when presented to the RVF. Results can be best explained by an interaction between task difficulty (leading to a right hemisphere superiority for the V-O condition) and the use of verbal coding allowing fast processing by the left hemisphere when task complexity increases. This interpretation is confirmed by signal detection analysis, which showed that different decisional strategies were used by the two cerebral hemispheres. The apparent superiority displayed by the left hemisphere for the more difficult task was the expression of more liberal judgmental standards, and not the greater competence of that hemisphere. The absence of overlap between results obtained with accuracy and RT suggests that these measures provide qualitatively different expressions of laterality differences. This experiment suggests that laterality studies should include testing with several simultaneous measures in order to distinguish between differences due to cognitive or to decisional strategies.

127.7 DIFFERENTIAL HEMISPHERIC PARTICIPATION: A CASE DEMONSTRATING VOLUNTARY SELECTION. E.C. Hughes, P.S. Gott,* and K. Whipple. Departments of Otolaryngology, Neurology, and Psychiatry, LAC-USC Medical Center, Los Angeles, CA 90033.

An extraordinary woman reported she could instantaneously, "at will" switch between two disparate mental and emotional states differentiated by features suggesting left hemisphere predominance in one, right in the other. She was extensively studied to elucidate characteristics and document the psychophysiological correlates of her separate states. The subject was an apparently normal female with no detectable neurological or psychiatric disorder.

Her self description of the two states provided a clear dichotomy of interests and capabilities. Formal psychological testing revealed no split personality traits. While her I.Q. and other broad mental capacities were superior and generally equal in both states, marked differences in performance between states were evident on specific tasks. In "State I" she was superior in sequential or verbal tasks, whereas in "State II" spatial tests were more accurate.

Electrophysiological tests documented differences in brain activity between states. Amplitude of the EEG alpha activity showed a large difference between states. Moreover, comparison of hemisphere alpha ratio (R/L) showed significant hemisphere differences correlated with the state held during spatial and verbal tasks. A Vibrotactile Somatosensory Evoked Response and the concomitant measurement of interhemisphere transmission time provided further data indicating differences between states.

Results support the probability that the subject can voluntarily place herself in one of the two states, I or II, associated with left or right hemisphere predominance, respectively. Her capacity for differential hemisphere participation may account for her somewhat exceptional talents in both spatial and verbal skills.

127.8 ELECTROPHYSIOLOGICAL AND NEUROPSYCHOLOGICAL CORRELATES IN PATIENTS WITH SECTION OF THE CORPUS CALLOSUM. Guy Geoffroy*, Maryse C. Lassonde, Hannelore Sauerweir* and Janine Flessas.* Ste-Justine Hospital, Montreal and Laboratoire de Neuropsychologie expérimentale de l'Université du Québec à Trois-Rivières.

It has often been suggested that sectioning of the corpus callosum prevents the spread of epileptic activity from one hemisphere to the other. In line with this assumption, lesion of the corpus callosum (callosotomy) and of many cerebral commissures (commissurotomy) have been performed therapeutically in patients suffering from intractable epilepsy. However, its effectiveness in reducing both the frequency and severity of the seizures has often been contested and neuropsychological sequelae have sometimes been reported. The present analysis focuses on nine patients, aged 5-16 years whose epilepsy resisted all conventional means of treatment. The purpose of the study was to examine, by employing extensive pre- and post-surgical evaluations whether or not this technique can really be considered as effective. Electroencephalography revealed multifocal activity in five and right-sided epileptic foci in four of the patients. The patients underwent a callosotomy according to the technique of Wilson. The method of investigation varied according to the physical and mental stage of the patient from observation and simple operations to complete neuropsychological examination, using the Michigan and the Reitan batteries as well as other tests designed to determine cognitive and perceptual skills, sensori-motor functions, cerebral dominance and interhemispheric communication. Postoperative EEGs revealed a significant reduction of epileptogenic activity in all but one of the patients. These findings were accompanied by an important reduction in both frequency and severity of epileptic seizures and a marked improvement in the general behavior of the patients. Furthermore, the reduction of anticonvulsive medication probably contributed to the observed improvement of memory, perceptual and motor functions in the majority of the cases. All patients achieved pre-operative levels of performance with regard to bimanual coordination and interhemispheric transfer of tactile and somesthetic information. However, post-operative tests of laterality showed temporary or persistent reversal of manual dominance in three and reversal of manual and ear preference in one of the patients. These results suggest that hemispheric disconnection with anterior commissure sparing can be successful in the control of epileptic seizures. Furthermore, the intervention seems to improve the patient's cognitive and motor behavior. Finally, the reversal of lateral dominance observed in some of the patients indicates that the corpus callosum may exert an inhibitory effect on the activity of the non-dominant pathway which may be neutralized by the callosal section.

127.9 INFORMATION PROCESSING FOLLOWING BRAIN BISECTION. J. D. Holtzman* and M. S. Gazzaniga. Division of Cognitive Neuroscience, Department of Neurology, Cornell University Medical College, New York, New York 10021

In a previous study, we reported that the commissurotomy patient has access to spatial information from both visual half-fields for the control of selective visual attention. We also found that, in contrast, when spatial information was required for explicit stimulus localization, the visual half-fields were disconnected: Significant impairments in performance were noted for tasks which required a comparison of the locations of stimuli appearing on both sides of the visual midline.

The present studies were designed to examine further the independence of perceptual processing in the disconnected hemispheres. The performance of commissurotomy patients at bilateral concurrent tasks has typically focused on tasks which include a bilateral motor component. Our studies assessed the information-processing capabilites of the two hemispheres independent of possible interactions at the output stage. On each trial two series of three items were presented sequentially, one series to each hemisphere via lateralized visual presentation, with an inter-item interval of 500 msec. On half of the trials, the two series contained the same elements in the same order; on half of the trials, a different series appeared in each field. Following a 1 sec delay, a probe item appeared in one visual field and the observer was required to indicate with a forced-choice key press whether the probe was an element of that field's list.

The performance of two commissurotomy patients did not differ significantly for our two conditions: There were no reductions in response latencies or errors when both hemispheres were concurrently engaged at processing identical lists. This finding contrasts with the performance of normal observers, who show facilitation in performance when the two visual half-fields contain identical information.

It was also determined that, although the hemispheres appeared to acquire the two lists independently, the performance of one hemisphere was found to vary when the cognitive load was manipulated in the other hemisphere. Our findings are consistent with the view that, while activation of the disconnected hemispheres is derived from a common resource pool, the cerebral hemispheres maintain functional independence at the level of stimulus processing. (Aided by USPHS Grant NS15053 and the Alfred P. Sloan Foundation.)

128.1 CHARACTERISTICS OF THE VENTROMEDIAL HYPOTHALAMIC OPIATE RECEPTOR ASSOCIATED WITH FEEDING AND TEMPERATURE RESPONSES IN THE RAT. F.S. Tepperman, M. Hirst and C.W. Gowdey*. University of Western Ontario, London, Ontario, Canada.

In rats, ventromedial hypothalamic (VMH) injections of small quantities of morphine initiate a protracted feeding response and hyperthermia. The present studies were undertaken to determine whether these responses require stereoselective, specific interactions with narcotic agonists.

Male Sprague-Dawley rats were housed individually and maintained on a 12 hr light-dark cycle. All were implanted stereotaxically with a cannula which extended to the right VMH. The animals became accustomed to being handled and sham-injected before experiments were initiated. On experimental days the free-feeding rats were injected between 12:15 and 12:45 p.m. with the appropriate drugs, always in a volume of 0.5 µl sterile, pyrogen-free saline. Food eaten and core temperature were recorded hourly for 3 hours.

In the first series, a Latin square design was used to arrange treatment of animals with levorphanol, its stereoisomer dextrorphan, and codeine. Levorphanol stimulated feeding significantly more than dextrorphan or codeine, but core temperature remained unaffected.

In a second series, another group of animals were treated in a Latin square design with saline, or equimolar doses of morphine, ketocyclazocine or phencyclidine. Only morphine significantly increased both feeding and temperature.

In a third series, animals were treated with saline, morphine, or various doses of D-ala^2-D-leu^5-enkephalin (a Type II opiate receptor agonist). Our data suggest that this peptide can increase food intake and core temperature over saline controls.

The results demonstrate that the opiate receptors in the VMH involved in feeding appear to exhibit stereospecificity and to be sensitive to µ (and perhaps δ) opiate agonists rather than to κ or σ agonists . Furthermore, Type II receptors may have a role in this response. Our findings also indicate that enhanced feeding need not be integrally related to increased temperature, for levorphanol can increase feeding without altering body temperature. (Supported by the Medical Research Council of Canada)

128.2 LACK OF OPIATE-SENSITIVE FEEDING SYSTEM IN HAMSTERS. M. T. Lowy* and G. K. W. Yim (SPON: L. Pellegrino), Department of Pharmacology and Toxicology, School of Pharmacy and Pharmacal Sciences, Purdue University, West Lafayette, Indiana 47907.

Endogenous opioids (EO) appear to be involved in consummatory behavior as opiate antagonists, such as naloxone, attenuate various types of feeding and drinking behavior, while opiate agonists stimulate food and water intake. Since most of these studies have used rats or mice, it was of interest to examine the effect of various opiate agents on the consummatory behavior of male golden hamsters. Four daily injections of the long-lasting opiate antagonist, nalrexone (NTX; 1 and 10 mg/kg) significantly decreased rat, but not hamster daily food and water intake. Hamsters increased food intake following insulin (50 U/kg) administration, but not after 24 hr food deprivation or 2-deoxy-D-glucose (800 mg/kg) injection. NTX, 1 and 10 mg/kg, had no effect on the feeding of these hamsters. However, NTX, 1 and 10 mg/kg, markedly attenuated hamster drinking induced by 48 hr water deprivation or hypertonic saline injection. Dexamethasone (DEX), a glucocorticoid which depletes pituitary β-endorphin and produces anorexia in rats had no effect on hamster daily food intake. Since the normal feeding profile of the hamster is similar to that of naloxone and DEX treated rats (Life Sci. 26: 2113; 27: 2553, 1980), hamsters appear to lack an opiate sensitive feeding system. In support of this, hamsters did not increase food or water intake following administration of morphine (6.25 - 800 mg/kg) or ketocyclazocine (0.26 - 16 mg/kg), opiate agonists which increase food and water intake in rats (Neurosci. Abs. 6: 528, 1980). In contrast, some aspects of hamster drinking behavior are opiate-sensitive. Thus, there are marked species differences concerning the involvement of EO in consummatory behavior. (Supported in part by Pharmacology/Toxicology Training Grant GM-709504 and American Cancer Society Grant CH-194.)

128.3 NALOXONE INDUCED SUPPRESSION OF FOOD INTAKE IS POTENTIATED BY NEONATAL ADMINISTRATION OF MONOSODIUM GLUTAMATE TO MICE. R. Dawson, Jr., and Z. Annau. Dept. of Environ. Hlth. Sci., Division of Toxicology, The Johns Hopkins University, Baltimore, MD. 21205.

Neonatal administration of monosodium glutamate (MSG) to rodents results in a massive loss of neurons within the arcuate nucleus of the hypothalamus and significant reductions in hypothalamic, immunoreactive β-endorphin, ACTH, α MSH and dopamine. These anatomical and neurochemical alterations result in a syndrome characterized by obesity, endocrine dysfunction and behavioral deficits. The present study was conducted to evaluate the anorectic potency of naloxone and fenfluramine in MSG-treated (4mg/g MSG on post-natal day 4) male mice.

The effect of naloxone and fenfluramine on food and water intake at doses of 1,5,10 and 20 mg/kg was compared to food and water intake following saline injections. Naloxone at 5,10 and 20 mg/kg was more effective ($p<.05$) in suppressing food intake in MSG-treated mice (n=14) than in controls (n=14). In contrast, naloxone suppressed water intake in controls at 5 and 10 mg/kg ($p<.05$) and produced only a slight drop in water intake in the MSG-treated mice. Both treated and control mice exhibited a significant suppression of water intake at 20 mg/kg. Fenfluramine produced a dose dependent decrease in food intake in both the MSG-treated and control mice. Water intake was suppressed at 5,10 and 20 mg/kg ($p<.05$) in controls, however only 20 mg/kg produced a significant decrease in water intake in MSG-treated mice.

In a separate study, plasma estrogen levels were measured in female MSG-treated (n=16) and control mice (n=16). Trunk blood from 4 mice was pooled to give enough plasma for one sample to be assayed in duplicate. MSG-treated mice (8.4±8.4 pg/ml) had significantly lower plasma estrogen than controls (36.3±4.2 pg/ml).

In summary, naloxone produced a specific and more pronounced suppression of food intake in MGS-treated mice, whereas fenfluramine was equipotent in suppressing food intake for both MSG-treated and control mice. MSG treatment did not alter the anoretic action of fenfluramine demonstrating the specificity of MSG treatment in its enhancement of naloxone-induced food intake suppression. The increased sensitivity to naloxone exhibited by the MSG-treated mice may reflect an alteration in endogenous levels of hypothalamic β-endorphin. MSG-treated mice also exhibited decreased plasma estrogen which parallels the marked ovarian atrophy found in MSG-treated mice.

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128.4 EFFECTS OF NALOXONE ON FOOD AND FLUID CONSUMPTION IN VAGOTOMIZED RATS. D. B. Clarkson*, R. D. Olson, B. M. King, R. C. Hemmer*, G. A. Olson, and A. J. Kastin. Department of Psychology, University of New Orleans, New Orleans, LA 70122

Several studies have shown that opiate antagonists like naloxone reliably suppress food and fluid intake under a variety of conditions. The site of action for this effect, however, is still not clear, although the vagal nerve has been implicated by other investigators as a possible mediator.

To more fully evaluate the role of the vagal nerve, testing was completed under the main conditions known to influence consumption in vagotomized rats. Accordingly, 40 male rats underwent either a subdiaphragmatic vagotomy (VAG) or a sham vagotomy (SHAM) and then were assigned to appetitive or deprivation-induced motivational conditions. All four independent groups of rats were then tested in a repeated measures design using a solid sucrose pellet or liquid 20% sucrose solution after being injected intraperitoneally with 0.0, 1.0, 2.0, 4.0, or 8.0 mg/kg of naloxone. The number of grams consumed in two consecutive 30-minute intervals was the primary dependent variable.

The results of the mixed analysis of variance indicate that the main effect for surgery was not significant, with VAG and SHAM rats consuming nearly idnetical amounts of liquids and solids. This finding shows that the vagal nerve is not a significant mediator of naloxone's effect on consumption. Further, the reliable interaction of VAG with liquids and solids showed that VAG rats drank more liquids and ate less solid food than SHAM rats, suggesting that surgery had an effect on ingestion independent of any action by naloxone. No other interactions involving naloxone were significant. The results also indicate a main effect for motivational condition, with rats tested under appetitive conditions ingesting less food and fluid. This is consistent with our previous results that appetitively-induced drinking is not as vigorous as deprivation-induced drinking. A main effect showing more liquids than solids consumed was also obtained. Test period was reliable, with more consumption in the first 30 minutes than in the second period. The main effect for naloxone was also significant, with consumption decreasing in a dose-dependent fashion as expected. Several interactions involving naloxone administration were obtained. Liquid consumption was noticeably suppressed in a dose-dependent fashion while solid consumption only decreased slightly, which is consistent with our previous data. Further, the test period by naloxone interaction showed that the 8.0 mg/kg dose led to maximum suppression in the first 30 minutes followed by a "rebound effect" on increased consumption in the second test period. In summary, the results show that the vagal nerve is not required for the mediation of naloxone effects.

128.5 EFFECTS OF NALOXONE, MIF-I, AND MIF-I ANALOGS ON FLUID CONSUMPTION IN RATS. R. D. Olson, R. C. Fernandez*, A. J. Kastin, G. A. Olson T. K. vonAlmen*, D. G. Erickson*, D. B. Clarkson*, and D. H. Coy*. Department of Psychology, University of New Orleans, New Orleans, LA 70122

We have shown previously that MIF-I suppresses fluid intake in rats in a manner comparable to that demonstrated by naloxone. Further, following high doses of naloxone, a "rebound effect" characterized by greatly increased consumption occurs after the initial suppression of drinking. The main purpose of this study was to determine the lowest dose of an opiate antagonist that would suppress consumption. A second purpose was to look at possible interactions with sex, which previous work in this laboratory has shown to be very significant.

Albino rats (48 male, 48 female) were randomly assigned to the appropriate experimental group, naloxone, Pro-Leu-Gly-NH_2 (MIF-I), Tyr-Pro-Leu-Gly-NH_2 (Tyr-MIF-I), or pGlu-Leu-Gly-NH_2 (pGlu), and received an intraperitoneal injection of either 0.01, 0.1, or 1.0 mg/kg of each test substance. A control group of 4 males and 4 females was also tested using the diluent vehicle. Consumption of a 20% sucrose solution was measured in grams every 30 minutes for 10 hours.

A mixed analysis of variance indicated the main effect for test substance to be significant. Scheffe's test for individual comparisons showed that rats injected with MIF-I and the pGlu analog drank the most followed by rats injected with naloxone, Tyr-MIF-I, and the controls. The main effect for dose was also significant, with consumption decreasing in a dose-dependent fashion. Dunnett's test showed all experimental groups to be reliably above the control except the groups receiving the 1.0 mg/kg dose of Tyr-MIF-I and the 1.0 mg/kg dose of naloxone, which were not reliably different from each other or the control. The main effect for sex was not significant, but the interactions of sex by peptides and also sex by dose were highly significant. Males drank more than females after injections of MIF-I or naloxone, about the same after injections of the pGlu analog, and less after injections of Tyr-MIF-I. In all tests, females drank the least at the 0.1 mg/kg dose while males drank the most at the same dose.

The data vary significantly from previous studies using larger doses of opiate antagonists in that the lowest doses in the current study all produced reliable increases in consumption rather than the typical decreases associated with such antagonists. The results show that MIF-I and its analogs have actions comparable to naloxone in modulating fluid consumption in rats.

128.6 **EFFECT OF ANORECTIC DOSES OF ETHANOLAMINE-O-SULFATE (EOS) ON REGIONAL BRAIN UPTAKE OF ^{14}C-2-DEOXY-D-GLUCOSE (^{14}C-2DG).** **J.N. Nobrega, D.V. Coscina, J. Chambers* and J.S. Yeomans (Biopsychol. Sect., Clarke Inst. Psychiat. and Psychol. Dept., Univ. of Toronto, Toronto, Ont., Canada).**

Central injections of EOS, an irreversible inhibitor of GABA transaminase, increases brain GABA levels while depressing food intake in a dose-dependent manner (Cooper et al, Life Sci, 1980, 26, 1997). EOS has also been shown to reverse the overeating induced by medial hypothalamic lesions, systemic 2-DG injection, or highly palatable diets (Coscina & Muir, East. Psychol. Assoc. Abs. 1981, 52, 108). In an effort to gain information on possible brain sites mediating these effects we investigated the regional brain uptake of ^{14}C-2DG by autoradiography and scintillation counting after intracerebral EOS. Under ether anesthesia, male rats (300-400 g) received one intracisternal injection of 400 ug EOS. This dose has been previously shown to induce a pronounced elevation of brain GABA and reliable anorexia for several days. Twenty-four hours later, rats received 20 uCi of ^{14}C-2DG i.p., and were sacrificed 45 min later. Brains were removed and frozen for subsequent sectioning or were dissected in situ into nine regions (cerebellum, hypothalamus, septum, hippocampus, striatum, thalamus, cortex, midbrain and hindbrain) for liquid scintillation counting. A marked reduction in ^{14}C-2DG uptake was found in EOS-treated brains (mean reduction in CPM /mg tissue: 78% ± 3%). This reduction was fairly uniform across the nine regions studied (range: 74% in hypothalamus to 84% in striatum). The generalized nature of this effect was confirmed in the autoradiographs. In all cases, sections from EOS-treated brains appeared homogeneously pale compared to corresponding sections from control brains exposed on the same X-ray film. These results suggest that anorexia-inducing elevation of GABA levels by EOS is associated with a profound and generalized decrease in brain glucose uptake. The uniformly massive decrease in ^{14}C-2DG uptake across brain regions might suggest that the behavioural deficits observed are associated with a general metabolic effect of GABA elevation as opposed to inhibition of specific neuronal circuits. Consistent with this possibility, Olgiati et al. (Psychopharm., 1980, 68, 163) have shown that the anorectic effects of EOS are not blocked by the GABA receptor blocker bicuculline.

J.N.N. is a Fellow of MRC of Canada and CNPq of Brazil.

128.7 REDUCTION OF LONG-TERM FOOD INTAKE FOLLOWING VENTRICULAR INFUSION OF ETHANOLAMINE-O-SULPHATE. P.Bishop* and J. Panksepp, Dept. of Psych., Bowling Green State University, Bowling Green, Ohio 43403.

Long-term body energy regulation may be mediated by the encoding of metabolic information via GABA shunt intermediates in neural circuits which inhibit feeding related behaviors. The GABA shunt provides an ideal mechanism by which inhibitory neuroactive information concerning the overall energy status of an organism could be generated from intermediary metabolism. To further evaluate this possibility, we have chronically infused the GABA-transaminase inhibitor, ethanolamine-O-sulphate (EOS), into the 3rd ventricle of freely fed Long-Evans female rats. Week long infusions of EOS with Alzet minipumps produced dose dependent reductions in feeding and body weight. 200 ug EOS/day inhibited all feeding behavior and animals died within 5 days. 100 ug/day led to a 67% reduction in daily food intake and a 27% reduction in body weight. Animals exhibited symptoms similar to those with lateral hypothalamic lesions. 50 ug/day led to a 45% reduction in food intake and a 16% decrease in body weight. The animal appeared normal. The GABA-antagonist, picrotoxin, was infused in a similar manner (24 ug/day) over the course of one week resulting in no reliable effects on either food intake or body weight. However, when this dose of picrotoxin was paired with EOS (50 ug/day), the reduction in food intake and body weight previously observed with the EOS alone was partially attenuated.

These data implicate the involvement of a GABA receptor system in the effects of EOS on food intake and body weight and provide further convergent evidence for the hypothesis that GABA exerts inhibitory control over long term body energy regulation via control of feeding behavior.

128.8 PROSTAGLANDIN E_2 SUPPRESSES SALINE INTAKE OF RATS WITH RESTRICTED ACCESS TO SALT. Karen E. Moe* and Nancy J. Kenney, Dept. of Psychology, University of Washington, Seattle, WA 98195.

Prostaglandin E (PGE) has been repeatedly implicated in the regulation of body fluid homeostasis. Administration of PGE either centrally (Kenney & Epstein, 1978) or peripherally (Kenney et al., in press) attenuates the water consumption that occurs in response to a variety of dipsogens, including angiotensin II, hypertonic saline, polyethylene glycol and water deprivation. Prostaglandin E may also be involved in the control of sodium excretion, though whether it is natriuretic (Bartha, 1977) or anti-natriuretic (Kirschenbaum & Stein, 1976) is still a controversy. The experiments reported here examined the role of prostaglandin E_2 (PGE_2) in the sodium appetite of rats with restricted access to salt.

Male rats were trained to consume a saline solution (1.5%) for two hours each day. Throughout the experiment, including the training period, the rats had continuous access to a sodium-deficient diet and de-ionized water. Thus, consumption of saline during the 2-hour period was their only means of obtaining salt. After saline consumption had stabilized, all animals received a daily intraperitoneal (IP) injection of either PGE_2 (10, 50 or 100 µg/ml/kg) or the PGE_2 vehicle (1 ml/kg) immediately prior to the NaCl access. Each prostaglandin treatment day was preceded and followed by a vehicle treatment day. Saline and water consumption were monitored during the entire 2 hours.

Saline consumption was significantly ($p < .05$) suppressed by IP injection of PGE_2 at all three doses used, though the degree and duration of the suppression varied with dose employed. In general, actual consumption was reduced for only the first 30 minutes. However, as consumption during the remaining 90 min was no different from that of vehicle days, cumulative saline intake remained depressed for a much longer time. Only the 100 µg/kg dose affected water intake, reducing it for the first 30 minutes relative to control values.

To determine if the PGE treatment inhibits saline intake by making animals sick, a second experiment utilized the conditioned taste aversion paradigm (Garcia & Koelling, 1966) to test for the presence of malaise. IP injection of PGE_2 (50 or 100 µg/kg) was paired with consumption of a novel-tasting substance to determine whether such PGE treatment will lead to the formation of a conditioned taste aversion. The resulting failure to elicit a taste aversion suggests that IP PGE_2 does not induce sickness. Therefore, the attenuation of NaCl consumption by IP PGE_2 is more likely the result of a specific inhibitory mechanism rather than general malaise or inability to respond.

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128.9 NOREPINEPHRINE METABOLISM IN MEDIAL HYPOTHALAMUS IS INCREASED BY ACUTE AND CHRONIC DEHYDRATION. Harry Klemfuss* and Lewis Seiden (SPON: M. Hollyday). Dept. of Pharmacol. & Physiol. Sciences, University of Chicago, Chicago, IL 60637.

Concentrations and turnover rates of hypothalamic catecholamines (CA) were altered after manipulations which interfere with water homeostasis in the rat. Chronic dehydration was produced by limiting access to water to a 10-min period starting at 1000 hrs. After 7 days of this treatment, rats were injected with either saline or 200 mg/kg α-methyl-p-tyrosine methylester HCl (AMT) at approximately 0800 hrs and were killed 2 hrs later. Brain regions were assayed for CA using high performance liquid chromatography with electrochemical detection. Turnover rate was determined by comparing CA levels after AMT pretreatment to matched controls treated with saline. Chronic dehydration reliably increased both levels and turnover of norepinephrine (NE) in the hypothalamus, but not in amygdala, mesolimbic area, septum, telencephalon, or brainstem. Within the hypothalamus, chronic dehydration did not affect NE metabolism in paraventricular nucleus, preoptic area, or lateral hypothalamus, but in medial hypothalamus NE concentrations increased to 144% of control ($p < 0.005$ level). NE turnover was also significantly increased ($p < 0.05$). Dopamine metabolism was unchanged by chronic dehydration in caudate, substantia nigra, limbic areas or any hypothalamic region studied. There are several stimuli which activate compensatory mechanisms for maintaining water homeostasis. Hypernatremia stimulates hypothalamic osmoreceptors. Hypovolemia is signalled by both peripheral nerves and by circulating angiotensin II. Hypernatremia and hypovolemia can be experimentally induced by injecting hypertonic saline or hyperoncotic colloid, respectively. Angiotensin II levels can be raised without altering plasma volume by ligating the inferior vena cava above the renal veins. In the rat, none of these manipulations altered NE concentrations in the medial hypothalamus. On the other hand, both hypernatremia and caval ligation increased the turnover of NE in the hypothalamus ($p < .05$). We are currently examining dehydration effects on dopamine and epinephrine metabolism in the rest of the hypothalamus. (Supported by PHS MH-11191; RSA MH-10562; MH-14274).

128.10 PHARMACOLOGICAL DEPLETION OF NOREPINEPHRINE AND FEEDING BEHAVIOR IN THE RAT. John Rossi III*, R. F. Davies*, A. J. Zolovick* and Jaak Panksepp. (SPON: F. G. DeEskinazi) Dept. of Psychology Bowling Green State University, Bowling Green, OH 43403

Both norepinephrine (NE) and serotonin (5-HT) have been implicated in the neurochemical control of feeding. Although previous findings suggest that manipulations which facilitate activity in brain NE systems can increase feeding and those which increase activity in the 5-HT system can decrease feeding, disagreement exists concerning feeding effects following reduction of activity in these systems. In the present study, feeding behavior was examined after acute pharmacological depletion of NE alone or in combination with specific 5-HT reuptake inhibition.

Dopamine-B-hydroxylase (DBH) inhibition with FLA-63 (10 mg/kg) disrupted free feeding behavior in satiated rats. While the average number of meals taken was not different than vehicle injected controls, meal size was reduced 58% in the first 9 hrs after treatment with FLA-63. In starved animals, FLA-63, produced little effect on feeding behavior, even though NE depletion was in excess of 40%. When FLA-63 was given in combination with the vesicular depleting drug RO4-1284 (5 mg/kg), feeding was reduced to 16% of control intake and NE was specifically depleted in excess of 98%. Starvation feeding was reliably reinstated in animals which received FLA-63 plus RO4-1284 with either dl-threo-DOPS, a metabolic precursor to NE, or direct intrahypothalamic injections of NE. These findings suggest that the feeding suppression observed after these manipulations was due to disruption of transmission in brain NE systems.

When starved rats are co-treated with FLA-63 (10 mg/kg) and the specific 5-HT reuptake blocker Ll10-140 (3.75 or 7.5 mg/kg) the suppression of feeding observed is much greater than when either drug is given seperately and is as great as that observed after nearly total NE depletion with FLA plus RO4-1284. Furthermore, neither FLA-63 nor Ll10-140 when given seperately or in combination reduced deprivation induced drinking behavior indicating that their interaction in the suppression of feeding may be specific.

Taken collectively, these findings suggest that the primary role of NE in feeding is maintenence of the consumatory response and that these effects are expressed in relation to activity in other neurochemical systems such as 5-HT.

128.11 BEHAVIORAL EFFECTS OF NOREPINEPHRINE AND/OR DOPAMINE DEPLETIONS. J. H. McLean, S. B. Hutson*, B. M. King, and R. M. Kostrzewa. Dept. Psychology, University of New Orleans and Dept. of Pharmacology, East Tennessee State University College of Medicine.

Various dosages of 6-hydroxydopamine were administered intraventricularly to rats on days 5 and 7 after birth to deplete selectively central norepinephrine (low NE), dopamine (low DA), or both (low NEDA); saline was administered similarly to control animals. Animals were studied at 3 months of age on a variety of behavioral tasks before being sacrificed at approximately 5 months of age. Significant differences between groups were found in activity, emotionality, daily food and water intake, nonprandial drinking, sucrose solution intake, and feeding in response to insulin. Significant differences were found more frequently between the treatment groups than between the control group and treatment groups. No significant differences were found between groups in active and passive avoidance, intake of quinine solution, response to hypertonic saline injection, and feeding in response to two 2-deoxy-d-glucose dosages. In comparison to saline animals, significant depletions of endogenous NE were found in the hippocampus, hypothalamus, and cortex for animals receiving one large dose of 6-hydroxydopamine (low NEDA) and animals receiving two small doses of 6-hydroxydopamine (low NE). Striatal dopamine was significantly depleted in all treatment groups. Norepinephrine content of the cardiac ventricle was not significantly affected in any group.

128.12 RESTORATION OF REGULATORY DRINKING IN RESPONSE TO HYPERTONIC SALINE IN 6-HYDROXYDOPAMINE-TREATED RATS: A TEST OF DOPAMINE RECEPTOR STIMULATION. C.T. Dourish*, R.S.G. Jones (SPON: J.D. McQueen) Psychiatric Research Div., University Hosp., Sask., Canada S7N OXO

Brain dopamine (DA) neurons have been implicated in the control of regulatory drinking responses since bilateral 6-hydroxydopamine (6-OHDA) lesions of the nigrostriatal bundle cause a syndrome of aphagia and adipsia which is similar to the deficits induced by bilateral electrolytic lesions of the lateral hypothalamus (LH). Following LH or 6-OHDA lesions rats do not drink in response to physiological stimuli such as hypertonic saline (HS) which elicits thirst in intact animals. It has been demonstrated (Marshall and Ungerstedt, Physiol. Behav., 17:817, 1976) that the systemic administration of a low dose of the DA agonist apomorphine can restore drinking in response to HS or isoproterenol in 6-OHDA lesioned rats, but not in rats with LH lesions. We have extended this observation and used a similar procedure to examine the postulated DA agonist properties of a number of drugs. We studied 3 groups of male Sprague-Dawley rats: unoperated controls (n=16), rats given bilateral 6-OHDA lesions of the ventral tegmentum (n=8) and rats given bilateral radio-frequency lesions of the LH. Following surgery lesioned animals were aphagic and adipsic and had to be fed intragastrically to maintain body weight. The rats were tested over a period of 7 days (beginning 2 days after surgery) for drinking responses to HS, apomorphine, piribedil, lergotrile, β-phenylethylamine and tyramine. Each drug was administered i.p. alone and in combination with HS. In control rats HS elicited a strong drinking response (x=7.5 ml) but had no effect in lesioned animals. When HS was followed by apomorphine, piribedil or lergotrile, rats with 6-OHDA lesions became activated and drank a similar volume of water (x=6.0 ml) to that consumed by controls. β-Phenylethylamine and tyramine did not induce drinking when administered with HS in 6-OHDA lesioned animals. Rats with LH lesions did not drink in response to HS regardless of whether it was administered in combination with apomorphine, piribedil or lergotrile.

These observations confirm the findings of Marshall and Ungerstedt and support the hypothesis that DA pathways are involved in the control of regulatory drinking. Since this study can be interpreted as a test of DA receptor stimulation, however, the results cast doubt on the proposed status of β-phenylethylamine (Antelman et al., Brain Res. 127:317, 1977) as a direct DA receptor agonist. Supported by Sask. Health and the M.R.C. of Canada.

128.13 NEUROCHEMICAL AND BEHAVIORAL EFFECTS OF MONOSODIUM GLUTAMATE ADMINISTRATION TO NEONATAL MICE. M.F. Callahan, R. Dawson, Jr. and Z. Annau. Dept. of Pharmacology, Chicago Medical School, North Chicago, IL. 60064 & Dept. Environ. Hlth. Sci., Division of Toxicology, The Johns Hopkins University, Baltimore, MD 21205

Monosodium glutamate (MSG) administation of neonatal mice results in destruction of the arcuate nucleus of the hypothalamus and reductions in hypothalamic and pituitary dopamine (DA). The neurochemical changes following MSG treatment contribute to a syndrome characterized by obesity, endocrine dysfunction and behavioral deficits. In the present study, hypothalamic DA and norepinephrine (NE) turnover was estimated in 20 adult, male mice treated with a single subcutaneous dose of MSG (4mg/g) on postnatal day 4 and an equal number of control mice utilizing α-methyl-p-tyrosone (α MPT) (400 mg/kg) and a sensitive radioenzymatic assay procedure. Groups of 5 animals were decapitated at 1,2 and 3 hours following the injection of α MPT.

Steady state levels of hypothalamic NE and DA were not significantly altered by MSG treatment. The turnover rate of NE in MSG-treated mice did not differ significantly from that of the controls. DA turnover rate in the hypothalamus of MSG-treated mice did not differ from the controls over the 3 hour time course of DA synthesis inhibition, however, DA levels in the MSG-treated mice were significantly lower than controls 1 hour after α MPT injection. This suggest a more rapid utilization of DA in MSG-treated mice. Further studies at different time points are being conducted to clarify this finding.

Taste reactivity testing, and activity levels were also evaluated. One hour consumption of a number of flavored solutions was evaluated in 14 male MSG-treated and 14 control mice. Both groups significantly ($p<.05$) increased their intake of 0.9% saline, however neither group increased their intake of a 0.1% sacchrin solution. Control mice drank significantly less ($p<.05$) of a 0.1% quinine solution, whereas MSG-treated mice exhibited a nonsignificant decrease. Activity levels were monitered in 7 male MSG-treated and an equal number of control mice at 65,90 and 200 days of age. The MSG-treated mice exhibited increased activity levels at all time points, reaching significance at day 65 ($p<.02$) and 200 ($p<.02$).

The neurochemical findings suggest that dopaminergic function may be altered in those DA neurons that survive MSG treatment. It is concluded from the behavioral studies that the hypoactivity reported in MSG-treated mice is a function weight gain and not of MSG-treatment per se. It also appears that MSG-treated mice do not exhibit the finickiness characteristic of damage to the ventromedial nucleus.

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128.14 NOCTURNAL FOOD-CONTINGENT HYPERDIPSIA IN THE SPONTANEOUSLY HYPERTENSIVE RAT. F.S. Kraly, A.F. Moore*, L.A. Miller*, L.J. Morrison* and A. Drexler*. Psych. Dept., Colgate Univ., Hamilton, NY 13346 and Norwich-Eaton Pharm., Norwich, NY 13815.

Male spontaneously hypertensive rats (SHR; 5 mo. of age, $n = 10$; Taconic Farms) ate the same in 24 hr. as normotensive Wistar-Kyoto (WKY; $n = 6$) rats with continuous access to standard laboratory pellets and tap water (Table 1). SHR rats drank more water, however, and this hyperdipsia was reflected in a higher water to food ratio (W:F) for SHR rats.

Table 1 (24-hr. day & night)

	WKY	p	SHR
Food (g/100g)	6.5 ± .2	>.20	6.6 ± .1
Water (ml/100g)	10.9 ± .4	<.02	12.9 ± .5
W:F (ml/g)	1.7 ± .05	<.05	2.0 ± .1

When rats ate in the day phase of a 12:12 light/dark cycle after 24-hr. food deprivation, SHR rats ate and drank the same as did WKY rats and SHR rats had a similar W:F in a 60-min. test (Table 2). When the same rats ate in the night phase after 24-hr. food deprivation, however, SHR rats were hyperdipsic: They ate the same as WKY rats, but SHR rats drank significantly more and had a higher W:F (Table 2). SHR rats were also observed to groom less ($p<.01$), to explore the home cage less ($p<.01$), to recline in the cage more often ($p<.001$) and to interrupt eating by drinking more often ($p<.05$) than WKY rats when tested eating and drinking at night.

Table 2

	WKY	p	SHR
Day Phase			
Food (g/100g)	1.6 ± .1	>.20	1.5 ± .2
Water (ml/100g)	2.1 ± .2	>.20	2.2 ± .2
W:F (ml/g)	1.4 ± .1	>.10	1.5 ± .1
Night Phase			
Food (g/100g)	1.8 ± .1	>.20	1.7 ± .1
Water (ml/100g)	2.5 ± .1	<.001	3.2 ± .1
W:F (ml/g)	1.4 ± .1	<.01	1.9 ± .1

This relative hyperdipsia reflects the increased ability of ingestion of food to stimulate drinking in SHR rats, because when food was absent for a 60-min. test at night SHR rats drank the same as did WKY rats (SHR: .8 ± .2 ml/100g; WKY: 1.0 ± .2 ml/100g; $p>.20$).

This nocturnal food-related hyperdipsia in SHR rats appears not to be mediated by peripheral angiotensin II (AII), because the AII converting enzyme inhibitor SQ 14,225 (50 mg/kg p.o. 60 min. prior to eating) failed to inhibit drinking ($p>.20$) or to reduce W:F ($p>.20$) in SHR rats.

128.15 LOW DIETARY PROTEIN AND THE FACILITATION OF AGGRESSIVE BEHAVIOR IN RATS. K.M. Kantak and B. Eichelman. Laboratory of Behavioral Neurochemistry, University of Wisconsin and William S. Middleton Veterans Hospital, Madison, Wisconsin 53705.

Dietary intake of protein is important for growth and physical and mental development. The effects of low protein intake on feeding behavior and body weight maintenance are well described. Since the ingestion of protein affects many neurochemical parameters, it is reasonable to expect that the intake of low levels of protein affects many other behavioral measures as well. In the present study, rats were maintained on either an 8% low protein diet or a 25% isocaloric normal protein control diet and tested for changes in aggressive behavior. For shock-induced fighting, which tests for defensive aggression, the low protein diet significantly elevated the level of fighting two weeks following its initiation with no effects 24 hr and 1 week following its initiation. After three weeks return to normal protein chow the levels of fighting returned to baseline levels. For mouse-killing behavior, which tests for predatory aggression, the low protein diet significantly reduced the latencies to sniff, attack and kill mice and significantly increased the number of mice killed one week and two weeks following its initiation in rats which were experienced spontaneous killers. In experienced non-killer rats, the low protein diet failed to affect the incidence of mouse-killing, indicating that 8% low dietary protein does not influence the initiation of mouse-killing but facilitates its maintenance. After three weeks return to normal protein chow mouse-killing was no longer facilitated in spontaneous killer rats. The facilitation of shock-induced fighting and mouse-killing in killer rats following ingestion of a low protein diet was independent of potential physical debilitating effects of the diet since body weight levels were maintained at pre-low protein diet levels. These data indicate that the intake of low levels of dietary protein can modulate behavior in rats which in the present study is a facilitation of both defensive and predatory aggression.

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128.16 EFFECTS OF ESTRADIOL ON SHORT-TERM AND LONG TERM FEEDING SYSTEMS C.W. Simpson,* C.Dudley,* and C. Deluca,* K. Moore.*
(Spon: J. Schaeffer) Dept. of Biology and Sch. of Medicine Univ. of Missouri-K.C., Kansas City, MO. 64110

Estrogen reduces body weight and ad. lib. feeding in normally cycling rats. We have shown in previous work that estrogen level alters catecholamine elicited eating at anterior hypothalamic sites. Norepinephrine (NE) elicited feeding is not disrupted and may be potentiated at these anterior sites in the presence of estrogen. Dopamine (DA) elicited feeding, at these same sites is inhibited in the presence of estrogen. Neither NE or DA significantly altered 24 hour food intakes or body weight following central injections. Rats at different levels of body weight were microinjected at catecholamine sensitive sites in the anterior hypothalamus under different estrogen conditions. Ovariectomized groups of rats at 3 significantly different levels of body weight were injected with NE and DA in the anterior hypothalamic sites. Ne and DA significantly increased 1-2 hours feeding in all weight groups. All groups were then reduced to lower absolute levels of body weight by daily estrogen injection 2µg/day in oil. All weight groups were again microinjected with NE and DA in anterior hypothalamic sites in the presence of estrogen. Under these conditions estrogen continued to reduce body weights and 24 hour food intake for all groups but did not alter the increases in 1-2 hour food intake following injections of NE or in this experiment DA. The mechanism of action for the estrogen effects on catecholamine eating was investigated by direct implantation of estrogen in estrogen sensitive sites as described by (Stumpf W. Am. J. ANAT. 129, 1970.) and concurrent catecholamine injections in the anterior hypothalamic sites. Animals were fitted with 2 sets of cannulas, one in the catecholamine feeding site and the other set in different groups of animals in LPO, VmH or anterior hypothalamic estrogen sensitive sites. Estrogen crystals in doses > 5µg/day but not cholesterol crystals, significantly reduced 24 hour food intakes and daily body weights when placed in LPO, VmH and anterior hypothalamic estrogen sensitive sites. While the boby weight loss and decrease in 24 hour intakes was consistent for all groups, estrogen effects on 1-2 hour feeding following NE or DA injections were differentially affected. NE elicited increases in short-term food intake irrespective of hormone condition at any site. Conversely DA elicited increases in 1-2 hour intakes only when estrogen was at the LPO site. When estrogen preceded DA in the anterior hypothalamic or VmH sites short-term food intake increases were inhibited.

128.17 FOOD PREFERENCES, EATING PATTERNS, AND CALORIC INTAKE IN NORMAL AND COMPULSIVE EATERS. B. J. Sahakian, S. Bingham*, P. Murgatroyd*, M. Lean*, and W.P.T. James*. Dunn Clinical Nutrition Centre, University of Cambridge and M.R.C. Dunn Nutrition Unit, Cambridge, CB2 1QE, England.

The food preferences, eating patterns, and caloric intake of human subjects with and without eating disorders were studied by means of a food machine (Silverstone, T. In: Recent Advances in Obesity Research : II, ed. by G. Bray, London: Newman, 421-432, 1978) and an ACORN microprocessor. Considerable differences in caloric intake (MJ/24h) in subjects with similar weight/height^2 indexes were found. Correlations of subjects' eating patterns, food choices, caloric intake, weight/height^2 indexes, and responses on scales of restrained vs. unrestrained eating (Polivy, J., Herman, C.P. and Warsh, S., J.Abnormal Psychol., 87, 497, 1978) were performed.

This method appears to be an excellent means of quantifying food preferences, patterns of eating, and caloric intake in human subjects in long-term studies, and therefore can be used to assess drug treatments in patients with eating disorders. After determination of baseline measures of preferences, patterns, and caloric intake in a patient with bulimia nervosa (Russell, G., Psychologic.Med., 9, 429, 1979), double-blind trials of naloxone, chlorpromazine, or placebo were undertaken to see whether these agents would suppress the patient's compulsive eating. The findings of these drug trials were negative. Trials with other subjects are currently underway to determine whether these or other drugs would prove effective in the treatment of patients with a different type of compulsive eating disorder, such as compulsive overeating in obesity.

129.1 SINGLE UNIT RESPONSES IN MLD OF THE REDWING BLACKBIRD. N. A. O'Connell and M. B. Sachs*. Dept. of Biomedical Engineering, Johns Hopkins University School of Medicine, Baltimore, Md. 21205

Responses of single cells in nucleus mescencephalicus lateralis pars dorsalis (MLD) to sound stimuli were recorded in redwing blackbirds. Mapping studies of MLD in anesthetized birds revealed a tonotopic organization with characteristic frequency increasing in the dorsal to ventral and anterior to posterior directions. Response properties of cells in anesthetized and awake birds will be presented. Stimuli included digitized recordings of blackbird and cowbird vocalizations as well as tones and broadband noise. Response maps showing regions of excitation and inhibition in the sound level-frequency plane were constructed. In anesthetized birds most response maps showed excitatory centers; maps for units with significant spontaneous activity often had inhibitory sidebands. A strong onset response to tones was the most common response type. Many units in unanesthetized birds had an excitatory onset response followed by inhibition for most tones. Response maps for onset and sustained responses of these units were mapped separately. The onset response was typically excitatory over a broad range of levels and frequencies. The sustained response was inhibitory over a wide range with no excitatory regions. This work was supported by a grant from the National Institute of Neurological and Communicative Diseases and Stroke. N. A. O'Connell is a National Institutes of Health Postdoctoral Fellow.

129.2 SPATIAL SELECTIVITY OF AUDITORY NEURONS IN THE CEREBELLUM. Jane C. Norris. Neurobiology Dept., Stanford University School of Medicine, Stanford, CA 94305.

The cerebellum receives a variety of sensory afferents including an auditory projection. Although the functional significance of this input remains unresolved, results of binaural interaction studies have suggested that these neurons may be involved in sound localization or in coordinating movements related to orienting to an auditory stimulus in space. Previous studies of the auditory centers of the barn owl have revealed a class of neurons distinguished by its selective responses to sounds originating only from a restricted area of space (Knudsen and Konishi, Sicence, 200, 1978). Such limited-field neurons could play an important role in sound localization.

The present investigation was designed to locate the auditory projection area in the owl cerebellum and to determine if cerebellar auditory neurons were selectively responsive to the spatial location of an acoustic stimulus. Extracellular recordings of auditory cerebellar unit activity in ketamine anesthetized owls were made. Stimuli consisted of noise or tone bursts delivered in a free-field from a speaker which was movable in both azimuth and elevation. The auditory receptive fields of neurons were obtained from spike counts collected as the auditory stimulus location was changed. Lesions made at locations where spatially sensitive auditory neurons were found allowed subsequent histological verification of the auditory projection area.

The results indicated that the majority of auditory units were located in folia VII and VIII. Most units were sensitive to sound location; that is, they had definite auditory receptive fields within which the spike rate markedly changed. Most of the limited field units demonstrated excitatory responses to sound but some had inhibitory fields. The size of the field (ranging from 18° to 43° in azimuth) was usually, but not always, independent of stimulus intensity. A smaller group of units preferred a certain spatial location but responded at a lower rate to all locations. Some of these units showed complex response patterns with both excitatory and inhibitory areas. Another small group responded in a spatially independent manner. These results suggest that precise auditory spatial information is available for processing in the cerebellum.

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129.3 IONTOPHORETIC APPLICATION OF PUTATIVE INHIBITORY NEUROTRANSMITTERS ONTO BINAURAL UNITS IN THE SUPERIOR OLIVE. Maurus Moore*, D.M. Caspary and D. Colleen Havey. Department of Pharmacology, Southern Illinois Univ. Sch. Med., Springfield, IL 62702.

The first conjunction of bilateral afferent pathways in the auditory system occurs in the superior olivary complex (SOC). Binaural neurons in these structures possess a two-dimensional dendritic input from opposite ears (Scheibel and Scheibel, Exp. Neurol., 43:339, 1974), and may be involved in dichotic sound localization (Erulkar, Physiol. Rev., 53:237, 1972). Many of these neurons may be excited by stimulating one ear, but this activity may be inhibited by simultaneous stimulation of the other ear (IE units, Goldberg and Brown, J. Neurophysiol., 31:639, 1968). This provides an opportunity to examine the interaction of synaptically mediated excitation and inhibition at the same neuron by manipulation of the acoustical input to each ear.

The present study examined the effects of iontophoretic application of putative inhibitory amino acid neurotransmitters and their antagonists onto IE neurons of the SOC. The experimental paradigm attempted 1) mimicry of the inhibitory input using GABA or glycine; 2) antagonism of the acoustically-evoked inhibitory synaptic input using bicuculline or strychnine; and 3) antagonism of the effects of the iontophoretically applied agonist. Sixty-nine IE neurons from the SOC were examined, 47 of which were extensively studied using iontophoretic application of candidate transmitters and their antagonists. Acoustically-evoked monaural excitation could be effectively inhibited by glycine in most cases. This inhibition could be antagonized by the glycine antagonist strychnine, but not by bicuculline. IE neurons generally display a binaural response that is markedly reduced from the monaural condition. In 28 neurons, strychnine application during binaural stimulation resulted in a response comparable to the monaural excitatory condition. Thus, it appears strychnine can effectively antagonize the acoustically-evoked inhibitory synaptic input to these neurons. At the peak of the strychnine antagonism of the synaptically-mediated inhibition, iontophoretic application of glycine had no effect. A number of neurons were inhibited by GABA, but antagonism of this effect and of inhibitory synaptic effects by bicuculline were rarely seen. Results of these studies suggest a glycinergic input to certain neurons of the superior olivary complex. (Supported by NIH grant #NS15640-02)

129.4 AUDITORY BRAINSTEM ANOMALIES IN ALBINO CATS. D. Creel, J.W. Conlee*, and T.N. Parks. VAMC, Salt Lake City, UT 84148; and Dept. of Anatomy, Univ. of Utah Coll. Med., Salt Lake City, UT 84132.

Abnormal routing of retinal fibers in albinos of 10 species of mammals has been described. The disorganization of visual pathways is correlated with reduced retinal pigmentation in mammals having mutations that cause ocular or oculocutaneous forms of albinism. The amount of melanin in the inner ear of pigmented mammals is positively correlated with the general pigmentation of the body and specifically with the amount of pigmentation in the eye. Pigment is absent in the inner ear of albino humans and animals. Electrophysiological evidence of significant differences in brainstem auditory pathways between albinos and pigmented humans has been reported.

Brainstem auditory evoked potentials (BAEP's) were recorded from 5 pigmented cats and 5 albino cats. The albinos were complete tyrosinase-negative (c), not the dominant white (W) variety. The cats were anesthetized and stimulated monaurally by 100 μsec clicks at a rate of 11.3 per second. Click intensities of 70,75,80, and 85 dB peak SPL (40-55 dB HL) were used. Potentials evoked by 1,000 clicks were averaged; the period of analysis was 10 msec from the onset of each click. Electrodes were placed 5 mm lateral to the midline at the vertex and were referred to the ipsilateral mastoid process.

In pigmented cats, asymmetry of BAEP's was limited to amplitude variations of 20-40% for components appearing between 2 and 3.5 msec, plus the expected faster ipsilateral latencies for components in the first 5 msec. Albinos, however, demonstrated a dramatic diminution of contralateral components appearing between 2 and 3.5 msec. At the lower click intensities (70,75 dB SPL), there were no contralateral components in this region.

These data indicate probable significant differences between the decussated and nondecussated auditory pathways at the level of the superior olivary nuclei and subsequent ascending pathways in the albino cat. This is further evidence of the anomalous decussation of brainstem auditory pathways in albinos.

129.5 MORPHOLOGY AND ORIGIN OF AXONAL ENDINGS IN NUCLEUS LAMINARIS OF THE CHICKEN. T.N. Parks, A.J. Robinson*, and J.W. Conlee*. Department of Anatomy, University of Utah College of Medicine, Salt Lake City, UT 84132.

The morphology of axon terminals in nucleus laminaris (NL), a 3rd-order auditory center, was studied in aldehyde-osmium fixed double-stained material from adult White Leghorn chickens. Structural correlates of physiological response properties and baseline data for evaluating normal development of NL and the effects of various experimental manipulations were sought. From montages of electron micrographs, 528 terminals on 16 cells at 6 posterior-to-anterior levels within NL were analyzed; in 20 of these endings, the packing density of synaptic vesicles and the width of the intercellular cleft separating ending and cell were also measured. 98% of all endings could be unequivocally assigned to one of two categories. Type M endings typically arise from large unmyelinated preterminal fibers; the mean (±S.E.M.) apposition length for these terminals is 2.39±0.21 μm, cross-sectional area is 6.17±1.83 μm^2, the area:length ratio is 5.94±1.75, the packing density of synaptic vesicles (mean±S.D.) is 41.7±13.9 per μm^2, and the mean width of the intercellular cleft is 35.7±14.7 nm. Large, clear, round synaptic vesicles cluster near small punctate synaptic junctions; both pre- and postsynaptic membranes bear pronounced densities. 54% of the type M terminals end on proximal dendrites--the rest end directly on cell bodies. Type M endings predominate on distal NL dendrites. In preliminary studies involving injection of horseradish peroxidase (HRP) into the crossed dorsal cochlear tract carrying axons from nucleus magnocellularis (NM) to NL, type M terminals are preferentially labeled; this suggests an origin in NM. The smaller, more elongated type D terminals have a mean (±S.E.M.) apposition length of 3.08±0.19 μm, a cross-sectional area of 2.42±0.21 μm^2, an area:length ratio of 1.38±0.04, a synaptic vesicle packing density (mean±S.D.) of 85.0±29.0 per μm^2, and an intercellular cleft width of 21.2±3.3 nm. In these endings, smaller round clear vesicles are arrayed behind a large symmetric synaptic contact, with very slight densities on both pre- and postsynaptic membranes. 81% of the type D terminals end on the cell body; 19% are found on proximal dendrites or capping short somatic processes or spines. Type D endings were not commonly found on distal dendrites; it seems likely that they supply descending inhibitory input. There were no significant regional variations in ending morphology or distribution along the posterior-to anterior axis of NL.

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129.6 ANURAN MIDBRAIN RESPONSES TO MULTIPLE TONE STIMULATION: INHIBITORY AND FACILITATORY INTERACTIONS. Z.M. Fuzessery* and A.S. Feng. Dept. Phyiology & Biophysics, Univ. of Illinois, Urbana, IL 61801.

Single unit recordings in the principle and magnocellular nuclei of the torus semicircularis of Rana p. pipiens show these neuronal populations exhibit a wide range of selectivity in both the spectral and intensity domains. At the extremes, some neurons are responsive to the entire audible range at intensities up to 120 dB SPL, while others have closed excitatory tuning curves limited to 200 Hz bands and 25 dB intensity ranges. Two-tone stimulation revealed that over 75% of neurons studied could be totally inhibited by frequencies lower and/or higher than the best excitatory frequency. Of this population, over 90% could be totally inhibited by lower frequencies, excluding neurons with excitatory ranges extending to the lower limit of the audible range. In nearly all cases in which neurons were inhibited by both higher and lower frequencies, the lower inhibitory threshold was at least 10 dB lower than that of the higher inhibitory frequency. Thresholds for total inhibition were sometimes lower than the excitatory threshold; such neurons were unresponsive to noise. In many cases, the inhibitory threshold was fixed, ie., once this threshold was reached, the neuron remained totally inihibited regardless of the intensity of the excitatory frequency. Such central inhibition is in marked contrast to peripheral two-tone inhibition, where only higher frequencies are inhibitory, and only at 10-20 dB above the excitatory frequency intensity. Furthermore, best higher inhibitory frequencies were often immediately adjacent to best excitatory frequencies in the torus, while in the periphery there is a 200-300 Hz separation between the two frequencies.

Many torus neurons responded to noise, but not to single tones. Such neurons were found to be highly selective for specific combinations of two or three tones, and were completely unresponsive if one of the tones was subtracted. A majority of such neurons were responsive to combinations of tones within the spectral peak of the species mating call, suggesting such neuronal specificity may play a role in the selective detection of this call.

129.7 AUDITORY INFORMATION PROCESSING IN THE TELEOST DIENCEPHALON. Stephen M. Echteler. Neurobiol. Unit, Scripps Instit. of Oceanog. and Dept. of Neurosci., U.C.S.D., La Jolla, CA 92093.

Behavioral experiments have established conclusively that, despite the absence of a structurally well differentiated auditory labyrinth, fish not only respond to sound but are capable of pitch discrimination and sound source localization. The Ostariophysi, a group of fishes with direct connections between the swimbladder and the inner ear, via a chain of specialized vertebrae, have especially well developed auditory capacities.

Electrophysiological studies of the teleost auditory system have concentrated on the inner ear microphonic potentials, primary VIII nerve afferents, medulla, and the torus semicircularis (TS) (a midbrain structure suspected to be homologous to the mammalian inferior colliculus). The precise locations of auditory centers in the teleost forebrain are not known.

In the present study averaged evoked potentials (AEPs) and multiunit activity (MUA) were recorded from the midbrain and diencephalon of an ostariophysian fish, Carassius auratus, in response to airborne clicks of 1 millisecond (ms) duration and 12-20 dB (re 1 μbar) intensity. In addition to simple repetitive stimuli, click pairs and click trains were presented to the animal to compare the adaptation characteristics of the acoustic centers in the midbrain and diencephalon. The Prussian Blue marking technique was used to verify the recording sites.

The TS field potential was biphasic with a characteristic negative wave (N-8) occurring 8 ms following stimulus onset. The N-8 wave reached maximum amplitude in the central and medial TS at depths of 1500-2500 μm below the surface of the caudal tectum. Frequently, the N-8 wave reversed polarity at depths of 2500-3000 μm. MUA phase locked to the click stimulus was usually recorded at the reversal depth of the N-8 wave.

In the diencephalon neuronal activity in response to clicks was especially strong at a depth of 1500-2000 μm below the tectal surface in an area of the caudal thalamus possibly equivalent to the central posterior thalamic nucleus (Northcutt & Butler, Brain Res., 190:33, 1980). After electrode penetration of this area the AEP underwent a sudden transformation with the N-8 wave, characteristic of the TS, being replaced by a new negative wave with a latency of approximately 18 ms (N-18). Just dorsal (within 100-200 μm) to the appearance of this N-18 wave MUA phase locked to the click stimulus was encountered.

In response to click pair and click train tests the auditory AEPs in the diencephalon showed a much more rapid onset and longer duration of adaptation than auditory AEPs recorded in the TS.

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129.8 ORGANIZATION OF NEWBORN RABBIT AUDITORY CORTEX. N. T. McMullen and E. M. Glaser. Dept. of Physiology, Univ. of Maryland School of Medicine, Baltimore, MD. 21201

Newborn (P-0) to three day old (P-3) New Zealand rabbits were used. The brains were removed following exsanguination and processed with the Van der Loos Golgi-Cox Nissl method. Frontal sections, 200 μm thick, through the presumptive auditory cortex were processed and counterstained with methylene blue. Well impregnated neurons were traced with either a camera lucida or a computer microscope. Cortex at Birth: The auditory cortex at birth is 63% of its adult thickness. The cortical plate is present and is characterized by densely packed radially aligned neurons. The Golgi method reveals that mostly pyramidal-type neurons are present within the cortical plate although a few nonpyramidal neurons are present at the top of the subplate zone. Upper cortical plate neurons exhibit multiple branched apical dendrite arbors extending into the marginal zone and almost no basal dendrites. Pyramidal neurons deep in the cortical plate have poorly developed apical dendrites with several collateral branches which invariably terminate with growth cones. Their basal dendrites are thin, relatively unbranched and exhibit terminal and preterminal growth cones. Numerous incipient branches sprout from the somatic surface. Spines are rarely seen. In the subplate zone, both pyramidal and nonpyramidal neurons are present. The somata and apical dendrites of larger pyramids exhibit profusely branched dendrites. Nonpyramidal neurons in the subplate, although not as developed as the large pyramids, have dendrites which are more developed than cortical plate nonpyramidal neurons. Cortex at P-3: The cortical plate has acquired a trilaminar organization. Neurons in the marginal zone and deep cortical plate are particularly more developed in comparison to their appearance at P-0. The bottom cell-dense layer of the cortical plate is populated with developing stellate neurons with local axons. In addition to growth cones, spine-type structures are present on the dendritic shafts. Subplate pyramidal neurons have well developed dendrites which also exhibit primitive spicules. We conclude that neuronal growth and differentiation in the rabbit auditory cortex begins well before the onset of hearing at approximately P-7. Data comparing the length, branching pattern and spatial orientation of dendrites for neonatal and adults will be presented.

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129.9 EFFECTS OF SONG LEARNING ON AUDITORY NEURONS IN THE FOREBRAIN OF AN AWAKE BIRD. H.-J. Leppelsack, Lehrstuhl für Allgemeine Zoologie, Ruhr-Universität Bochum, 4630 Bochum, West Germany.

White-crowned sparrows (Zonotrichia leucophrys) as with many other songbirds have to learn their conspecific song from a tutor (Konishi, M., Z. Tierpsychol., 22:770-783, 1965). The way this usually happens is that the young bird at the age of a few weeks hears the song of the father and keeps it in a memory. At age several months, it starts developing the individual song by comparing its own sound production with the memorized pattern. Deafening a bird at this stage will inhibit the development of a species-specific song. It is possible to influence the development of the individual song by experimental modifications of the tutor song. White-crowned sparrows do not change their song for their life time.

These behavioral findings indicate processes of neural plasticity in the auditory pathway during ontogeny. The aim of this study was to find functional indications of this plasticity on the single unit basis. 1200 auditory units were studied by glass micropipette recording in 18 awake birds. The animals had learned models of conspecific songs in which the direction of frequency modulation in the trill segment was going either upward or downward. Adequate song versions were used as stimulus during the recording experiments. 11.3% of all auditory neurons respond to only one of the two alternative trill versions. Of these units usually more can be found that only respond to the song which was learned than to the alternative one. There is a preference of 2:1 and 3:1 for the song that was learned. Statistical tests show a high significance of differences between the alternative groups of birds. This indicates some causal relationships between the number of specialized neurons and the song learning. Most of the neurons are highly specialized and respond to only a narrow range of steepness in the modulation.

The neurons described here are located in the Neostriatum caudale pars mediale, the central part of which is tonotopically organized. A relatively high number of specialized neurons is found between the isofrequency lines of 2 and 5 kHz.

129.10 PATTERNS OF CAT AUDITORY CORTICAL FIELD PROJECTIONS TO THE BASAL GANGLIA. Richard A. Reale* and Thomas J. Imig (SPON: R. Bleier). Dept. of Neurophys. and Waisman Ctr. on Ment. Retard. and Hum. Dev., Univ. of Wisconsin Med. Sch., Madison, WI 53706.

We have studied anatomical projections from auditory cortical fields by combining microelectrode and autoradiographic techniques. In these experiments best frequency (BF) maps are obtained to delimit four auditory cortical fields each of which contain a complete and orderly tonotopic representation. These fields have been designated as anterior (A), primary (AI), posterior (P) and ventroposterior (VP) (Reale and Imig, JCN, 192:256-92, 1981). By varying the amount and injection site of the radioactive amino acid, we found each field to be a source of projections to the homolateral basal ganglia. Cortical terminations were always restriced to the caudal one-half of the putamen following each injection. Additionally, fields A, AI and the low-to-mid BF(s) of field P each project to the body of the caudate nucleus. By comparison, field VP and the mid-to-high BF(s) of field P were the only sources found to produce labeling in the lateral amygdaloid nucleus. The organization of these projections is compatible with previous descriptions of a widespread corticostriatal projection system. The dorsal portion of the cortical-projection zone in the basal ganglia is strongly connected with the most anterior and dorsally situated cortical fields (A & AI), while the ventral portion of the cortical-projection zone is strongly related to the posterior and ventrally situated fields (P & VP).

When the source of the projection is confined to a portion of a cortical field labeling often appears as dense patches of silver grains separated from each other by areas of less dense labeling. In frontal sections, the dense patches of silver grains are distributed within a single elongated band curving from dorso medial to ventrolateral. This curving band can include adjacent regions of the caudate and putamen (separated by the internal capsule) or adjacent regions in the putamen and lateral amygdaloid nucleus (separated by the external capsule). In horizontal sections, the patchy distribution of cortical inputs is elongated from anteromedial to posterolateral. Two dense bands of label are seen when the lowest BF and highest BF representations were each used as injection sites in either fields AI or P. Thus in three dimensions the cortical-projection zone can be viewed as a laminated region with each lamina compressed mediolaterally.

When the sources of cortical projections includes most or all of an auditory field more complex patterns of labeling occur. The distributions of silver grains labeling the axon terminals are irregular, circular, ellipsoidal or annular and can vary in form along the extent of the projection.

(NS-05459, HD-03352, BNS76-19893)

129.11 AUDITORY CORTICAL CONNECTIONS IN THE KITTEN. J. Z. Feng* and J. F. Brugge* (SPON: C. N. Woolsey). Dept. of Neurophysiology and Waisman Ctr., Univ. Wisconsin, Madison, WI 53706.

Tritiated proline or HRP was injected into the middle ectosylvian cortex of one hemisphere in kittens during the first few weeks postpartum. Survival time was 24-96 hrs. The TMB (Mesulam) method was used to process HRP material. By the first postnatal day (PND 1) most neurons retrogradely filled in the contralateral auditory cortex form a densely packed, continuous band that presumably represents mainly layer III. This continuous pattern is in contrast to that seen in the adult cat where callosal neurons tend to segregate into clusters. Other retrogradely-labelled cells are more sparsely distributed in deeper layers with the greatest accumulation being in layer VI. At this time callosal neurons are elongated, with their long axes parallel to radial cell columns. In autoradiographs on PND 2, silver grains are seen throughout the thickness of the cortex indicating that callosal axons have reached the upper layers of the gray matter. Neither the alternating vertical columns nor the laminar bands of callosal afferents that characterize this projection in the adult is clearly seen in kittens of this age. By PND 1 the pattern of projections to the medial geniculate body and inferior colliculi are very similar to those in the adult. In several experiments small injections of HRP (conjugated to WGA) were made into middle ectosylvian cortex in kittens on PND 2-6. The distributions of retrogradely-filled neurons within small, restricted regions of the MGB were essentially coextensive with the distributions of anterogradely-labeled axonal terminals, suggesting the presence of topographically organized, reciprocal patterns of connections between cortex and MGB, as is seen in the adult. Projections are also seen to the putamen, caudate nucleus and pons. By PND 8-12 the segregation of callosal afferents into vertical columns and horizontal laminae is evident in the gray matter; by PND 16 these patterns are well developed. On the other hand, the somata of callosal neurons of layer III retain their juvenile continuous distribution beyond PND 18, even though they have by this time developed their adult shapes. Preliminary results suggest that the projections to subcortical structures from auditory cortex develop considerably earlier than auditory callosal projections and that the adult distribution patterns of auditory callosal sources and terminals first appear at different times postpartum. BNS7912939, HD03353, NS12732.

129.12 CONNECTIONAL DIFFERENCES BETWEEN AUDITORY FIELDS IN A CF-FM BAT J.B.Fritz*, J.Olsen, N.Suga and E.G.Jones (SPON: J. Wallach) Depts. of Biology, and Anatomy & Neurobiology, Washington Univ., St. Louis, MO 63110

We have been studying the auditory system of the Panamanian mustasched bat, *Pteronotus parnellii rubiginosus*, which uses echo location to orient, and capture prey. Its auditory cortex is composed of several functional areas which have been defined neurophysiologically. They include the DSCF, FM-FM and CF/CF areas, each of which is specialized for the systematic representation and processing of different features of the bat's biosonar signal and echo; such as target size, location, distance and velocity (Suga, O'Neill and Manabe Science 203:270, 1979). In order to investigate the anatomical basis of these functional areas, we carried out experiments with anatomical tracing techniques. In 15 bats, tracer was injected in separate cortical loci under electro physiological guidance. In the autoradiographic studies, we made small localized injections of ^{3}H-proline/leucine (5-25μCi). The horseradish peroxidase (HRP) injections were also small (.05-.1μL of 20% HRP solution). Retrograde and anterograde transport of HRP was visualized using tetramethylbenzidine.

Our results show that the FM-FM and CF/CF areas are reciprocally connected with their contralateral homologues and ipsi- and controlaterally connected with three other cortical areas. The DSCF area has reciprocal connections with its contralateral homologue and ipsi- and contralateral connections with only one other cortical area. We have yet to characterize the heterotopic cortico-cortical projection areas physiologically. Injections into the FM-FM area resulted in anterograde labelling of a large number of cells within a dorsal division of the medial geniculate body (MGB). A few scattered cells were also found within a medial division. Anterograde label within the MGB was coextensive with the retrograde label. The pattern of thalamic labelling after CF/CF injections was similar to that seen in the FM-FM case. However a different pattern of labelling in the MGB was observed following injections into the DSCF area; this is connected to a ventral division of the MGB. All areas projected to the pontine nuclei, the inferior and superior colliculi, the striatum and had reciprocal connections with the claustrum. In addition, the DSCF area was found to project bilaterally to the basolateral amygdala. It is known that the DSCF area responds optimally to single tones and that the FM-FM and CF/CF areas show facilitation for paired auditory stimuli. Our results indicate some neuroanatomical correlates of this neurophysiological distinction.

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129.13 ACTIVITY IN AUDITORY CORTEX AND ITS THALAMIC AFFERENTS ELICITED BY HUMAN SPEECH SOUNDS IN THE MONKEY. Mitchell Steinschneider*, Joseph C. Arezzo and Herbert G. Vaughan, Jr. Dept. of Neuroscience, Albert Einstein College of Medicine, Bronx, N.Y. 10461.

To determine whether phonetic features are reflected in the patterns of neural activity in the auditory cortex and its thalamic afferents, responses to 3 synthetic syllables: /da/, /ba/, and /ta/, which vary in their place of articulation or voice onset time (VOT), were recorded in awake monkeys. The stimuli were presented at 80 dB SPL to the ear contralateral to the recording sites. /Da/ and /ba/ were identical except during the 40 msec of formant transitions. /Ta/ differed from /da/ by an increase in the VOT from 0 to 80 msec. Averaged multiple unit activity was obtained from electrodes which passed vertically through the superior temporal plane and into the underlying white matter. When single units could be isolated, PST histograms were constructed. Cortical activity was differentiated from activity in afferent fibers on the basis of response latencies to click stimulation and histological verification of recording sites.

VOT was related to the discharge patterns of thalamocortical axons and cortical units. 'On' responses to /ta/ differed from 'on' responses to /da/ and /ba/ in that a second activity burst 80 msec after the first was associated with the onset of the voiced portion of the stimulus. Phase-locked responses to the vowel fundamental frequency were recorded to all 3 stimuli. Whereas the phase-locked responses to /da/ and /ba/ began near stimulus onset and continued throughout the duration of the syllables, the periodic response to /ta/ was delayed by an interval that reflected the VOT.

Differential responses related to the consonants' place of articulation were also seen. Short-latency 'on' responses were sometimes noted to /da/ and /ta/ but not to /ba/; while occasionally the opposite pattern was seen. During the acoustically identical steady-state portions of /da/ and /ba/, phase-locked activity was very similar for the 2 syllables. However, during the early formant transition period, where the frequency compositions of the 2 stimuli were different, phase-locked activity could occur to one but not the other.

Activity patterns in thalamocortical axons and cortical cells also differed. While cortical neurons, especially in layer 4, demonstrated phase-locking to the fundamental frequency of vowels, this type of activity was more pronounced and consistent in thalamocortical axons. Cortical cells, on the other hand, more often responded with sustained activity and/or to the transients of the speech stimuli.

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129.14 SINGLE UNIT ACTIVITY RELATED TO SENSORY-MOTOR ASSOCIATION IN THE AUDITORY CORTEX OF A MONKEY. E. Vaadia*, Y. Gottlieb* and M. Abeles* (SPON: M. H. Goldstein, Jr.). Dept. of Physiology, Hadassah Medical School, Jerusalem, Israel.

Stimulus induced behavior has been classically interpreted as an interaction between "sensory systems" and a "motor system" through the involvement of an "association system." In this research we studied some aspects of the role of the auditory cortex in the process of sensory motor association.

The activity of 146 units in the auditory cortex of a rhesus monkey was recorded during performance of auditory discrimination reversal task in which each of two stimuli was associated with each of two learned motor responses. Seventy-two units were in the primary cortex (areas Kam and Kalt), seventy-four units were in the lateral portion of the belt area (Pa Alt). The units were recorded from 40 recording sites, 2-6 single units were recorded simultaneously in each site. We found that:

(A) The spike activity of a fraction (9-17%) of the units in the auditory cortex reflects the process of sensory motor association.

(B) These neurons have a component of their evoked response which is sensory and a component which reflects sensory motor association.

(C) Units exhibiting association activity did not show any clustering.

(D) On the average, units of the koniocortex tend to respond strongly and with shorter latency to sound stimuli compared to the units of the lateral belt area. However, units exhibiting sensory motor association activity are equally distributed in both areas.

On the basis of these findings it is suggested that the association system need not be separated anatomically from the cortical sensory system. We suggest that the mechanism by which sensory-motor association occur may be better studied in further research, on the basis of the premise that a single neuron can participate in the performance of different functions.

129.15 NEUROPHYSIOLOGICAL MECHANISMS OF ANTICIPATION IN THE AUDITORY CORTEX OF THE MONKEY. S. Hocherman*, A. Itzhaki*, (SPON: R. Ashkenazi). Dept. of Physiology & Biophysics, Faculty of Medicine, Technion, Israel Inst. of Technol., P.O.B. 9649, Haifa, Israel.

Evoked unit activity was recorded in the auditory cortices of rhesus monkeys. Two types of auditory stimuli were used. One consisted of 100 msec burst of white noise, and the other was a 100 msec burst of a pure tone, of any frequency. The monkeys were trained to discriminate between these two types of auditory stimuli by making a corresponding lever push. In most trials, a light flash that preceded the auditory stimulus by 700 msec indicated to the monkey the type of the following auditory stimulus. Occasionally, however, the wrong auditory stimulus was made to follow the light signal. Analysis of the monkeys' behaviour ascertained that they indeed anticipated the nature of the auditory stimuli on the basis of the visual signals.

Quantitative analysis revealed that the evoked activity of about half of the 92 units that were studied was affected by the monkeys' anticipation of the auditory stimulus. The affected units could be grouped in two categories. 1. Units that showed response facilitation to correctly anticipated auditory stimuli, and response inhibition to wrongly anticipated auditory stimuli. 2. Units that responded at a base line intensity level to correctly anticipated stimuli, but that showed strong response facilitation to wrongly anticipated auditory stimuli. These findings are indicative of the neurophysiological processes that may underly the mechanism of anticipation.

129.16 LESIONS OF RESTRICTED FREQUENCY REPRESENTATIONAL SECTORS WITHIN PRIMARY AUDITORY CORTEX PRODUCE FREQUENCY DEPENDENT SOUND LOCALIZATION DEFICITS. W.M. Jenkins and M.M. Merzenich*. Coleman Memorial Lab., University of California, S.F., San Francisco, CA 94143.

It has long been known that auditory cortex contributes to the ability to localize a sound source. Recent evidence has indicated that even unilateral lesions of auditory cortex produce severe permanent contralateral hemifield sound localization deficits.

We have attempted to answer the questions: 1) Is sound localization dependent upon the integrity of primary auditory cortex (AI)? 2) How is sound localization behavior affected by partial lesions of this auditory field, e.g., lesions of restricted frequency representational sectors, or of given binaural bands? To answer these questions, microelectrode best frequency and binaural response maps of AI cortex were obtained under sterile conditions. After definition of boundaries, frequency organization and internal binaural structure, restricted lesions were effected by coagulation of cortical surface microvasculature. These adult cats' sound localization ability was evaluated in a seven-choice free sound field apparatus, using tonal and broad-spectrum stimuli before and after such lesions.

Preliminary results indicate that restricted lesions within the frequency domain produce predicted frequency-dependent deficits in sound localization ability. The ability to detect these deficits is dependent upon selection of an appropriate behavioral test, and on use of stimuli of brief duration. Continuing experiments are directed toward definition of the behavioral band(s) of AI that is (are) requisite for normal localization in the contralateral hemifield. (Supported by NIH grant NS 10414 and the Coleman Fund.

130.1 INHIBITION OF SENSORY TRANSMISSION AND DEPRESSION OF BLOOD PRESSURE BY ELECTRICAL STIMULATION WITHIN THE MEDULLARY RAPHE NUCLEI OF THE CAT. P.S. Blum, Department of Physiology, Thomas Jefferson University, Philadelphia, PA 19107.

Previous studies have shown that electrical stimulation within the medullary raphe nuclei (nucleus raphe magnus, nucleus raphe pallidus, nucleus raphe obscurus; MRN) can both change arterial blood pressure and inhibit sensory transmission in the spinothalamic tract (ST). The present experiment was designed to determine if these two effects are interrelated. The experiments were performed in four adult cats anesthetized with alpha-chloralose and paralyzed with gallamine. Sensory transmission was measured from the amplitude of averaged evoked potentials in the ST following stimulation of the superficial radial (SR) nerve. Mean arterial blood pressure (MABP) was measured via an intra-arterial catheter. In each experiment up to 10 MRN sites (total= 31) were selected for stimulation. These sites were spread evenly in a region from the pyramidal tract to the fourth ventricle and from the pontomedullary border to the obex. At each site, single pulse electrical stimuli were used to determine if stimulation within the MRN reduced the amplitude of SR-evoked ST potentials and 10 sec trains of stimuli were tested for the ability to change MABP. Fourteen sites (46%) were identified where electrical stimuli inhibited SR-evoked activity in the ST. Stimulation at the same 31 sites produced elevation of MABP at 2 sites (6%) and depression of MABP at 10 sites (33%). Seven sites were both inhibition-producing sites and depressor sites. These represent half of the inhibition-producing sites (7 of 14) and 70% of the depressor sites (7 of 10). Five of these dual effect sites were clustered in the rostral portion of the MRN, just dorsal to the trapezoid body. The other two sites were located approximately 2 mm caudally. These data support the concept that single neurons located in the rostral MRN can influence sensory transmission and blood pressure, probably via a descending projection to the sensory neurons of the dorsal horn and preganglionic sympathetic neurons.

130.2 MODULATION OF LATERAL CERVICAL NUCLEUS NEURON RESPONSES BY PERIAQUEDUCTAL GRAY AND RAPHE MAGNUS STIMULATION. J.O. Dostrovsky and P. Chang*. Dept. of Physiology, University of Toronto, Toronto, Ontario M5S 1A8, Canada

Stimulation of the periaqueductal gray (PAG) and nucleus raphe magnus (NRM) is known to inhibit nociceptive responses of spinal and medullary dorsal horn neurons. Recent studies have also shown that stimulation of these regions can inhibit non-nociceptive responses in spinal cord, trigeminal nuclei oralis and candalis, and dorsal column nuclei. The present study examines the effects of PAG and NRM stimulation on the transmission of information in the spinocervicothalamic pathway.

Experiments were performed on 8 chloralose anesthetized cats. Extracellular single unit recordings of lateral cervical nucleus (LCN) neurons were obtained using carbon fiber microelectrodes. Most of these neurons could be antidromically activated from the thalamus and had low threshold cutaneous receptive fields on the fore-or hind-limb. Conditioning stimuli consisted of 100 ms, 500Hz trains and were delivered to bipolar electrodes located in subsequently histologically verified loci in the PAG and NRM. In some experiments a microstimulation electrode was inserted in the dorsolateral funiculus (DLF) at L1. Stimulation of PAG and NRM 130 ms prior to delivering an electrical stimulus to the neuron's receptive field was found to powerfully inhibit the responses of 31 of 34 LCN neurons. This inhibition could be produced using stimulation currents in the range 50 to 250 uA which are comparable to intensities found necessary to inhibit spinal cord nociceptive and non-nociceptive neurons. However, these conditioning stimuli failed to block or increase the latency of the antidromic spikes in all but 3 of 28 units tested. Furthermore, they produced no effect on the responses produced by DLF stimulation in 5 units and only a weak inhibition at high stimulation intensities in the remaining 5 units tested. These latter findings imply that there is only a limited, or no direct inhibitory input from PAG and NRM to LCN. In summary, this study has shown that stimulation of the PAG and NRM markedly inhibits transmission of sensory information in the spinocervicothalamic pathway and that the inhibition is primarily mediated by an action at the spinal level.

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130.3 IS THERE A CROSSED INNERVATION OF THE ANTERIOR TEETH? P.M. Fuller, S. Wilson, J. Winfrey* and P. Johns* Departments of Anatomy and Oral Biology, University of Louisville School of Dentistry, Louisville, Kentucky 40232.

In the past few years there has been a re-newed interest in the question of a possible crossed or "transmedian" innervation of the anterior teeth. The use of various neuroanatomical and neurophysiological methods have led some investigators to conclude there is an extensive crossed innervation of both the maxillary and mandibular teeth, that extends as far laterally as the first premolar teeth. These conclusions are supported by data from the clinician who often is confronted with incomplete anesthesia of the anterior teeth after a unilateral mandibular nerve block. This leads the clinician to support the theory of a crossed innervation. In an effort to determine the precise innervation of the anterior teeth and to attempt to resolve the question of a possible crossed innervation, the present study was undertaken. The first set of experiments consisted of making a cavity preparation in either the maxillary or mandibular canine tooth in 15 adult cats. The second set of experiments concerned the incisor teeth. In this series, a cavity preparation was made in one of the mandibular incisor teeth, for a total of 18 experiments. In each of the experiments, 0.5-3.0 µl of fresh Horseradish Peroxidase (Sigma, type VI; 15% concentration) was placed in the cavity preparation. After 10 minutes the cavity was filled with gelfoam and capped with dental acrylic. Survival times ranged from 12-96 hours. The animals were then perfused with 0.1M cacodylate buffer, followed by an equal amount of fixative. The following tissues were quickly removed and placed in fresh fixative: the ipsilateral and contralateral trigeminal ganglia, the inferior alveolar nerve and the nerve to mylohyoid. Frozen serial sections were cut at 50µ and processed according to the histochemical procedure of Mesulam and then counterstained with Neutral Red. The results from the series of 15 experiments on the canine teeth showed that all of the labeled cells were in the ipsilateral trigeminal ganglia, and the number of labeled cells ranged from 96-248. The experiments with the mandibular incisor teeth also showed that all of the labeled cells were in the ipsilateral ganglia. In addition, a portion of the sensory fibers to the incisor teeth in the cat reach the teeth by way of the nerve to mylohyoid. Our data clearly shows that the mandibular and maxillary canine teeth and the mandibular incisor teeth in the cat are supplied entirely from the ipsilateral side of the mandible, and there is no basis for the claim of a crossed innervation of the anterior teeth in the cat.

130.4 PROPERTIES OF CELLS ACTIVATED BY VIBRISSAL MOVEMENT IN THE SUPERIOR COLLICULUS OF THE RODENT. J. McHaffie* and B.E. Stein (SPON: S.J. Goldberg). Department of Physiology, Medical College of Virginia, Richmond, VA 23298.

The vibrissae are specialized hairs which can play a significant role in the orientation and exploration behaviors of rodents. They are well represented in the intermediate and deep laminae of the superior colliculus, a structure intimately involved in these behaviors. The present study was an attempt to determine the properties of vibrissa-activated cells in the superior colliculus and to compare these properties to similar cells elsewhere in the central nervous system.

Single-unit recordings were made in the superior colliculi of urethane-anesthetized rats and hamsters. Cells activated by the displacement of the vibrissae were studied using an electronically-controlled moving coil vibrator with which stimulus velocity and amplitude could be precisely regulated. Generally, colliculus cells were activated by several adjacent contralateral vibrissae and were very sensitive to small displacements of any one of these. In each instance, a single vibrissa was inserted into a hollow stylus and moved at various velocities and amplitudes. In all cases a transient response was evoked, even to sustained displacements. Unlike elsewhere in the nervous system, no 'slowly adapting' vibrissal units were located. Furthermore, unlike vibrissa-activated cells in the cortex and thalamus, colliculus cells seemed poorly suited for coding stimulus amplitude and velocity. While most of the cells studied were exquisitely sensitive to small displacements and low velocities, they did not show a regular increase in impulse frequency to increasingly large displacements or higher velocities. Vibrissa-activated colliculus cells in these rodents seemed well-adapted to signaling the presence and location of a stimulus but not the specific characteristics of that stimulus.

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130.5 RESPONSES OF CELLS IN CAT CUNEATE NUCLEUS TO ITERATIVE STIMULATION OF FOREARM NERVES. R.J. Weinberg* and A.L. Towe (SPON: T.T. Kennedy). Dept. of Physiology and Biophysics, University of Washington, Seattle, WA 98195.

Standard single unit techniques were used to record from neurons in the cuneate nucleus of chloralose-anesthetized, paralyzed cats. Most microelectrode tracks were made 1-4 mm. caudolateral to the obex. The responses of cells to natural stimulation of the skin and to electrical stimulation of three major forearm nerves by implanted silastic cuffs were observed. Particular attention was directed to the responses to different iterative stimulus rates. An array of bipolar stimulating electrodes was placed in the medial lemniscus just caudal to the thalamus to identify cuneothalamic projection (CTP) cells by antidromic activation; less than a third of the sample tested met the criteria for thalamic projection.

The sample was partitioned into units that responded only to one nerve (Type I; 2/3 of the sample) and cells that responded to two or more nerves (Type II; 1/3 of the sample). Fully 3/4 of the Type I units, but only 1/3 of the Type II cells, were isolated in the dorsal half of the nucleus. Furthermore, 2/5 of the Type I units, but only 1/10 of the Type II cells tested qualified as CTP neurons. Attention was focused on the maximum iterative stimulation rate that each cell would faithfully follow (f_{max}). More than 1/2 of Type I units tested showed f_{max} >50/sec., whereas only 1/5 of the Type II cells exceeded this value. Among the Type II cells, when f_{max} was high (or low) for one nerve it was usually high (or low) for the other nerve(s). There was a clear gradient in mean f_{max} as a function of depth of isolation, grading from high f_{max} dorsally to low f_{max} ventrally in the nucleus.

When a Type II neuron was driven to exhaustion by prolonged iterative stimulation of one nerve near f_{max} for that cell, it remained responsive to stimulation of the other nerve(s) (though usually not as briskly as prior to the conditioning stimulation.) This implies that some portion of the frequency-following failure is due to homosynaptic depression. The mild reduction in responsiveness to the second nerve points to the possibility that an orthodromic inhibitory system with high f_{max} could play a role in frequency-following failure. Prolonged iterative antidromic activation of CTP neurons had little effect on their responsiveness to orthodromic input. This implies that a recurrent inhibitory mechanism involving CTP neurons cannot account for the frequency-following failure.

Portions of this research were supported by NIH grants NS05136 and GM07108.

130.6 WHITE NOISE ANALYSIS OF RECEPTIVE FIELD PROPERTIES OF SINGLE UNITS IN THE CUNEATE NUCLEUS OF CATS. D.J. Surmeier, Jr.*, A.L. Towe, and R.J. Weinberg*. Dept. of Physioloby and Biophysics, University of Washington School of Medicine, Seattle, WA 98195.

White noise analysis was used to describe the temporal patterns of excitability produced in cuneate neurons by cutaneous stimuli that were varied in strength and position, and to characterize the manner in which two spatially separated stimuli interact to produce neuronal activity. Single unit recordings were obtained from chloralose-anesthetized domestic cats using tungsten microelectrodes and standard amplification equipment. Following isolation, each unit's sub-modality sensitivity was determined, and its cutaneous excitatory receptive field (ERF) mapped using fine mechanical probes. In some experiments, units were tested for antidromic activation from the medial lemniscus. Bipolar needle electrodes were placed in the center of the ERF and at loci inside and outside the ERF boundary. Two independent 10/sec. pseudo-Poisson process impulse trains were used to simultaneously drive stimulators connected to the two needle electrodes. The spike train elicited was led into a window discriminator, the digital output of which, along with stimulus event pulses, was fed into an on-line computer that stored the information on magnetic disk. Upon completion of a noise run, spike train stationarity was tested, and first- and second-order Wiener kernels were computed using a crosscorrelation technique. This procedure was repeated at several stimulus intensities.

Analysis of initial results showed that: (1) The majority of neurons, regardless of sub-modality class, responded to a stimulus within their ERF with an initial period of increased excitability, followed by a clear decrease in excitability. The most notable exception was a subset of the hair-sensitive class. (2) Short latency facilitation and/or inhibition following stimulation outside the ERF was common to all sub-modality groups; apparently only the hair-sensitive class displays an exclusively inhibitory surround. (3) Stimulation of the margin of the ERF significantly altered the first- and second-order kernels derived from the center, indicating the existence of circuitry requiring spatial summation for activation. (4) Units driven by muscle, tendon, or joint afferents often received a cutaneous input, usually facilitatory-inhibitory in nature, but rarely frankly excitatory.

Portions of this research were supported by NIH grants NS05136 and GM7108.

130.7 SOMATOTOPIC ORGANIZATION OF SUBCUTANEOUS PROJECTIONS FROM TAIL, HINDLIMB AND ABDOMEN TO NUCLEUS Z IN FOX SQUIRRELS. E.-M. Ostapoff*, J. I. Johnson and B. C. Albright. Neuroscience Program and Psychology, Biophysics and Zoology Depts., Michigan State University, East Lansing, MI 48824; and Dept. of Anatomy, Univ. of North Dakota Medical School, Grand Forks, ND 58202.

Nucleus z wraps around the rostral pole of the gracile nucleus, and can be distinguished from the gracile by cytoarchitecture (e.g., smaller cells), input pathway (via lateral rather than dorsal spinal columns), and dominant physiological projections (from hindlimb muscles rather than skin), in primates and carnivores. In a similar region in fox squirrels (Sciurus niger) arboreal rodents, we mapped responses to mechanical stimulation using tungsten microelectrodes and urethane anesthesia. We found two types of projections (z vs. gracile) corresponding to distinct cytoarchitectural regions. While the ranges of cell (perikaryal) diameters of the z and gracile populations overlapped (n = 10 cells per region in 4 separate samples = 40; range of z = 12-32 μm, range of gracile = 16-34 μm) all but 3 of the 40 z cells were smaller than the mean of the 40 gracile cells (this mean of gracile was 25.9 μm, mean of z was 20.0 μm). For these measures the boundaries between gracile and z were established by the location of contrasting projections along tracks of electrode penetrations (54 responding loci in 17 electrode penetrations from 5 animals). The z type projections were located within a volume whose maximal extent was 0.6 mm in any dimension; these projections were all from subcutaneous receptors and were somatotopically organized. Projections from the tail were located rostromedially, lateral to these were projections from the hind limb and foot, and most lateral were projections from the abdomen. The contrasting projections to the gracile (large-celled) region were primarily from cutaneous surfaces of hind limb, tail, and posterior trunk, but did include some subcutaneous input. There was a break in somatotopic continuity as electrode penetrations passed from gracile to z regions. These results indicate that fox squirrels possess a region physiologically and cytoarchitecturally similar to the nucleus z of other species;that in addition to the hindlimb projections reported in other species it receives projections from tail and abdominal regions; and that these projections are somatotopically organized. (Supported by NSF grant BNS - 7903421).

130.8 INDEPENDENT PROJECTIONS FROM THE DORSAL COLUMN NUCLEI TO THE TECTAL AREA, PRETECTAL AREA AND THALAMUS IN CATS. M.S. Bull*, S.K. Mitchell* and K.J. Berkley (SPON: D.R. Kenshalo). Dept. Psychol., Fl. St. Univ., Tallahassee, FL 32306.

When ablations or injections of ^{3}H-leucine are made in the dorsal column nuclei (DCN), projections can be traced from DCN not only to the thalamus, but also to specific targets in discrete portions of the tectal area (external and pericentral inferior colliculus, intercollicular area, deep layers of superior colliculus) and of the pretectal area (anterior pretectal n., pars compacta and a small region in the middle of the posterior pretectal n.). The purpose of the present experiments was to characterize, anatomically, the neuronal populations within DCN that project to each of these three major terminal areas.

A double retrograde labeling strategy was used in which two different tracers were injected into any two of the three terminal areas in the same cat. The tracers were: horseradish peroxidase (HRP), tritiated inactivated HRP (^{3}H-apo-HRP), HRP conjugated to wheat germ agglutinin (WGA:HRP) and tritiated acetyl-WGA (^{3}H-acetylWGA). The usual combinations were ^{3}H-apoHRP with HRP and ^{3}H-acetylWGA with WGA:HRP. Following 24-72h survival times, a set of sections was processed for autoradiography and HRP histochemistry using diaminobenzidine. One of the adjacent sets of sections was processed only for autoradiography; the other only for HRP histochemistry using tetramethylbenzidine.

Double-labeled neurons were only rarely observed in DCN in experiments involving accurately-placed, non-overlapping injections. Analysis of the adjacent sections suggested that this lack of double-labeling was not due to insensitivity of the methods. In agreement, many double-labeled neurons were clearly observed in other experiments where the injections overlapped or invaded another of the territories.

The neurons projecting to the tectal or pretectal areas were of mixed morphology and tended to occupy similar positions within DCN. The two groups formed a loose network around the edges of and between the gracile n. and cuneate n. in regions generally unoccupied by the relatively homogeneous population of large and round thalamic-projecting neurons. The few double-labeled neurons observed in each experiment were usually located at the border between the cuneate n. and spinal trigeminal n.

These results demonstrate an almost complete independence of the neuronal populations in DCN which project to the three target areas. The results also suggest, however, that the tectal and pretectal-projecting neurons may be functionally related to each other, but different from the thalamic-projecting neurons.

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130.9 A SOMATIC SENSORY PROJECTION SYSTEM INVOLVING THE DORSAL COLUMN NUCLEUS, PRETECTAL AREA AND INFERIOR OLIVE AS VISUALIZED WITH THREE DIFFERENT BIDIRECTIONAL TRACERS IN CATS. (SPON: H.H. Molinari). S.K. Mitchell*, M.S. Bull* and K.J. Berkley. Dept. Psychol., Fl. St. Univ., Tallahassee, FL 32306

Following ablations or injections of ^{3}H-leucine into the dorsal column nuclei (DCN) of cats, distinct and densely-focused projections can be traced from DCN to specific portions of the contralateral anterior and posterior pretectal nuclei as well as to the dorsal and medial accessory divisions of the contralateral inferior olive. Relevant to these findings are the results of a number of authors demonstrating that neurons in different portions of the pretectal area appear to project to different portions of the ipsilateral inferior olive. These results suggest that the DCN-recipient zones located in the pretectal area and inferior olive might themselves be specifically connected.

In order to determine if this triadic somatic sensory projection exists, three different tracers were injected into the anterior pretectal area of different cats. The tracers used were: tritiated inactivated horseradish peroxidase (^{3}H-apoHRP), tritiated acetyl-wheat germ agglutinin (^{3}H-acetyl-WGA), and wheat germ agglutinin conjugated to HRP (WGA:HRP). Following a survival period of 72h, the tissue was processed for autoradiography and/or horseradish peroxidase histochemistry using both tetramethylbenzidine and diaminobenzidine as chromagens. As expected, retrogradely-labeled neurons were observed in specific portions of DCN in every case. In addition, dense, orthogradely-labeled terminal zones located in the DCN-recipient portions of the inferior olive could also be observed in every case.

These results demonstrate that ^{3}H-apoHRP, ^{3}H-acetyl-WGA and WGA:HRP can all be used effectively as orthograde as well as retrograde tracers. In addition, the results firmly establish the existence in the cat of a well-organized, somatic sensory-related projection system involving specific neurons in DCN and specific portions of the pretectal area and the inferior olive.

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130.10 SOMATOSENSORY INFORMATION PROCESSING WITHIN THE RACCOON THALAMIC VENTROBASAL COMPLEX. Susan Warren*, and Benjamin H. Pubols Jr. Department of Anatomy, College of Medicine, Pennsylvania State University, Hershey, PA 17033.

The differential role of neurons of the thalamic ventrobasal complex in tactile information processing has been examined by comparing the properties of such neurons with those of single neurons of the cuneate neucleus (Rowinski, Haring, and Pubols, Brain Res., 1981; Haring, Pubols, and Rowinski, J. Physiol., 1981) and primary afferent fibers of the cuneate fasciculus (Pubols and Pubols, J. Neurophysiol., 1973), previously studied utilizing similar procedures. Tungsten microelectrodes have been used to detect extracellular unit responses to mechanical stimulation of glabrous surfaces of the forepaw in methoxyflurane or barbiturate anesthetized raccoons, and all recording loci have been histologically verified as falling within the ventrobasal complex.

Twenty-five units have been classified according to their response to controlled mechanical stimuli as rapidly adapting (RA; N = 18), slowly adapting (SA; N = 3), or Pacinian (Pc; N = 4). Percentages derived from these figures are similar to those found at levels of both the cuneate nucleus and the upper cervical cuneate fasciculus.

Peripheral receptive field (RF) areas are smaller on digital surfaces (median = 30 mm^2) than on palmar surfaces (median = 105 mm^2). These values are approximately 4 X the corresponding values for the cuneate nucleus, which, in turn, are 40 X (digital RFs) and 100 X (palmar RFs) values for primary afferents.

The relationship between instantaneous spike frequency during displacement ramp stimulation, and ramp velocity, was examined in 1 SA and 7 RA units. Although a power function provided the best fit in 6 of the 8 cases (least squares method), both the exponents of the power function equation (b), and indices of goodness of fit (r) were considerably lower than values for units of both the cuneate nucleus and cuneate fasciculus. Most values of r were between .67 and .76, while 6 of the values of b were < .35, in contrast to values of b of .40 or higher at the two lower levels.

A comparison of neural properties at the three levels of the dorsal column-medial lemniscal system suggests that there is a greater loss in fidelity of spatial information transmission by single neurons within the cuneate nucleus, but a greater loss of quantitative information transmission capability by single neurons within the ventrobasal complex. (Supported in part by research grant NS-13418, USPHS.)

130.11 A THALAMIC TERMINUS FOR THE LATERAL CERVICAL NUCLEUS. D.C.N. da Costa*, R.S. Metherate*, P. Herron and R.W. Dykes (SPON:L.O'Kelly). Microsurgery Lab. Depts. of Surgery, Neurology and Neurosurgery, and Physiology, McGill University, Montreal, Quebec H3A 1A1

When horseradish peroxidase (HRP) was injected into the feline posterior thalamus at sites centered on the ventroposterior inferior nucleus (VPI), retrogradely labelled cells were located in the posterior part of dorsal column nuclei (DCN) and in the lateral cervical nucleus (LCN). The VPI was known to receive input from predominantly Pacinian corpuscles, hence several questions arose: (i) Does LCN project to VPI or did the VPI-centered HRP injection spread to an adjacent region of the posterior thalamus that serves LCN? (ii) Does the LCN relay Pacinian information, as does posterior DCN, or are its neurons conveying another class of afferent input?

To answer these questions experiments were performed on nembutal-anaesthetized mongrel cats using glass-coated tungsten electrodes and/or NaCl-filled glass micropipettes. Single units were recorded in the LCN and identified on the basis of their large cutaneous rapidly-adapting receptive fields; they were characterized as being post-synaptic cells by their inability to follow high frequency electrical stimulation applied to the center of the receptive field. Electrode trajectories were reconstructed in 80 um thionin-stained frozen sections. Of 53 penetrations, 15 were histologically verified as having passed through LCN. Thirty-four LCN units were found within the cytoarchitectonic limits of the LCN. In each case, their response properties demonstrated a lack of Pacinian afferent input and a convergence of information from numerous hair-associated, rapidly-adapting fibers.

A subsequent HRP injection into the medial part of VPI failed to label the LCN and exploration of the region around the VPI with metal electrodes provided evidence that a discrete volume of the posterior thalamus beneath the lateral geniculate nucleus receives input predominantly from hair-associated rapidly-adapting cutaneous afferent fibers. The cells in this region have very large receptive fields and appear to be submodality-specific; stimuli to deep structures or sustained or noxious cutaneous stimuli did not activate the cells. In these respects the neuronal response properties parallel the response properties of cells in the LCN. Presumably this thalamic site receives a major excitatory input from the LCN. Generally large thalamic receptive fields are associated with posterior group neurons having non-lemniscal and nociceptive inputs. Our experiments suggest that at least a part of the posterior thalamus contains neurons receiving submodality-specific input devoid of convergence from nociceptors. (Supported by the Medical Research Council of Canada)

130.12 DISTRIBUTION OF TRIGEMINOTHALAMIC SYNAPSES ON IDENTIFIED THALAMOCORTICAL RELAY NEURONS IN THE VENTROBASAL COMPLEX OF THE MOUSE. Gary R. Belford and Edward L. White. Dept. of Anatomy, Boston Univ. Sch. of Med., Boston, MA 02118

Early attempts to identify thalamocortical relay (TCR) neurons relied on indirect evidence: the free disappearance within the section of the axons of Golgi-impregnated neurons. Presumably, these axons enter myelin sheaths and are not impregnated. With the advent of retrograde labeling of neurons with horseradish peroxidase (HRP), it became possible to inject HRP into the cortex and thereby label the somata of TCR neurons. However, since only the most proximal portions of dendrites were labeled, the full extent of the dendritic trees could not be visualized (e.g., Ralston and Sharp, BR 62:273, '73; Saporta and Kruger, JCN 174:187, '77). Recently, the retrograde transport of HRP was used to label entire dendritic trees of neurons in cortex (White et al., Neurosci. Let. 19:149, '80). In the present study this technique has been coupled with lesion-induced degeneration to study the distribution of trigeminothalamic synapses on unequivocally-identified TCR neurons in the ventrobasal complex (VB) of the mouse.

The vibrissal area of the principal trigeminal nucleus was lesioned in adult male CD-1 mice. One day later, HRP was injected into the corresponding 'barrel' region of the contralateral SmI cortex. Two days after this the animals were perfused and their brains sectioned and reacted for HRP. After light microscopic evaluation, sections containing labeled TCR cells were embedded and serially thin sectioned.

TCR neurons, as identified by HRP labeling, have 4 to 10 primary dendrites, which may branch into tufts of secondary dendrites, branch only once or not at all. Dendrites may extend hundreds of microns from the soma. Each of 4 TCR cells examined with the electron microscope forms trigeminothalamic synapses on its soma or primary dendrites. Those on somata tend to be near the bases of primary dendrites; those on dendrites, near branch points. Nearly all of 4 dendrites belonging to one labeled TCR cell have been reconstructed from an unbroken series in excess of 1000 sections. One dendrite (101 μms in length) forms no trigeminothalamic synapses. 75% of the trigeminothalamic synapses formed by the other dendrites occur within 15 μms of the soma. Although trigeminothalamic synapses (8/ 303 μms dendritic length) comprise only a small proportion of the synapses formed with the reconstructed dendrites, they are well situated to dominate the electrical activity of the thalamocortical relay neuron.

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130.13 CONNECTIONS OF THE VENTROPOSTERIOR INFERIOR NUCLEUS OF THE THALAMUS IN CATS. P. Herron* and R.W. Dykes. Departments of Physiology, Surgery, Neurology & Neurosurgery, McGill University, Montreal, Quebec, Canada H3A 1A1.

Based on neurophysiological evidence, several investigators have hypothesized that the inputs of each mechanoreceptor class are spatially segregated in the dorsal column-medial lemniscal pathway (DC-ML). Recent neurophysiological studies of nuclear areas in the DC-ML have shown that the segregated sites of Pacinian inputs are the caudal poles of the dorsal column nuclei (DCN), ventroposterior inferior nucleus (VPI) of the ventroposterior nuclei of the thalamus, and somatosensory area II (SII).

In this study, we tested the anatomical implications of this hypothesis; we injected horseradish peroxidase (HRP) into VPI in order to determine the organization of its connections with the DCN and somatosensory cortical areas I (SI) and II. The areas of Pacinian inputs in SII and VPI were carefully delineated with tungsten microelectrodes and electrolytic lesions were made on the boundaries of the Pacinian-input zone. Pipette electrodes were filled with a solution of HRP in tris buffer and then used to record the Pacinian responses again. Following identification of the Pacinian-input zone, a single small iontophoretic injection of HRP was injected.

The results indicate that the VPI nucleus and a portion of SII are principal sites that are activated by Pacinian inputs in the thalamus and cortex, respectively. When HRP was placed in VPI labeled cell bodies were observed in SI, SII, and the DCN. There were a greater number of labeled cell bodies in SII than there were in SI. The labeled cell bodies observed in the DCN were localized in the cytoarchitecturally distinct caudal poles of the gracile and cuneate nuclei; the labeled cell bodies were located in the same region previous experiments have shown was activated by Pacinian inputs. In addition, labeled cell bodies were observed in adjacent thalamic nuclei. Injections of HRP on the lateral border of VPI resulted in labeled cell bodies in both the caudal poles of the DCN and the spinal lateral cervical nucleus.

We conclude that discrete regions in the medulla relay afferent information from Pacinian corpuscles to the VPI nucleus and, assuming reciprocity of thalamocortical and corticothalamic connections, the VPI nucleus relays Pacinian information to the SII cortex. This study provides another example of a submodality-specific pathway for cutaneous afferent signals to be delivered to the cerebral cortex.

(Supported by the Medical Research Council of Canada.)

130.14 FACIAL AND DENTAL PULP-EVOKED POTENTIALS IN THE THALAMUS OF CYNOMOLOGUS MONKEYS PERFORMING A SHOCK-AVOIDANCE THRESHOLD TASK. Ronald F. Young, M.D., Kent M. Perryman, Ph.D.* Division of Neurosurgery, UCLA School of Medicine, Harbor/UCLA Medical Center, Torrance, California 90509

Macaca fascicularis monkeys were behaviorally trained to depress a lever which terminated a brief period of electrical stimulation of facial skin or the dental pulp. The animal's pain (escape) threshold was then determined using a random presentation of ten stimulus current intensities which ranged from innocuous to noxious levels. Slow-wave evoked potential (SEP's) were simultaneously recorded from bipolar concentric macroelectrodes chronically implanted in the contralateral ventralis postero-medialis (VPM) and centrum medianum (CM) thalamic nuclei during the behavioral testing sessions. Computer-averaged evoked potentials were obtained at each of the ten different stimulus intensities. Recording sites were histologically confirmed.

SEP's recorded from CM evoked by dental pulp or cutaneous facial stimulation were characterized by two prominent early components with 20 and 50 msec latencies. Both components increased in amplitude with increasing stimulus intensity. Dental stimulation evoked potentials in CM which were of significantly greater amplitude than those evoked by cutaneous facial stimulation. Additionally a long latency, long duration (max. 125 msec) delayed potential was sometimes recorded in CM at stimulus intensities above escape thresholds.

SEP's recorded from VPM showed early components evoked by both dental and cutaneous stimulation which were similar in latency and configuration to these seen in CM. A delayed potential was also sometimes seen in VPM but its duration was shorter than the similar potential recorded in CM.

These recordings are unique because of the lack of anesthetic agents and the correlation with behavioral parameters. They will serve as a basis for future studies of single unit activity and the effect of ablative lesions in a similar primate model. Supported by National Institute of Dental Research Grant DE05208.

130.15 NEURONAL MECHANISMS OF INFORMATION PROCESSING AND THEIR PHARMACOLOGICAL MODULATION IN THE SOMATOSENSORY THALAMUS (VPm). K.-M. Gottschaldt, C. Vahle-Hinz* and T.P. Hicks*. Max Planck Institut for Biophys. Chem., Dept. Neurobiol., 34 Göttingen, FRG

We have developed a method which yields DC-recordings with steel microelectrodes in an "Imminent Membrane Contact" (IMC) electrode position from functionally identified neurons in the trigeminal component of the ventrobasal thalamus in the cat. With this technique, changes in intracellular potential can be recorded from an extracellular electrode position and the activity of single neurons in response to quantitative stimulation of sinus hairs or skin can be studied for long periods of time.

In most neurons afferent impulses elicit small all-or-nothing prepotentials, reflecting presumably dendritic spike components, which trigger full-blown soma spikes. While the transmission of afferent impulses into prepotentials is very stable, the conversion rate of the prepotentials into soma spikes varies with the peripheral stimulus condition. The receptive field of prepotentials may differ from that of soma spikes, as may the characteristics of the prepotential response from those of the soma spike response. The analysis of over 200 IMC-recordings suggests that the control of input-output relations in single neurons operates mainly through a change in the rate of conversion of prepotentials into soma spikes and that this mechanism differs from the classic presynaptic and postsynaptic control mechanisms in neuronal information processing.

To test this hypothesis we have combined steel microelectrodes with multibarrel glasspipettes for iontophoretic administration of various inhibitory and excitatory agonists and their antagonists. The results, again obtained with IMC-recordings, support our original hypothesis that the elicitation of soma spikes is independent of the amplitude of the prepotentials. With the administration of GABA, the amplitude of prepotentials may increase while soma spikes are blocked. However, when additional glutamate is given, the amplitude of the prepotentials remains enlarged but the discharging of soma spikes is facilitated. Bicuculline decreases the prepotential amplitude and antagonizes the GABA-elicited blockade of soma spikes.

The results, although preliminary, suggest that GABA and glutamate act upon different mechanisms which can independently modulate the elicitation of soma spikes and thus the input-output relationship of thalamic neurons. The combination of IMC-recording with microiontophoresis is a promising technique for studying the bases of the neuronal control mechanisms in operation during information processing under natural stimulus conditions.

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130.16 SOME CONNECTIONS OF SI AND SII IN THE RAT. C.S. Lin, and J.K. Chapin. Dept. Anatomy, Duke Univ. Durham, N.C. 27710 and Dept. Cell Biology, The Univ. of Texas Health Science Center, Dallas, TX. 75235.

The aim of this study was to identify sources of cortical and subcortical projections to somatosensory areas I and II in the rat. Body parts in SI and SII were mapped electrophysiologically prior to iontophoretic HRP injections (2-3 uAmp for 15-30 minutes) from glass microelectrodes (tip size 20-30 um) containing a mixture of L-lysophosphatidyl choline (1-3%) in a 20-30% HRP (Sigma VI) solution. Brain sections were pretreated with $CoCl_2$ and then reacted with DAB. This technique allowed visualization of the detailed dentritic morphology of the cells projecting to the injection sites.

Following injections of the vibrissa area in either SI or SII, the zones of labelled cells in the ventrobasal nucleus (VB) of the thalamus were organized into rostro-caudal columns. However, the labelled neurons comprised less than 60% of the total population of cells in these columns. The soma size of the labelled neurons varied from medium to large. Following injections in either SI or SII, labelled neurons were also found in the intralaminar nuclei and posterior nuclear complex. A zone (UZ) in which cells are unresponsive to light cutaneous stimuli received afferents from neurons located dorso-medially to VB. Cytoarchitectonically, this labelled region appears to be continuous with the posterior nuclear complex.

The ipsilateral cortical neurons labelled after injections in SII were pyramidal cells located primarily in layers II/III and V in SI. Most of the completely back-filled cells were located near the lower border of layer III. The neurons in SII labelled after injections in SI are also found in layers II/III and V (Neuroscience Abst.,6:62 '80). These results suggest the presence of precise reciprocal connections between cells in layers II/III and V of SI and SII. Finally, the neurons labelled in the contralateral SII after injections in SII were also located in layers II/III and V.

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130.17 THALAMIC AFFERENTS TO THE ANTERIOR ECTOSYLVIAN SULCUS IN THE CAT. J.M.Roda* and F.Reinoso-Suárez (SPON: I. de Andrés). Departamento de Morfología. Facultad de Medicina. Universidad Autónoma. Arzobispo Morcillo 2. Madrid 34. Spain.

The cortex of the anterior ectosylvian sulcus (AES), that has been included in the proximal association cortex (Graybiel, 1972), is the main and constant source of cortical afferents to the superior colliculus (Tortelly, Reinoso-Suárez and Llamas, 1980). The AES has been described to receive thalamic projections from the posterior nuclear group (Po) and the lateral posterior-pulvinar complex (LP-Pu; Heath and Jones, 1971, Graybiel, 1972, 1973), but a systematic description of its afferents has not been reported heretofore. The aim of this report is to investigate the thalamic afferents to both banks of the AES. In adult cats multiple or single small injections of a 50% aqueous solution of HRP were placed in AES. After a survival period of two days, the animals were perfused and processed in accordance to Mesulam technique (1978). All the experimental animals showed a large amount of labeled neurons in the nucleus ventralis medialis thalami (VM). In the case with multiple injections of HRP, which affected the entires AES cortex, in addition to VM retrograde labeling, labeled cells were observed in: lateral medial-supragenicuiate complex, magnocellular division of the medial geniculate, intermediate and lateral division of Po and ventral and caudal part of the LP-Pu. Some HRP positive cells were also found in: VL,VA, VPl, MD, intralaminar nuclei, medial division of Po and VPi. The results in cases with small single injections allow to point out several topographical differences in neuronal distribution with respect to which bank of AES was injected. The main differences are: 1) The VM labeled cells after AES dorsal bank injections are located more medially and extend more caudally than those cells labeled after ventral bank injections; 2) The most caudal labeled neurons are situated dorsolaterally after AES ventral bank injections and ventromedially after dorsal bank injections.

130.18 MANUAL DISCOORDINATION AFTER DCN LESION. D.E. Teodoru, T.A. Tran*, A.J. Berman. Bronx V.A. Hospital, Bronx, N.Y. 10468

Monkeys tested with vision after dorsal column nuclei (DCN) lesion exhibit poor coordination of hand and fingers (Teodoru, Tran, & Berman, 1978). A similar deficit ("optic ataxia" and "finger agnosia") is found after parietal lobe lesion (Damasio & Benton, 1979). This symptomatology has been attributed to poor visuo-somatosensory integration, consistent with the view that DCN & posterior parietal cortex are part of the same system controlling guidance of hand movements (Stein, 1978; Iwamura, 1979). This explanation, however, has been challenged by Hartje & Ettlinger (1973) who reported similar incoordination in monkeys after parietal lesion when tested both in light and dark. On the basis of this study, Ettlinger (1978) argued that touch was dominant over vision in the guidance of distal forelimb movements.

Berman, Teodoru, and Tran (1980), reported that monkeys tested with vision after DCN lesion recovered distal coordination only when dorsal rhizotomy (DR) was superimposed, indicating that the original deficit resulted from distortion of somatosensory guidance of the hand due to DCN lesion. To test the hypothesis that vision did not play a role in the recovery process, four monkeys were trained to seize small food pellets from a narrow platform and from a dexterity board while blindfolded. After bilateral DCN lesion, they were retested. Distal discoordination was present, identical to that seen in DCN-lesioned monkeys tested with vision. Extensive practice did not result in any improvement. Testing on the dexterity board revealed the same deficits as after S-I lesion (Cole & Glees, 1954), supporting the conclusion that it is tactile and proprioceptive information that are poorly integrated after lesion at any level of the lemniscal system (Norrsell, 1980). Since the distal discoordination resulting from DCN, parietal cortex or S-I lesion is unaffected by the presence or absence of vision, it is suggested that tactile information is dominant over visual information in guiding distal forelimb movements. Furthermore, even though vision is known to exert an inhibitory effect on DCN (Atweh & Jabbar, 1974), this is not sufficient to overcome the dominance of somatosensory guidance even when this guidance is defective. This is supportive of the view that the lemniscal system may integrate the submodalities of somesthesis but does not integrate somatosensory with visual information.

131.1 DIFFERENCES IN SPECIALIZATION OF FAST AND SLOW-TWITCH CHICK MUSCLES AND WORK-DEPENDENT CYTOSOLIC CREATINE KINASE ONTOGENY. O. Ramírez and Margarita Hernández*
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Development of isometric twitch contractions is not the same in all skeletal muscles of higher vertebrates. In the chick, much work has been performed with the slow anterior latissimus dorsi (ALD) and the fast posterior latissimus dorsi (PLD) in regard to electrical and mechanical properties during development, in vivo and in vitro. It is suggested that specific differentiation of the two muscles is determined by their innervation (Purves, R.D. and Vroba, G., J. Cell Physiol. 84; 97, 1974). It seems to be that a good correlation exists between the speed of contraction of skeletal muscles and their content in phosphotransferases in the adult rat (Shainberg et al., Develop. Biol. 25:1 1917). However, 17 day-old Rhode Island chick embryos showed that 120 msec for PLD and 280 msec for ALD were the times for isometric twitch contraction, whereas creatine kinase (CK) specific activities for the same state averaged 91.5 units* for PLD and 148.6 for ALD. Previously at day 14, PLD averaged 32.1 units and ALD 36.7. On the other hand, at hatching, PLD had 1072.9 units and only 329 were in ALD. In ovo, ALD and PLD do not seem to work very much during development, for anatomical reasons. Since it was thought that CK activity could be work-dependent, time-courses of CK in the proper embryonic muscles showed that indeed this was the case. Breast (white-fast) non-working muscle and leg (mixed) working muscles began to differ from day 12 of incubation and at hatching 1220 CPK units were in favour of the working leg muscles. These findings in vivo are in agreement with those in cultures of skeletal muscle cells of the chick embryo, subjected to repetitive electric stimulation. The contraction increased mostly the synthesis of proteins associated with the contractile apparatus, as the myosin-heavy chain.

On the other hand, this work also showed that membrane and cytosolic differentiation in skeletal muscle occur separately during development.

*nmoles of substrate transformed/min/mg protein.

131.2 DEVELOPMENTAL INCREASE IN ACETYLCHOLINE RELEASE FROM CHICK EMBRYO NEURAL RETINA. Jeffrey M. Thompson and Clint Makino*. Laboratory of Neurosciences, Gerontology Research Center, National Institute on Aging, Baltimore, MD 21224.

When the neural retina layer is removed from the embryonic chick, it will take up [^{3}H]-choline, synthesize [^{3}H]-acetylcholine ([^{3}H]-Ach) and release [^{3}H]-Ach in response to KCl or glutamate. These properties can be used to determine developmental changes in the functional release of acetylcholine from retina neurons. After incubation in [^{3}H]-choline, the retina layers are washed and placed in a multi-channel perfusion system. The perfusate is collected in timed samples and the amount of [^{3}H]-choline and [^{3}H]-Ach in each sample measured before and during addition of 80 mM KCl or 5 mM glutamate to the perfusing medium. Six-day retina do not release [^{3}H]-Ach in response to either KCl or glutamate, 8-day retina show a moderate response to the drugs and older retina show further increases. Sixteen-day retina, the oldest age tested, released the largest amounts of [^{3}H]-Ach. The developmental increase in Ach release parallels the developmental increase in choline acetyltransferase activity (Puro et al., Proc. Natl. Acad. Sci. USA, 74, 4977-4981, 1977; Bader et al., Proc. Natl. Acad. Sci. USA, 75, 2525-2529, 1978). However, when the release data is normalized to eliminate differences in uptake and synthesis, a developmental increase in release is still observed. Thus, the total release reflects a summation of synthesis and release processes.

The developmental changes reported for synapse formation between chick embryo retina neurons and muscle cells in culture (Ruffolo et al., Proc. Natl. Acad. Sci. USA, 75, 2281-2285, 1978) are not correlated with the observed changes in ACh release. Eight-day retina neurons have the greatest capacity to form cholinergic synapses on muscle, while 16-day retina neurons do not form any synapses. Acetylcholine can be released from 16-day retina in large quantities as shown in this study, yet synapses are not formed. Thus, the mechanisms for synapse formation and neurotransmitter synthesis and release are not coupled in the developing chick retina.

131.3 ACETYLATION OF POLYAMINES IN THE MOUSE BRAIN. Ortiz, J.G., Giacobini, E. and Schmidt-Glenewinkel, T. (Sponsor, M. Wilson)
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Putrescine, spermidine and spermine have been shown to be acetylated in several mammalian tissues (Seiler, N. and Al Therib, M.J., Biochem. Biophys. Acta. 354:206, 1974, Blanckenship, J. and Walle, T., Arch. Biochem. Biophys. 177:237, 1977). The acetylation of these compounds and of cadaverine in the mouse brain has been examined in our laboratory.

Polyamine Acetyltransferase (PAT) activity could only be detected in brain nuclear and microsomal fractions. Substrate kinetics with the nuclear preparations, indicate that these compounds are acetylated at physiological concentrations. The Km's for putrescine, cadaverine, spermidine and spermine are 3.0 mM, 5.2 mM, 1.2 mM and 3.5 mM, respectively. The microsomal fraction has comparable levels of activity and appears to have similar substrate preference.

Regional studies with spermidine indicate that the olfactory bulb and cerebellum have the highest activity (per mg protein), followed by cortex, midbrain and brainstem. Preliminary results with putrescine and cadaverine suggests that their acetylation follows a similar regional distribution. Interestingly, ornithine decarboxylase activity has a very similar regional pattern as the acetylation of polyamines (Rochel, S. and Margolis, F.L., J. Neurochem. 35:850, 1980). In some regions, the cell number (DNA levels) may account for the regional distribution observed.

The possible relationship between Polyamine Acetyltransferase and Ornithine Decarboxylase activities in brain is discussed.

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131.4 INTRACELLULAR ACCUMULATION OF ACHE INDUCED BY EARLY OLFACTORY BULB LESIONS: INTRALITTER SIMILARITIES. Celeste R. Wirsig, Joan Morasco* & Christiana M. Leonard. Dept Neuroscience, College of Medicine, University of Florida, Gainesville FLA 32610.

Removal of the olfactory bulb (OBx) in the 5 day old hamster causes cellular changes in the ventral forebrain (VF) which are accompanied by abnormalities in neonatal thermoregulatory and social behavior. Work in our laboratory suggests that the behavioral pathology may be due to lesion-induced abnormalities in cholinergic function (Leonard, Williamson & Freund, this volume). Twenty-four hrs after the lesion, a number of cells in the ipsilateral caudal anterior olfactory nucleus (AON) and olfactory tubercle (OT), collectively the VF, show an increased amount of AChE intracellularly. These reactive cells may project centrifugally to the OB and/or anterior AON and thus had their axons severed by the lesion. Muscarinic cholinergic receptor binding density assessed with QNB in the ipsilateral VF is also elevated by the lesion. The number of reactive cells varies greatly between pups from different litters (as does QNB binding), but correlates with the amount of QNB binding in the pooled VFs of littermates. This suggested that the variability we have found in both histochemical and biochemical measures might reflect an underlying genetic variability in cholinergic function. The aim of the present study was to determine whether the number and distribution of reactive cells is comparable in littermates.

The brains of 6 pairs of pups (3 pairs ROBx & 3 pairs LOBx) from 6 litters of the out-bred Charles River strain were examined. Animals were bulbectomized on day 5 and perfused 24 hrs later. The brains were sectioned (80 μ) and stained for AChE using the Tsuji method. Each section was drawn and the reactive cells were counted caudal to the lesion. All animals demonstrated reactive cells throughout the caudal AON and in most cases in the OT. Reactive cell density generally peaked at the beginning of the OT. Although the number and distribution of cells in the OTs among litters differed greatly (cell # range = 1-112), the number and distribution of these cells were highly correlated in littermates ($r=.83$, $p<.02$; $r=.91$, $p<.01$, respectively). To date no left-right differences in cell distribution have been seen.

The differential location of these cells along the rostrocaudal axis of the VF may reflect differences in developmental status among litters or may be due to genetic variation in the cellular distribution. We are presently examining the possibility of the former by comparing the effects of bulbectomies performed at various postnatal ages.

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131.5 MESOLIMBIC LESIONS DELAY DEVELOPMENT OF SOCIAL HUDDLING IN GOLDEN HAMSTER PUPS. Dorothy K. Burt*, Cynthia L. Rodriguez*, Neal R. Kramarcy and Christiana M. Leonard. Department of Neuroscience, Univ. Fla. Coll.Med., Gainesville, FL 32610.

During the first week of postnatal life, golden hamster pups use thermal rooting (R), an active behavior characterized by dives and displacements in a tightly knit group, as a form of behavioral thermoregulation. Quiet huddling (S) replaces R during the second week. The pups crouch together in a loose association and activity is limited to head movements and grooming.

Previous work in our laboratory has implicated the olfactory system, particularly the olfactory tubercle (OT), in behavioral changes occurring between the first and second week. Since the olfactory tubercle is a major target of mesolimbic dopaminergic (DA) projections arising in the ventral tegmental area (VTA) we decided to investigate the effect of 6-OHDA placed in this area at 5 days on huddling behavior measured at 10 days of age.

Eighteen litters of 7 or 8 pups were assigned to either 6-OHDA (D), sham (S) or untreated control (U) groups. Pups in the D and S groups were pretreated with desipramine hydrochloride (DMI: 20 mg/kg, ip, 60 min before surgery). Either 6-OHDA (2 µg/µl) or 0.2% ascorbic acid vehicle (1 µl; 0.5 µl/min) was infused into VTA on the left side through a 33 g cannula (placement determined stereotaxically). The behavior of three-pup groups (run blind) was videotaped in 8 min tests on a mild thermal gradient (20 to 30°C). Thermoregulatory and social contact behaviors were categorized every 15 sec and rectal temperature was monitored throughout by means of a thermistor inserted in one of the pups. On day 18 pups were sacrificed and DA levels in OT assayed with HPLC.

The pups that had received 6-OHDA were much more active than shams or controls. They displayed thermal rooting 60% of the time compared to 18% for the controls and 34% for the shams (D vs S, $p < .05$). Conversely, controls displayed quiet huddling 52% of the tme, shams 42% and D 20% (D vs S, $p < .05$). The groups did not differ in weight, body temperature, time spent in the warmth or amount of total social contact.

We conclude that the maturation of VTA projections into OT may play a role in the onset of quiet huddling. The effect of the lesion is not permanent, however, as behavior and dopamine levels were similar in all groups at sacrifice. Whether dopamine is significantly depleted at the age when behavior is abnormal remains to be determined.

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131.6 ASYMMETRICAL EFFECTS OF EARLY OLFACTORY BULB LESIONS: IS THE HAMSTER BRAIN LATERALIZED? C.M. Leonard, M. Williamson*, G. Freund. Dept. of Neuroscience and Dept. of Medicine, Univ. Florida Coll. Med. Gainesville, FL 32610.

Language, species specific calls, and songs are social behaviors controlled and decoded by the left hemisphere. Although the right hemisphere in humans is thought to specialize in nonverbal communication, interpreting and organizing emotional responses, the possibility that the right hemisphere in animals might also specialize in directing nonverbal social interactions has not been proposed. Since even solitary species like the golden hamster Mesocricetus auratus have a number of stereotyped social behaviors with a presumed communicative function, we decided to investigate whether control of their behavior was lateralized.

We used neonatal hamsters because the type and duration of their social interactions can be precisely controlled by varying thermal conditions in the environment. When placed on a thermal gradient pups maintain their body temperature by varying the percent time they spend in asocial thermotaxis (strung out singly on the warm side), social huddling (a loose aggregation somewhat resembling a campfire circle) and exploration. Since huddling is under olfactory control we compared the effect of left and right olfactory bulbectomy (OBx) on day 5 after birth on social huddling measured on day 12. Six litters of LOBx and 6 litters of ROBx were compared to a control group of 3 sham operated and 3 intact litters.

The behavioral strategy used to maintain temperature was strongly affected by the side of the lesion. After LOBx, pups increased their time huddling in the warmth while ROBx pups spent more time in asocial thermotaxis. Mean huddling/ thermotaxis ratios were 1.6 for LOBx, 1.3 for controls and 0.9 for ROBx.

Since the ventral forebrain (VF) projections of OB are characterized by large amounts of acetylcholine, we determined the density of cholinergic muscarinic receptor binding with labelled QNB in VF. We found an L/R asymmetry in binding density which was increased after LOBx but reversed after ROBx (L/R ratios for controls = 1.12; LOBx = 1.23, ROBx - .91). The ratios for individual litters correlated positively with their H/T scores ($r = .47$, $p < .05$) and time huddling was negatively correlated with binding density in the right but not the left VF ($r = -.64$, $p < .01$).

These results suggest that the two hemispheres are asymmetrically involved in the control of social behavior in the golden hamster pup. Whether the asymmetry is a characteristic of sensory, motor, or motivational processes remains to be determined.

Supported by NINCDS (NS 13516 to CML) and the VA (GF).

131.7 DEVELOPMENT OF NEUROTRANSMITTER SYNTHESIS AND ACCUMULATION IN PRIMARY AND SECONDARY NEURAL CREST CULTURES. G. D. Maxwell, P. D. Sietz* and C. E. Rafford*, Dept. of Anatomy, Univ. of Connecticut Health Center, Farmington, CT 06032.

Primary cultures of quail trunk neural crest cells, without exogenously added non-neural crest cells, develop histochemically demonstrable catecholamine (CA)-containing cells as well as dopamine-ß-hydroxylase and choline acetyltransferase enzyme activities (Cohen 1977 PNAS 74: 2899; Kahn et al., 1980 Dev. Biol. 77: 340).

In order to further characterize the ontogeny and control of neuronal phenotypic expression of neural crest cells in vitro, we have examined the capacity of such cultures to synthesize and store putative neurotransmitters from [^{3}H]tyrosine, [^{3}H]choline and [^{3}H]tryptophan using the screening procedure of Hildebrand et al., (1971 J. Neurobiol. 2: 231).

In primary cultures, the synthesis and storage of [^{3}H]CA per mg protein from [^{3}H]tyrosine is negligible until 3 days in vitro, and rises 4-fold between 3 and 7 days. A similar developmental increase is seen when [^{3}H]CA is normalized to cell associated [^{3}H]tyrosine present at the end of the incubation. This suggests that the increase in [^{3}H]CA synthesis and storage is not due to increased tyrosine uptake. The time course of the increase in [^{3}H]CA synthesis and storage agrees well with published data on dopamine-ß-hydroxylase activity and histochemically detectable CA-containing cells. The CA synthesized in these cultures include [^{3}H]dopamine, [^{3}H]norepinephrine, and sometimes [^{3}H]epinephrine. These compounds were identified using ascending chromatography following electrophoresis and independently by HPLC analysis (performed by Dr. Thomas O. Fox).

Secondary cultures, prepared from 2-day primary cultures, show no [^{3}H]CA accumulation on the first day after subculture. [^{3}H]CA synthesis and storage increases between 3 and 8 days after subculture with the amount of [^{3}H]CA per mg protein reaching levels comparable to those seen in primary cultures.

Acetylcholine (ACh) synthesis and storage from [^{3}H]choline is also observed to develop in primary cultures. Little [^{3}H]ACh is produced for the first 3 days in vitro. [^{3}H]ACh accumulation per mg protein rises 7-fold between 3 and 7 days in vitro.

Primary cultures exhibit little or no ability to synthesize and accumulate [^{3}H]5HT from [^{3}H]tryptophan over the period of 2 to 10 days in vitro.

(Supported by NIH grant NS 16115 and Basil O'Connor Starter Grant 5-289 from the March of Dimes Birth Defects Foundation).

131.8 EARLY DEVELOPMENT OF CHOLINERGIC FUNCTION IN RAT RETINA. B-A. Battelle, D.G. Puro and K.H. Hansmann.* Laboratory of Vision Research, National Eye Institute, NIH, Bethesda, MD. (Spon: H. H. Hess).

To examine aspects of cholinergic development in rat retina, acetylcholine (ACh) synthesis, choline acetyltransferase (CAT) activity and ACh release were assayed. ACh synthesis from ^{3}H-choline (15µM) was assayed in intact, isolated retinas incubated in vitro. Labeled ACh formed was quantified following high voltage electrophoresis of extracts from retinal homogenates. In these assays, carnitine acetyltransferase was not a source of confusion since NVP, a specific inhibitor of CAT, blocked greater than 90% of the choline acetylation without affecting carnitine acetylation. Release of ACh was assayed in a cell culture system utilizing rat striated muscle cells as postsynaptic targets for cholinergic neurons (Puro et al. PNAS 74, 4977: 1977). Synaptic input to myotubes was measured by intracellular recordings one day after addition of trypsin-dissociated retinal cells.

By embryonic day 17 (E-17) ACh synthesis and CAT activity are detected. Spontaneous release of ACh can be demonstrated using E-18 retinal neurons. As CAT activity increases 9 fold between E-17 and E-19, the rate of ACh synthesis rises from 0.29 to 6.88 pmoles/mg protein/hr. These events correlate with formation of the inner plexiform layer and occur more than one week before morphological synapses have been reported. ACh release could be evoked from retinal neurons by glutamate only after E-19. Microiontophoresis of glutamate onto E-19 retinal neurons did not increase the frequency of depolarizations measured in any of the innervated myotubes tested (0/19). When the same assay was done with cells from postnatal day 0 (P-0) retinas, 73% (8/11) of the innervated myotubes showed evoked synaptic input. The basis for this maturation of glutamate-evoked response is uncertain; however, preliminary evidence indicates that glutamate receptors are present on some E-19 cholinergic neurons.

The rate of ACh synthesis increases sharply after P-6. Two factors may contribute to this: 1. a marked increase in CAT activity (from 0.35 to 1.74 nmoles/mg protein/min between P-6 and P-12) and 2. an increase in high affinity choline uptake. When retinal incubations were done in the presence of a low concentration of ^{3}H-choline (0.65µM), and (50µM) HC-3, a specific blocker of high affinity choline uptake, ACh synthesis was reduced approximately 40% in P-9 retinas but by only 25% in P-4 retinas. The development of cholinergic function during the second postnatal week corresponds to the period when morphological synapses appear in the retina.

131.9 INTRAVENTRICULAR NEONATAL 6-OHDA: EFFECTS ON THE ONTOGENY OF SPONTANEOUS MOTOR ACTIVITY AS A FUNCTION OF ENVIRONMENTAL CUES. Sonya K. Sobrian. Dept. of Pharmacology, Howard University College of Medicine, Washington, DC 20059.

Developing rats exhibit a characterist ontogenetic pattern of spontaneous locomotor activity (SMA) when tested in isolation. Activity levels are low at birth, increase to maximal levels at day 15 and then decline to reach adult levels between 25 and 30 days of age. Systemic injections of 6-hydroxydopamine (6-OHDA) on postnatal day 0-3, while producing a selective depletion in NE in brain regions innervated by the ascending dorsal adrenergic system, do not alter the normal ontogenetic pattern of SMA. In contrast to these findings are the alterations in SMA following neonatal intraventricular 6-OHDA.

Sprague-Dawley rat pups were injected in the lateral ventricles with 50 ug of 6-OHDA on postnatal days 1 and 2, and SMA was measured every 5 days from days 10-30. Pups were tested either in isolation (without shavings) or in the presence of shavings from their home cages. Unlike the differential pattern of regional changes seen after neonatal systemic 6-OHDA, intraventricular injections of both NE and DA throughout the brain of the 30 days of rats. Cortical, cerebellar, hippocampal and hypothalamic NE was reduced to 5-60% of control levels; striatal DA was reduced to 5% of control values. Behavioral changes were also more striking. In the isolated condition, intraventricular 6-OHDA altered the levels but not the developmental pattern of SMA. Treated pups inhibited at a significant but transient hyperactivity between 15 and 25 days of age.

The presence of home cage shavings in the testing apparatus markedy altered the development of SMA in control pups. Activity levels remained low and relatively unchanged between 10 and 30 days of age. In contrast, this test situation exaggerated the hyperactivity seen in 6-OHDA treated pups between days 15 and 25. Activity levels were not only significantly increased over control values, but were also higher than the level of drug treated pups tested in isolation. Preliminary data suggests that this enhanced hyperactivity in a familiar environment may reflect an olfactory deficit in pups treated intraventricularly with 6-OHDA. Treated pups exhibited no preference for the odor of home cage shavings at an age (Day 8) when controls spend 90% of their time over this odor. These data indicate that environmental factors can influence the effects of neurochemical changes in modulating developing behavior. (Supported by NSF Grant #BNS-80-26765)

131.10 NALOXONE DURING INFANCY INCREASES OPIATE BINDING WITHOUT ALTERING MONOAMINE SYSTEMS. M.T. Bardo, R.K. Bhatnagar and G.F. Gebhart. Department of Pharmacology, University of Iowa, Iowa City, IA 52242.

We recently reported that chronic administration of naloxone from birth to 21 days of age increases opiate binding in rats. Since strong evidence indicates that at least some opiate receptors are located on monoamine-containing neurons, we hypothesized that naloxone-induced alterations in opiate binding might produce a concomitant alteration in monoamine systems. In the present experiments, we examined the effects of chronic naloxone treatment on levels of norepinephrine, dopamine and serotonin, and on opiate receptor binding during different periods of postnatal development. Sprague-Dawley rat pups were injected subcutaneously with either naloxone hydrochloride (1 mg/kg) or 0.9% NaCl vehicle twice daily at various intervals after birth. In one experiment, animals were injected for one week beginning at either 1, 8, or 15 days of age. These animals were decapitated on the day following the last injection (i.e., day 8, 15 or 22), and the brains and spinal cords were removed; brains were dissected into medulla-pons, midbrain, hypothalamus, striatum and cortex. Opiate receptor assays were performed on homogenized tissue incubated with 1 nM ^{3}H-naloxone in the presence or absence of 100 nM levallorphan. In another experiment, animals were injected with either naloxone or saline from 1 to 21 days of age. On the day following the last injection, each animal was subsequently injected intraperitoneally with either α-methyltyrosine (250 mg/kg), an inhibitor of tyrosine hydroxylase, or saline vehicle. Three hours later, animals were decapitated and the brains regionally dissected as above. Norepinephrine, dopamine and serotonin assays were performed using standard radioenzymatic procedures with ^{3}H-S-adenosyl-methionine. α-methyltyrosine-induced decreases in catecholamine content was used as an index of amine turnover.

Naloxone treatment during the first postnatal week increased ligand binding in spinal cord, hypothalamus and striatum. An increase in ligand binding was also evident following naloxone treatment during the second and third postnatal weeks, although not in striatum. Steady-state levels of catecholamines and serotonin, and turnover of catecholamines, were unaffected by the naloxone treatment. Thus, while opiate receptors may be located on monoamine neurons, these results indicate that changes in opiate receptors do not necessarily produce changes in monoamine systems.

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131.11 THE ONTOGENY OF ENKEPHALIN-LIKE IMMUNOREACTIVITY IN THE DEVELOPING AVIAN CILIARY GANGLION AND THE NUCLEUS OF EDINGER-WESTPHAL. Brian M. Davis, Jonathan T. Erichsen and Harvey J. Karten. Dept. of Neurobiology & Behavior, SUNY, Stony Brook, New York 11794.

Leucine-enkephalin-like immunoreactivity (ELI) has been demonstrated in the preganglionic terminal endings upon both ciliary and choroid neurons in the avian ciliary ganglion (Karten et al., Neurosci., submitted). Lesion studies have shown that enkephalinergic neurons in the nucleus of Edinger-Westphal (EW) are the source of these immunoreactive terminals (Erichsen et al., 1981). Since neurons of EW are also known to be the source of cholinergic input to the ciliary ganglion (CG), enkephalin may coexist with acetylcholine and play an ancillary role in synaptic transmission. The present study describes the ontogeny of ELI in both the CG and EW of the chick.

ELI first appears in both EW and the CG at St 25 shortly after cell proliferation ends in the CG (Landmesser & Pilar, 1978). The first functioning synapses in the CG are reported to occur at St 26 (Landmesser & Pilar, 1972).

From St 25 through 36, ELI staining in EW is punctate and diffuse in appearance, revealing no obvious enkephalin-positive cells. Enkephalin-labeled neurons appear in EW at St 37 and continue to show a similar pattern of ELI through St 45, although the number of labeled neurons diminishes after St 40.

In the CG, ELI staining is weblike in appearance between St 25 and St 33 and shows no obvious morphological specializations around individual neurons. By St 35, shortly before enkephalin-positive cells first become evident in EW, delicate enkephalin immunoreactive fibers completely surround most cells in the CG. The first recognizable immunoreactive calyceal-like terminal endings appear at St 37, near the time calyces are known to start forming but before transmission by these synapses is reported (St 39) (Landmesser & Pilar, 1972). By St 41, terminal endings on both choroid (boutons) and ciliary (calyces) cells show ELI in the CG. The pattern of ELI remains essentially the same through St 45.

The development in the pattern of ELI of the preganglionic terminal endings within the CG correlates well with the described sequence of events during synaptogenesis. Significantly, enkephalin appears immediately before the first functioning synapses, and subsequently, recognizable calyces show prominent ELI before they are reported to become synaptically active. These results suggest that enkephalin plays an essential role in synaptogenesis and/or neurotransmission in the CG.

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131.12 LOCOMOTOR HYPERACTIVITY IN NEONATAL RATS FOLLOWING LESIONS OF MESOCORTICAL DOPAMINERGIC NEURONS. Alfred Heller, Thomas Heffner, Connie Kotake*, Frederick Miller* and Lewis Seiden. Dept. of Pharmacol. & Physiol. Sci., Univ. of Chicago, Chicago, IL 60637.

In neonatal rats, destruction of brain dopamine (DA) neurons by ivt injections of 6-hydroxydopamine (6-HDA) results in locomotor hyperactivity (Shaywitz et al. Sci.191:305,1976; Erinoff et al. Br. Res. 164:195,1979). As an approach to determining which DA projections are responsible for this effect, we examined locomotor development in rats after ablation of either mesocortical or nigrostriatal DA neurons at 4 days of age. A single electrolytic lesion in the medial ventral tegmental area (VTA) or bilateral lesions of the substantia nigra (SN) were made by use of stereotaxic procedures (Heller et al. J. Neurosci. Meth. 1:41, 1979). Locomotion in control rats (measured for 1 hr daily in stabilimeter cages) averaged 38 counts per hr from days 22-24 of life. Rats with lesions placed in the medial VTA displayed a 3-fold increase in locomotion relative to controls during this time whereas rats with lesions placed in the SN displayed no change in locomotion. The VTA lesions depleted DA in frontal cortex (FC) by 45%, septum (S) by 57% and nucleus accumbens (NA) by 29%, but did not alter DA content of the caudate-putamen (CP). The SN lesions reduced DA in the CP by 60% but had less effect on DA within FC (-33%), S(-18%), and NA (-20%). The SN lesions produced substantial bilateral loss of cells within the pars compacta. In rats that displayed hyperactivity following a VTA lesion, the ablated area involved the midline tegmentum immediately dorsal to the interpeduncular nucleus generally extending above the rostral 1/2 to 2/3 of this nucleus. In rats that did not display hyperactivity after a VTA lesion, the lesion spared this midline area. In an attempt to produce less nonspecific destruction of cells other than DA neurons within the medial VTA, 6-HDA (2 µg/0.4 µl) was injected into the midline VTA site in 4 day old rats pretreated with desipramine. These rats displayed a 2-fold increase in locomotion relative to controls during days 22-24 of life. The depletions of DA within the FC (-54%), S(-44%), and NA (-44%) in these rats were similar to those seen in rats that displayed hyperactivity after an electrolytic lesion in the VTA. Although VTA 6-HDA injections produced depletion of DA in the CP (-54%) this depletion did not exceed that seen in rats that failed to display hyperactivity after electrolytic lesions in the SN. These results suggest that destruction of DA cells within the midline VTA results in locomotor hyperactivity in the neonatal rat. Destruction of these cells may account for the hyperactivity seen following ivt 6-HDA injections. Thus, DA cell groups within the medial VTA may be essential for the normal ontogeny of locomotor behavior. (USPHS-12324; MH-10562; MH-14274).

131.13 EFFECTS OF NEONATAL X-IRRADIATION ON SELECTED STROMAL AND PARENCHYMA CELLS IN RAT BRAIN. L.K. Gerbrandt, R.B. Chronister and G.C. Palmer. Neurosci Res. Prog., M.I.T., Boston, MA. 02116 and Univ. So. Ala. Col. Med., Mobile, Alabama 36688.

We have recently shown that neonatal x-irradiation directed to the hippocampus produces not only anatomical alterations (diminution of the granule cell layer), but also subsensitivities of biogenic amine (norepinephrine, dopamine and histamine) receptors coupled to adenylate cyclase. The purpose of the present study is to examine in similar animals, the effects of x-irradiation on cyclic AMP systems and histological changes in other areas of the CNS.

Long-Evans derived hooded rats were given unilateral x-irradiation as follows: 200 rads on neonatal days 2, 3 and 150 rads on alternate days 3-15. Partial schedules were given identically but the procedure was stopped after day 7. Tissue was removed from animals at 3 months of age.

The irradiation produced a marked decrease in the skin pigment of the head and clearly showed the outline of the irradiation. The eye was also smaller, a midline shift of the head was toward the x-irradiated side, and the lacrimal gland was hyperplastic. Histological examination showed striking changes in the retina. The rod and cone nuclei were diminished and occurred in clusters. Several layers of bipolar cells were present. Concomitantly dopamine activation of adenylate cyclase was augmented. The pia overlying the posterior cortex was hyperplastic but the stimulation of adenylate cyclase by norepinephrine (NE) and/or GTP analogs were reduced. Similarly the posterior cortex showed a decrement in NE-adenylate cyclase without remarkable histological change. The capillaries in the posterior cortex were unchanged. The hippocampus displayed again a lessened sensitivity of NE-adenylate cyclase even when the damaged gyrus was removed. The partial irradiation schedule revealed only a slight reduction of NE enzyme sensitivity in the hippocampus.

These findings show that the effects of neonatal x-irradiation are complex, area specific and vary in direction. (Supported by NSF PCM 7911782.)

131.14 THE MONOSODIUM L-GLUTAMATE (MSG) SYNDROME IN MICE DEVELOPS INDEPENDENTLY OF HOUSING CONDITION. June E. Barnhart* and William J. Pizzi. Neuropsychology Lab, Northeastern Ill. Univ., Chicago, Illinois, 60625.

The sequelae of neuroendocrine, somatic, and behavioral events following neonatal MSG treatment has been well documented, including obesity and decreased locomotor activity. Recently, however, Dawson & Lorden (J. Comp. Physiol. Psych., 95: 71,1981; Abstr. Soc. Neurosci., 5: 215, 1979) reported that several features of the MSG syndrome only appear in group-housed animals. While these findings are difficult to reconcile with the existing MSG literature, they could be of theoretical importance if verified. The present studies were designed to systematically explore the effects of housing conditions on the MSG syndrome.

HaICR mice (n=193) were treated with MSG or a control vehicle for 10 days (days 2-11 after birth) according to a dose schedule described by Potts et al. (Amer. J. Ophthalmol., 50: 900, 1960). The dosage ranged from 2.2 mg/g b.w. on day 2 to 4.4 mg/g b.w. on day 11. Animals were assigned to either an individual-housing condition (1 animal/cage)or a group-housing condition (4-6 animals/cage) at weaning (29 days of age). Growth curves were recorded until the mice reached 200 days of age. On day 200 a random sample of group-housed males were switched to the individual-housing condition, and followed for 20 days in order to simulate the conditions of Dawson & Lorden. Male mice in each housing condition were tested for locomotor activity levels between 190 and 200 days of age.

All MSG-treated male and female mice, regardless of housing condition, displayed the extreme obesity typical of the MSG syndrome ($p < 0.001$). Regardless of housing condition, all MSG-treated animals were hypoactive ($p < 0.001$). When the group-housed male mice were switched to individual housing at day 200, they failed to show any disruption in their growth curves.

These findings fail to show any effects of housing condition on the development of the MSG syndrome. In this study animals were assigned to the housing conditions well before any obesity occurred, and no disruption of the growth curve was observed. Animals which became obese in the group-housed condition also failed to show any weight loss when switched to the individually-housed condition. We conclude that housing variables play no role in the production of the MSG syndrome in mice.

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131.15 THE PERINATAL DEVELOPMENT OF NEUROTRANSMITTER RECEPTORS STUDIED BY AUTORADIOGRAPHIC METHODS. M. Lewis, J.M. Palacios, J.R. Unnerstall, D.L. Niehoff, M. Molliver and M.J. Kuhar (SPON: W.S. Young, III). Dept. of Neuroscience, Johns Hopkins Univ. Sch. of Med., Balto., MD 21205.

Using light microscopic autoradiographic techniques for the localization of neurotransmitter receptors after *in vitro* labeling of mounted tissue sections, we have studied the ontogeny of several receptor types, e.g., opiates, dopamine, norepinephrine, serotonin, GABA and benzodiazpines. Goals of this study were to correlate the appearance of receptors with 1) the maturation of particular cell populations, 2) the appearance of responses to agonists and antagonists of these receptors and 3) maturation of the presynaptic input.

The different receptor types exhibit dissimilar patterns of development. Many of the receptors develop mainly during postnatal life, whereas in the fetus, opiate receptors are found in high concentrations in particular regions of the brain. In some cases, receptors were present in young animals in areas where they are absent in the adult. This age-related "elimination" process was observed in several cases: opiates, serotonin and GABA receptors. Association of receptor development with the maturation of some specific cell types was observed. For example, undifferentiated cells in the external granule layer of the cerebellum do not exhibit any receptor binding. But, as these cells mature and migrate to the granule cell layer, a concomitant increase is seen in the density of GABA receptors. However, no clear relationship could be established between the maturation of either cell types or neurotransmitter systems and their receptors. For example, opiate receptors in some areas develop before measurable quantities of enkephalins are found; beta-adrenergic receptors, on the other hand, develop much later than the noradrenergic input. The results indicate that the initial development of specific receptors is, in most cases, not dependent upon the ingrowth of axons that contain the appropriate neurotransmitter. Rather, the pattern of receptor development is an expression of the individuality and the distinctive membrane differentiation of different neuron types. Later in ontogeny a complex process of receptor remodelling leads to the mature receptor profiles of different sets of adult neurons.

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131.16 DEVELOPMENT OF ADRENAL MEDULLARY AND CARDIAC RESPONSES TO SYMPATHETIC STIMULATION IN RATS. R. F. Kirby* and R. McCarty. Dept. of Psychol., Univ. of Virginia, Charlottesville, VA 22901.

The ability of the sympathetic nervous system to increase ornithine decarboxylase (ODC, EC 4.1.1.17) activity in the heart and to stimulate catecholamine secretion from the adrenal medulla requires a functional system of innervation to these tissues (Bareis and Slotkin, J. Pharmacol. Exp. Ther., 205: 164, 1978). In the present study, we were interested in determining the age at which functional sympathetic innervation of the adrenal medulla and heart occurs. Insulin was administered to developing rat pups to stimulate a centrally-mediated increase in sympathetic activity and increases in heart ODC activity and depletion of adrenal catecholamines were used as measures of tissue responses to sympathetic stimulation. Litters of Sprague-Dawley rats were reared in our laboratory. At 12±1 days of age, pups were injected with insulin (1, 10, 20 or 30 IU/kg, s.c.) and sacrificed at 1, 3 or 4 hours post-injection to determine the dose of insulin and time of sacrifice which yielded maximal stimulation of heart ODC and maximal depletion of adrenal catecholamines. Heart ODC activity was found to be more sensitive to increases in sympathetic stimulation than the depletion of epinephrine (EPI) from the adrenal medulla. To assess the development of sympathetic responses in heart and adrenal medulla, rat pups (4, 8, 12, 16 days old) were injected with saline or insulin (30 IU/kg, s.c.) and sacrificed 3 hours later. Administration of insulin resulted in a significant increase in heart ODC activity at 12 and 16 days of age (Table 1).

Table 1. Heart ODC Activity (nmoles/g/hr, $\bar{x}$±SE)

Age (days)	4	8	12	16
NaCl	8.17±1.94	8.69±1.63	6.63±1.18	4.41±.80
Insulin	12.22±3.77	11.31±2.98	19.19±3.06*	10.57±2.34*

In contrast, administration of insulin resulted in a significant depletion of EPI from the adrenal medulla as early as 8 days of age (Table 2). At no age was there a significant depletion of norepinephrine.

Table 2. Adrenal Epinephrine (µg/pair, $\bar{x}$±SE)

Age (days)	4	8	12	16
NaCl	.495±.067	1.009±.144	2.390±.233	2.436±.386
Insulin	.526±.055	.615±.096*	.979±.146*	1.201±.241*

These data suggest that the development of functional sympathetic innervation to the adrenal medulla of rats occurs prior to the development of functional sympathetic innervation to the heart.

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131.17 DEVELOPMENTAL CHANGES IN CONCENTRATIONS OF BIOGENIC AMINES IN THE BRAINSTEM NUCLEI OF THE RABBIT. M. Colleen McNamara and E.E. Lawson.* University of North Carolina, Chapel Hill, NC 27514.

Neurotransmitter levels were studied in specific brainstem nuclei of postnatal rabbits at various developmental ages (1,3, 7,14, and 21 days). Animals were killed by decapitation; brains were rapidly removed and immediately frozen in ice chilled isopentane at -70°C. Six brainstem regions, zona compacta of the substantia nigra (SN-A_9); nuclei parabrachiales (npb); nucleus raphe dorsalis (dr); locus coeruleus (LC-A_6); nucleus ambiguus (n. Amb.); and the nucleus tractus solitarius (nts-A_2), were removed according to the micropunch technique of Palkovitz (Brain Res., 59:449, 1973). Serotonin (5HT) was measured using the radioenzymatic method outlined by Saavedra, et al. (J. Pharm. Exper. Therap., 186:508, 1976). Dopamine (DA) and norepinephrine (NE) were determined by a modification of the method of Versteeg, et al. (Brain Res., 113:563, 1976).

As in the rat, we found low levels of 5HT at birth in all areas sampled. These concentrations gradually increased, and by day 21 the dr and n. Amb. showed the most dramatic changes, 511% and 468% respectively. Concentrations of DA also were very low at birth, especially so in the pons and medulla (less than 5pg/µg). The highest concentrations were observed in the LC 12pg/µg. By 21 days of age, there were moderate increases in DA, the largest being 366% in the SN.

In contrast, high NE levels were present at birth in the SN 31pg/µg, npb 48pg/µg, the LC 32pg/µg and the nts 23pg/µg; by day 21 the NE had dropped an average of 60%. The largest decrease was 94% in the LC.

These data demonstrate dramatic changes in neurotransmitter concentrations in the brainstem nuclei during postnatal development, and suggest that changes in neurotransmitter synthesis accompany the known changes in brainstem neurologic function. This work thus represents an essential first step in determining the roles and interrelationship of neurotransmitters with the function of neuronal circuits.

131.18 VINBLASTINE INJECTED NEONATALLY PRODUCES A PARTIAL BUT PERSISTENT PERIPHERAL SYMPATHECTOMY IN RATS. G. Jaim-Etcheverry and L. M. Zieher*. Instituto de Biología Celular, Fac. de Medicina, Buenos Aires, Argentina.

The systemic injection of the vinca alkaloid vinblastine (VIN) to adult rodents, seems to destroy the terminal portions of their peripheral sympathetic neurons leaving unaffected the noradrenergic neurons in the brain. The morphological and biochemical alterations in the periphery gradually recover because the cell bodies of the sympathetic neurons are not irreversibly damaged. However, many of these cell bodies are destroyed when VIN is injected to newborn rodents.

To further characterize the alterations produced by VIN in the development of noradrenergic neurons, we studied the long-term effects of a single systemic injection of VIN to rats at 2 days of age (0.25 µg/g, sc) on the concentration of noradrenaline (NA) in the periphery as well as in the brain. Already at 15 days of age, the absolute amount of NA in the superior cervical ganglion (SCG) was depleted but normal values were obtained for NA when expressed per unit weight. This indicates that part of the cells in the SCG were destroyed while those remaining had a normal content of NA. These changes became more evident at 30 and 60 days of age when the content of NA was reduced by 47 %. Marked depletions of NA were found in the heart, salivary glands and spleen at 15 days, an effect that persisted at 60 days when the reduction of peripheral NA was, on average, more than 70 % in comparison with controls. In several brain regions (cortex, brain stem, cerebellum and spinal cord), NA levels were not modified 30 or 60 days after systemic neonatal VIN.

Thus, a single systemic injection of VIN to newborn rats produces a partial but persistent peripheral sympathectomy without affecting noradrenergic neurons in the brain. These seem to be insensitive to the actions of VIN even when the compound is given directly into the brain. Similarities and differences between the effects of VIN and those of neurotoxins that alter the development of central and peripheral noradrenergic neurons, may help to understand the mechanisms involved in this process. (Supported by grants from CONICET, SECYT and Secretaría de Salud Pública, Argentina).

131.19 Neonatal Desmethylimipramine(DMI) and Methysergide(MSG) treatment: Neurochemical responses of serotonergic neurons to L-5-hydroxytryptophan(5-HTP). H. Taub* and D.A.V.Peters*(SPON:R.J.Boegman). Dept. of Pharmacology, Sch. of Med.,Univ. of Ottawa, Ottawa,Ont.

Previous studies in our laboratory showed that several psychoactive drugs as haloperidol, chlorpromazine and lithium alter the development of central 5-hydroxytryptamine(5-HT)-containing neurons in the rat when administered during early postnatal period. In the present study DMI and MSG exposure served as a model with which to investigate further the responses of the brain to potential toxic insults occurring during specified developmental stage.

Newborn Sprague-Dawley rats were given once daily intravenous injections of DMI (15 µg/g), MSG (5 µg/g), or saline (1 µL/g) on days 1 and 2 after birth. No other treatment was given until 21 days of age when subgroups of rats were injected with 5-HTP (30 mg/Kg, i.p.) or its vehicle and killed 60 minutes later. The rat brain was dissected into 8-12 discrete regions and assayed spectrophotofluorometrically for 5-HT and 5-hydroxyindoleacetic acid (5-HIAA).

DMI treatment and to a lesser degree MSG treatment reduced the body growth rate of the offsprings, adrenal, whole brain and cerebellar weights. DMI treatment produced small (20-40%) but significant increases in the 5-hydroxyindole (5-HI) levels in midbrain, thalamus, motor cortex, olfactory tubercle and cerebellum when examined at 3 weeks of age. In contrast, MSG produced small but significant decreases in 5-HI levels of the above regions as well as in the striatum and hippocampus.

To assess the possible adaptive changes in the synthesis of 5-HI following drug exposure, 5-HTP was used. While 5-HTP produced marked elevations in 5-HI levels in all regions examined, the magnitude of the increases were significantly modified by the neonatal drug treatment. The 5-HTP-induced stimulation of 5-HI synthesis was significantly greater (25-127%) in all regions of DMI-exposed rats, and to a lesser extent (29-87%) in several regions of MSG-exposured rats. The data suggests that exposure to DMI and MSG (drugs presumed to differentially modify serotonergic function) during the neonatal period may alter the development of serotonergic neurons differentially. However,it may be tentatively concluded that the underlying enhanced sensitivity of serotonergic neurons may reflect a common mechanism of neurochemical adaptation. One cannot exclude the possibility that DMI and MSG, additionally, modify the development of other central neurotransmitters, i.e. catecholamine-containing neurons, and their possible interaction with serotonergic neurons may be responsible for the observed effects.

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131.20 DYNAMICS OF HEAVY METAL CONCENTRATION BY HIPPOCAMPAL MOSSY FIBERS. Richard L. Roth* and John H. Peacock (SPON: D. Kennedy). Hopkins Marine Station of Stanford Univ., Pacific Grove, CA 93950 and Dept. of Medicine, Univ. of Nevada Medical School, Reno, NV 89507.

We have used Timm's sulfide silver method in a semiquantitative way to study postnatal accumulation of heavy metals by hippocampal mossy fibers of laboratory mice. As part of this effort, six logarithmic dilution series of $ZnCl_2$ (range: 9.0-0.001 mM) were mixed with equal volumes of 4% agar and fixed with sulfide-containing formalin. Slices of these zinc standards were processed by Timm's method, and the time required for each specimen to acquire a standard tinctorial quality was recorded. For brain samples, we minimized the influence of factors other than the intended experimental variable by processing the brains of 6-12 animals in tandem, using the same batches of sulfide-containing fixative, buffers and staining solutions.

Over a wide range of concentrations, the time required for visible silvering is related to zinc concentration in an almost perfectly inverse fashion--viz., a doubling of concentration results in an approximate halving of staining time. This stoichiometric relationship permits reliable comparisons of different tissue samples; by concurrent staining of brain slices and zinc standards actual tissue-zinc concentrations can be estimated.

The heavy metal content of mossy fibers is detectable in neonates, increases rapidly between the 5th and 30th postnatal days and continues to increase at a slow rate through at least the 8th postnatal month. Overall there is at least a ten-fold increase in heavy metal content between birth and the 8th month, the most rapid increase occurring between the 5th and 12th postnatal days.

By subcutaneous injection or cardiac perfusion of zinc-containing solutions and by incubation of 0.5 mm brain slices in similar solutions, we have found that the capacity to sequester exogenous zinc is present by the 18th day of gestation, increases slowly over the first 4 postnatal days, increases very rapidly between the 5th and 10th postnatal days and slowly thereafter. Throughout postnatal life hippocampal mossy fibers are capable of taking up several times their normal metal content within 1 min of exposure to 0.75 mM $ZnCl_2$ made up in any of a wide variety of physiological saline solutions.

The energy dependence of heavy metal uptake is indicated by its temperature sensitivity, its diminution by 0.001 mM CCCP and its abolition by 0.01 mM CCCP. 30 mM Ca^{++} modestly inhibits zinc uptake; 4 mM La^{+++} and 0.2 mg% ruthenium red have a strong inhibitory effect, and uptake is abolished by 0.2 mg% verapamil. Mossy fibers appear to be incapable of distinguishing Zn^{++} from many other divalent cations since Cd^{++}, Co^{++}, Cu^{++}, Fe^{++}, Hg^{++}, Mn^{++}, Ni^{++}, Sn^{++}, Sr^{++} and UO_2^{++} are also sequestered.

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131.21 BEHAVIORAL TOXICITY OF ETHYLNITROSOUREA AND CORRELATION WITH TRANSPLACENTAL CARCINOGENICITY. S. M. Lasley, R. L. Bornschein*, J. Manson*, and L.S. Rafales*. Kettering Laboratory, University Cincinnati, College of Medicine, Cincinnati, Ohio 45267.

Direct-acting alkylating agents are the most active known transplacental carcinogens, and of these the most potent are the alkylnitrosoureas. Few studies have assessed the developmental and behavioral effects of ethylnitrosourea (ENU) or have compared tissue pathology data to such findings as was done in this work.

On day 16 of pregnancy dams received an i.p. injection of 20 mg/kg ENU dissolved in a trioctanoin oil vehicle or the vehicle alone. Litters were weighed at birth, and individual weights were recorded at days 12 and 22 (weaning), and at other points up to 150 days. Behavioral testing was initiated at 70 days of age and repeated at 4-week intervals--offspring were placed in individual photocell activity cages and responses monitored at 5 minute intervals for 3 hours, the last 2 hours following a 1 mg/kg s.c. dose of D-amphetamine. At death autopsies were performed and major organs retained for gross evaluation. At 9 months of age all remaining animals were sacrificed to obtain a proportion of treated animals that did not display overt toxicity for contrast with animals that had died previously.

At the point of termination of the study 59% of the treated animals had died with a median age at death of 199 days. Neurogenic tumors were localized in the brain (45%) largely related to the trigeminal nerve, and in the spinal cord (43%) almost exclusively in the lumbar region. Analyses of growth curves uncovered treatment effects that differentiated males from females and were more prominent at later ages. Photocell activity measures on animals at 114 days showed that the decline in activity during the 2-hour post-D-amphetamine period was altered in treated animals, and that the activity of males was less than that of females and diminished at a faster rate. Additional analyses showed the decline in activity effect to be present in treated males only. A covariance analysis with body weight as the linear covariate did not alter these findings. The protocol for behavioral testing appears therefore to have value in detecting the neurocarcinogenic effect of ENU before overt toxicity is present.

131.22 DEVELOPMENTAL CHANGES IN PHARMACOLOGICAL RESPONSIVITY OF THE ACOUSTIC STARTLE REFLEX. D.W. Gallager, J.H. Kehne*, and M. Davis. Dept. Psychiat., Yale Univ. Sch. Med., New Haven, CT 06508.

Several ontogenic studies have found a general caudal to rostral sequence of neurochemical development of various neurotransmitter systems in the CNS. Data described here using acoustic startle measurements suggests that the pharmacological responsivity of this reflex behavior at different postnatal (P) ages may reflect this caudal to rostral developmental sequence.

In adult rats (P 55 days) systemic administration of the GABA antagonist, picrotoxin (PICRO) produces a dose-related inhibition of acoustic startle. In adult rats intraventricular administration of PICRO produces a marked inhibition of startle. In contrast, intrathecal infusion of PICRO onto the spinal cord enhances acoustic startle. The inhibition of startle observed following the systemic administration of PICRO appears to be a summation of inhibitory supraspinal and excitatory spinal effects of the drug. If caudal systems develop before rostral ones, PICRO should enhance startle responses in the immature rat, reflecting its activity in the spinal cord. To test this, the effects of PICRO were tested at various postnatal ages. At P 15 days, littermates were tested before and after i.p. PICRO or saline. Increases in startle amplitude occurred following 1 mg/kg of PICRO. However, by P 35 days this same dose depressed acoustic startle in the same rats. These data indicate that enhanced startle in immature animals after PICRO probably reflects the spinal actions of the drug.

To test the generality of these findings, comparable studies were done using drugs that act on central 5-HT systems. Recently, different functional effects of 5-HT in rostral and caudal 5-HT systems have been described. In both electrophysiological and acoustic startle experiments 5-HT has inhibitory effects in the forebrain but facilitates transmission in the spinal cord. p-Chloroamphetamine (PCA) releases 5-HT shortly after administration and concomitantly depresses startle in adult rats. If caudal to rostral maturation of the 5-HT system occurs during ontogenesis, increased 5-HT release should activate only the spinal (excitatory) system at ages prior to the completion of forebrain 5-HT synaptogenesis. Consistent with this expectation, 5 mg/kg of PCA increases startle response in P 15 day rats during the 5-HT release period. This effect is completely blocked by pretreatment with the 5-HT synthesis inhibitor PCPA. In contrast this same dose of PCA depresses startle in adult rats during the 5-HT release period, and this effect is blocked by PCPA. Taken together, these results suggest a functional caudal to rostral developmental sequence as measured by pharmacological responsivity of the acoustic startle reflex to drugs acting on two different neurotransmitter systems.

132.1 FIBER ORDER IN THE XENOPUS OPTIC NERVE AFTER QUADRANTIC GRAFTS OF EMBRYONIC RETINA. J.W. Fawcett* and R.M. Gaze* (SPON: S.C. Sharma). National Institute for Medical Research, London NW7 1AA, U.K.

The developing Xenopus eye acquires positional properties, which will later determine the nature of the retinotectal fiber projection, before any axons have grown from the eye rudiment. Shortly afterwards, grafting part of the eye to a different position in another eye has no obvious effect on these properties. Thus fibers from a temporal retinal quadrant will still connect with rostral tectum even when that quadrant has been transplanted elsewhere in the eye. We have been studying the behaviour of fibers from retinal quadrants moved 180° from their original positions.

Xenopus embryos at stages 28 to 31 had ventral, temporal or nasal quadrants removed from their right eyes and grafted in place of the left dorsal, nasal or temporal quadrants of a host of the same age; thus the quadrants were transplanted 180° from their original positions. The animals were grown to metamorphosis, mapped electrophysiologically to check that the implanted fragment was still present, and then had Horseradish peroxidase applied to the implant. Serial sections of the optic nerve and brain were taken and developed with benzidine dihydrochloride to show the course of axons to the tectum.

Fibers from temporal quadrants, transplanted to nasal positions, rotated in the optic nerve relative to the other fibers from the eye and took up a position at the chiasma normally occupied by fibers from temporal retina. Fibers from nasal quadrants, transplanted to temporal positions, in most cases occupied the two areas at the chiasma usually occupied by nasal fibers. However, fibers from ventral quadrants transplanted to dorsal positions showed no consistent behaviour in the optic nerve, often being randomly spread within it at the chiasma. These fibers, however, occupied only the medial brachium of the optic tract. To this extent, at least, they had regained their correct pathways between the chiasma and the tectum.

The optic chiasma must have properties which allow ingrowing fibers from different positions along the nasotemporal dimension of the eye to get to different parts of it. Organization of fibers from different positions along the dorsoventral retinal dimension may occur more centrally in the optic tract.

132.2 VISUALLY GUIDED ORIENTING BEHAVIOR BY RATS WITH EXPANDED IPSILATERAL VISUAL PATHWAYS. G. C. Midgley*(SPON: John P. J. Pinel) Department of Psychology, Univ. of Western Ont., London, Ont. N6A 5C2.

The behavioral and anatomical consequences of neonatal enucleation and subsequent expansion of the intact retinas' ipsilateral visual pathways were examined. The left eye of albino rats (Sprague-Dawley strain) was enucleated on either postnatal day 1 or 21 and their visual behavior made dependent upon the ipsilateral retinal projections by a lesion of the contralateral optic tract as adults. Visually guided orienting behaviors were examined following training to remain motionless for a food reward in a modified perimetry. Slow motion video tapes of the rats orienting behavior to food and other stimuli presented in the four quadrants of the animals' visual field were assessed for accuracy and direction of orienting responses and reaching responses to the food stimuli. The striate cortex ipsilateral to the intact eye was ablated and perimetry testing repeated.

Expansion of the ipsilateral pathway and the extent of the lesion of the contralateral pathway were assessed by injections of [^{3}H] proline in the intact eye and autoradiography. Enucleation on day 1 resulted in significant expansion of the ipsilateral projections while enucleation on day 21 did not. Both the day 1 and day 21 enucleated rats oriented toward and reached for food presented in the upper nasal visual field. The day 1 enculeated rats also oriented and reached for food presented in the lower nasal and lower temporal fields. The initial orienting component of the reaching response was misdirected on most occasions. When the striate cortex ipsilateral to the aberrant retinal projections was ablated the rats enculeated on day 1 made significantly more orienting and reaching responses to food presented in all but the upper temporal field. Misdirected turning was not observed following striate cortex removal. The day 21 enucleated rats did not show this recovery with striate lesions.

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132.3 REGENERATION OF A BANDED RETINOTECTAL PROJECTION IN GOLDFISH. S. C. SHARMA and A. D. SPRINGER (SPON: F. E. HORVATH). Depts. of Ophthalmology and Anatomy, New York Med. Coll., Valhalla, N.Y. 10595.

After one tectal lobe is removed in adult goldfish, the severed optic nerve fibers from the contralateral eye regenerate and innervate the remaining ipsilateral tectal lobe. Therefore, the remaining tectal lobe is innervated by both eyes. Initially, efferents from the two eyes are characterized by continuous, overlapping projections over the surface of the tectum. Within 70 days at 30°C these two projections become segregated into alternating "eye-specific" bands oriented rostrocaudally across the tectum. Once the bands have formed, the pattern of banding observed appears to be independent of how long the fish have survived with two eyes innervating one tectal lobe. Segregation of optic fibers into "eye-specific" bands, rather than maintained intermingling of the two sets of retinal fibers, suggests that the retinal fibers of one eye are distinct from the retinal fibers of the opposite eye. Experiments were designed to determine whether the banded retinal projections could impart their differential labels onto tectal cells. Goldfish in which two eyes projected to one tectal lobe for periods ranging from 112-1660 days were used in these experiments. In one group of fish (A), the contralateral optic nerve was crushed and the ipsilateral eye was left intact. In two other groups of fish, the contralateral optic nerve was crushed and the ipsilateral eye was removed at the same time (B), or just before the regenerating fibers reached the tectum (C). The pattern of optic nerve regeneration was examined over a period ranging from 11-70 days post-crush using [^{3}H]proline radioautography. The fish were maintained at 30°C to accelerate regeneration. Regenerating optic nerve fibers in Group A initially formed a continuous projection over the tectum, which later became banded. However, the regenerating optic nerve fibers formed a continuous projection over the tectum at all time points examined in groups B and C. Since a regenerated banded projection was not observed when the ipsilateral eye was removed, it appears that the putative differential retinal labels are not imparted onto the tectal cells.

132.4 FIBER-FIBER INTERACTIONS IN DUALLY INNERVATED TECTA FOLLOWING BILATERAL OPTIC NERVE REGENERATION IN XENOPUS WITH ONE COMPOUND EYE. C. Straznicky and D. Tay* Dept. of Human Morph. Sch. of Med., Flinders Univ. of S.A., Bedford Park, 5042, Australia.

Right compound eyes were formed in Xenopus at stage 32 by the fusion of two nasal (NN) or two temporal (TT) halves of the eye blastema. The left eye was kept intact. Two to four weeks after metamorphosis both the right and the left optic nerves were cut close to the chiasm to facilitate optic fiber regeneration from each eye to both tecta. One to 12 months after bilateral optic nerve sections the retinotectal projections to the dually innervated tecta were assessed anatomically and electrophysiologically. ^{3}H-proline was administered either into the right or left eye of the animal. In a few cases one eye had ^{3}H-proline the other eye had HRP injections. Twenty four to 48 hours after isotope and HRP administration the visual projections from each eye to both tecta were mapped electrophysiologically.

Retinotectal projections from TT eyes, determined autoradiographically and electrophysiologically, failed to cover the entire extent of the contralateral and ipsilateral tecta. The caudomedial area of each tectum (normally receiving nasal retinal fibers) was consistently uninnervated. The NN projection in some of the animals extended across the entire tectum, while in others they were restricted to the caudomedial part which is the normal destination of nasal fibers. The left normal eye projections extended over the entire tecta in all TT and NN eye animals. Both the compound eye and normal eye maps were orderly, projecting retinotopically in a superimposed fashion to the tecta. In areas where the compound eye and the normal eye projections overlapped eye-specific terminations bands were found. Double ^{3}H-proline and HRP labeling revealed mutually exclusive right and left eye termination bands extending in a rostrocaudal direction over part of the tecta. No banding was observed in areas which were innervated only by the left intact eye. The patterns of retinotectal projections were similar in animals with shorter or longer regeneration time.

These observations show that the tectal distribution of fibers from a compound eye is selective and that at least the greater part of the overlapping projections are in register as far as their retinotopic order is concerned. We suggest that the underlying mechanism is fiber-fiber interactions in the forms of recognition of the like fibers and of competition for preferred tectal termination. Supported by an Australian Research Grants Committee grant to CS.

132.5 WHOLE MOUNT VISUALIZATION OF BAND DEVELOPMENT IN THREE-EYED TADPOLES. M. Constantine-Paton, M. I. Law and P. Ferrari-Eastman*. Dept. of Biology, Princeton Univ., Princeton, NJ 08544.

Addition of a third eye primordium to the forebrain region of embryonic frogs (Rana pipiens) causes two retinas to innervate one optic tectum where their terminals segregate into highly sterotyped eye-specific stripes (Constantine-Paton, M. and Law, M. I., Science 202: 639, 1978; Law, M. I. and Constantine-Paton, M., J. Neurosci., in press, 1981). We have studied the development of this pattern in tadpole and young frog brains after horseradish peroxidase (HRP) application to the cut stump of either the normal or the supernumerary optic nerves.

We have used a diaminobenzidine histochemical procedure (Fugisawa, H. et al., Brain Res. 206: 21, 1981) that allows visualization of HRP filled retinal axons and terminals in whole brains. The development of the frog's visual pathway using this method was identical to that reported with 3H-proline autoradiography (Currie, J. and Cowan, W. M., Devel. Biol. 46: 103, 1975). In the earliest brains examined, the retinal projection was restricted to the anterolateral pole of the tectal lobe. It expanded with age in a medial and caudal direction to fill all but a caudomedial strip of the tectal surface by metamorphosis.

Three-eyed tadpoles show the same expansion of retinal projections within their doubly innervated tectal lobes. Supernumerary or "normal" eye projections were distributed as ≃ 200 micron wide stripes in the anterior tectal regions even in the youngest animals examined. These stripes were oriented perpendicular to the expanding edge of the retinal terminal zone but they ended abruptly at its caudomedial border. At higher magnifications, this region showed low densities of uniformly distributed optic fibers. Lack of segregation in such immature tectal areas may be due to low innervation density and to the lack of differentiated tectal cells.

In a few three-eyed animals, a small complement of supernumerary eye fibers entered the second tectal lobe. Banding did not occur in these partial projections at low innervation densities. At high innervation densities, these second tectal lobes showed a few bands. At intermediate levels non-continuous "patches" of supernumerary eye terminals were observed. These patches were arranged in a pattern that had the same orientation and spacing as stripes. Thus, given a certain threshold level of optic nerve fibers, bands seem to progressively form around discrete elements that are probably intrinsic to the optic tectum.

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132.6 MORPHOMETRIC EXAMINATION OF COMPETING RETINAL PROJECTIONS IN THREE-EYED FROGS. M. I. Law and M. Constantine-Paton. Dept. of Biology, Princeton University, Princeton, NJ 08544.

The effects of competition were morphometrically examined in the optic tecta and retinas of three-eyed tadpoles. The supernumerary eye in these animals co-innervates one tectal lobe and disrupts the normal eye's continuous projection, producing 200μ wide, eye-specific bands of neuropil (Constantine-Paton and Law, 1979, Science 202: 639-41). Volume measurements indicate that super-innervated tecta are ~ 30% enlarged, with most of the hyperplasia confined to cellular tectal layers. Moreover, volumes of neuropil occupied by either co-innervating retina are reduced by 46-59% relative to the neuropil volume of the singly-innervating optic tract in the same animal (Law and Constantine-Paton, 1981, J. Neurosci., in press). These results suggest that retinal ganglion cells (RGC) of competing eyes must either compress their terminal arbors or die at higher than normal rates.

Cell counts which sampled all three retinas in several frogs revealed fewer RGCs in co-innervating eyes compared to the host's normal retina. A reduction of 9-21% was observed in the competing host eye versus the non-competing eye of the same animal. This contrasts with less than 1% difference in the two normal retinas of an animal whose third optic nerve did not grow into the brain. The RGC reduction in co-innervating eyes is not sufficient to account for the large decrease in the neuropil volume occupied by their terminals. This suggests that some increase in optic fiber density may also be present in doubly innervated lobes.

There are potentially two components of competition in these tecta: competition between compressed fibers of the same projection and competition between similarly-specified axons from the two different eyes. The latter would be expected to occur primarily in areas of overlap between the eye-specific bands. We have now directly measured this overlap by differentially labelling the two projections with ^{3}H-proline autoradiography and HRP. The average area of overlap ranged between 100-800μ^2 in different animals. This represents ~ 5% of any single band's total area.

Overlap values may vary with the relative densities of the two projections or with the visual environment in which the animal is raised. Thus, this information will provide a background necessary to determine if the effects of binocular competition are separate from the effects of binocular segregation in three-eyed frogs.

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132.7 "OCULAR DOMINANCE" COLUMNS IN GOLDFISH, ONTOGENY AND EFFECT OF VISUAL ENVIRONMENT. Ronald . L. Meyer. Dept. Developmental-Cell Biol., Univ. Calif., Irvine, CA 92717.

Fascicles of optic fibers supplying dorsocaudal optic tectum were teased free of surrounding tectum and back to the anterior tectal pole near their exit from the medial brachium of the optic tract. These were then deflected into a large mediolateral incision made across the anterior end of the contralateral "host" tectum. This latter incision also severed the host fibers (cf Meyer, JCN 183: 883, 1979). Regeneration of host and deflected fibers was followed with autoradiography by injecting one eye with tritiated proline at 2 to 12 weeks after surgery. At 2-4 weeks both sets of fibers were spread throughout dorsal tectum, completely overlapping. At 6 weeks deflected fibers began to show local condensations of label and host fibers showed regions of lighter than normal label. By 8 weeks most deflected fibers were restricted to several clumps in dorsocaudal tectum while in this same region host fibers showed gaps in their otherwise continuous distribution of label. Some light label outside the clumps (deflected) or within the gaps (host) persisted but by 12 weeks had decreased to that seen previously at longer intervals (Meyer, ibid.). Thus host and deflected fibers appeared to be largely or completely segregated. These fish had been maintained in diurnal illumination.

To examine the effect of visual stimulation, fish with identical surgery and autoradiography were subjected to three different visual environments. One group was kept in total darkness in a standard aquarium for 2-12 weeks. In two other groups the individual fish were maintained for 8 weeks in 1000 ml round bottom boiling flasks having long necks and turned upside down. Fish lived in the spherical portion and were subjected to one of two environments. In one, the inside of the flask was painted with black polka-dots of various size against a white translucent background. Illumination was continuous. In the other group the flask was carefully made to be a homogeneous translucent white. The sole illumination was an overhead strobe operating at 6 hz. The autoradiographic results from these 3 groups could not be distinguished from that of the diurnal group.

It is concluded that segregation represents active sorting out of left and right optic fibers and that the mechanism is resistant to changes in the pattern of impulse activity and may not be due to impulse activity at all. An underlying left-right neurospecificity of optic fibers should be entertained as a serious possibility. (Supported by PHS Grant NS15381.)

132.8 ALTERATIONS IN THE CROSSED RETINOTECTAL PROJECTION DURING DEVELOPMENT OF THE CHICK. S. C. McLoon. Dept. of Anatomy, Medical University of South Carolina, Charleston, S.C. 29425.

Previous work has shown that a transient ipsilateral retinofugal projection is present during early chick development (McLoon and Lund, '80). This suggested that the visual system may initially develop broadly distributed projections which are subsequently refined to the adult pattern. It was not known whether this early projection was broad only with respect to laterality or if there was also a broad distribution of fibers within a target nucleus as well. This was approached in an earlier study in which partial ablation of an optic cup resulted in no retinal projection to a portion of the contralateral tectum at 18 days of development (Crossland et al., '74). The region of the tectum lacking innervation corresponded topographically to the ablated region of the eye. We have examined embryos at earlier developmental stages which had received similar ablations to determine if transient projections develop from the remaining portion of the eye to inappropriate regions of the tectum. A superior-nasal or inferior-nasal retinal quadrant of embryos was ablated on the third day of incubation. On embryonic day 10, 12, 14 or 16 an injection of horseradish peroxidase (HRP) was made into the partial eye. After an appropriate survival time the embryos' brains were fixed, sectioned and reacted with tetramethyl benzidine and hydrogen peroxide. Based on the distribution of HRP reaction product, a significant projection from the injected eye was identified in the contralateral tectum. For 10 and 12 day embryos the heaviest projection was to portions of the tectum corresponding topographically to the remaining portion of the eye. However, a light projection was also found in the tectal quadrant corresponding to the ablated retinal quadrant. By 14 days of incubation no projections could be identified in the region of the tectum corresponding to the retinal ablation. In another series of 10 and 12 day old embryos the partial eye ablations were made 0, 12 or 24 hours prior to the HRP injection. In these animals a projection was also identified from the remaining retina into areas of the tectum appropriate for the ablated retinal quadrant. These results suggest that during early development of the chick visual system, areas of the retina project broadly across the contralateral tectum. The results from the partial retinal ablations made just prior to the HRP injection suggest that these broad projections are present normally and were not induced by early lesions. Between 12 and 14 days of incubation, the retinotectal projection is refined leaving a tightly ordered topographic map. The broad distribution of retinal fibers on the tectum during early developmental stages suggests that an orderly ingrowth of optic fibers alone is not a sufficient mechanism for establishing the proper topography in the retinotectal system. (Supported by grant EY03314 and EY03713 from the National Institutes of Health)

132.9 DEVELOPMENT OF THE NUCLEUS ISTHMI IN XENOPUS LAEVIS. Susan B. Udin. Div. Neurobiol., State Univ. of NY, Buffalo, NY 14214.

The nucleus isthmi (NI) is a relay for the ipsilateral visuotectal projection in the frog, Xenopus. The nucleus receives retinotopic input from the tectum and projects topographically to both tecta. The topographic pattern of the crossed isthmotectal projection is influenced by visual experience during development; for example, mid-larval eye rotation induces abnormal isthmo-tectal topography (Keating and Udin, 1979, J. Physiol., 300:63P). To help understand how orderly connections between the NI and the tecta arise, the development of the NI in normal Xenopus is being studied.

The NI can be unambiguously identified by stage 53, when it acquires the characteristic structure of a "shell" of cells surrounding a relatively cell-sparse core. There are about 200 cells in the NI at stage 53. By stage 57, there are over 1000, and by the early 60's stage, just prior to metamorphic climax, the adult number of about 2000 cells is reached.

To study cell birthdays, tadpoles of different ages were injected with ^{3}H-thymidine and allowed to survive past metamorphosis. Autoradiographic results show that there is a spatiotemporal gradient of development, with cells born at about stage 29 occupying ventral NI and those born at stage 56 occupying the dorsomedial NI. (Similar results are reported by Tay and Straznicky, 1980, Neurosci. Lett., 16:313.) However, a few cells born between stages 52 and 56, plus many cells born between stages 58 and 62, occupy positions scattered throughout the nucleus. These cells may be glia.

Injections of horseradish peroxidase (HRP) show that there are connections between tectum and NI as early as stage 53. The tecto-isthmic and uncrossed isthmo-tectal projections are quite substantial, but the crossed isthmo-tectal projection is still sparse at this stage. Thus, the anatomical connections for ipsilateral visuatectal activity are present--in limited numbers--well before such activity is first found electrophysiologically, at around stage 60. The first cells which project from the NI to the opposite tectum lie in the ventral NI, the earliest-born region. However, cells in this position do not normally project to the earliest-born parts of the contralateral tectum, and a simple scheme of matching newly-generated NI cells with newly-generated tectal cells during growth cannot be invoked to explain normal connectivity of the crossed isthmotectal projection.

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132.10 CHANGES IN RESPONSE PROPERTIES OF SINGLE UNITS IN THE SUPERIOR COLLICULUS OF STROBOSCOPICALLY-REARED RABBITS. Helen E. Pearson and E. Hazel Murphy. Department of Anatomy, The Medical College of Pennsylvania, Philadelphia, PA 19129.

Our laboratory has recently reported that restricting early visual experience to a stroboscopically illuminated environment results in a modification of receptive field properties in the striate cortex of the rabbit with the most striking change being a loss of direction selectivity in orientation selective cells (Pearson, H. E., Berman, N. and Murphy, E. H., Devel. Brain Res. 1981). In the present study, we have investigated the effects of early stroboscopic rearing on receptive fields in the rabbit superior colliculus.

Single unit recordings were made from 194 neurons in the superior colliculus of rabbits raised from birth in a stroboscopically illuminated environment. These data were compared with 265 neurons recorded in the superior colliculus of normal rabbits.

The receptive field properties of strobe reared rabbits were significantly different from those of normals. In strobe reared rabbits there was a profound loss of direction selectivity. There was also an increase in the percentage of units preferring the offset rather than the onset of flashed stationary stimuli, and an increase in receptive field size. In addition, the two groups differed in their responses to stroboscopic stimulation. Compared to normals, the units recorded in strobe reared rabbits showed an increase both in the percentage of units which responded to stroboscopic flashes, and in the percentage of units whose responses to their optimal stimuli were inhibited by the simultaneous presentation of stroboscopic flashes.

Our study indicates that strobe rearing causes a significant alteration of response properties in the rabbit superior colliculus. These changes are strikingly similar to those which we have previously reported (Graham, Berman and Murphy, ARVO Abstr. 1981) in the superior colliculus of the rabbit following ablation of the visual cortex. This suggests that modification of corticotectal influences plays a major role in strobe-induced alterations of rabbit superior colliculus response properties.

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132.11 NEONATAL VISUAL CORTICAL ABLATIONS ALTER RETINOTECTAL PROJECTIONS IN HAMSTER. R. W. Rhoades, D. C. Kuo, and J. D. Polcer*. Dept. of Anatomy, CMDNJ-New Jersey School of Osteopathic Medicine and Rutgers Medical School, Piscataway, NJ 08854.

Autoradiography and the anterograde transport of horseradish peroxidase (HRP) were used to examine retinotectal projections in normal hamsters and in animals subjected to ablation of most of the dorsal, posterior neocortex at 1, 3, 6, 10 or 120 days of age. The results obtained from the normals were in good accord with previous reports from this and other laboratories. In both the autoradiographic and HRP material, label was observed throughout the superficial laminae of the colliculus contralateral to the injected eye, and was most dense in the dorsal two-thirds of the stratum griseum superficiale. In the autoradiographic material the ipsilateral projection was restricted to a series of discrete patches in the rostral third of the stratum opticum and a few labelled fibers in the zonal layer. In the tissue processed for HRP the ipsilateral projection appeared much more extensive, and labelled fibers could, in most cases, be traced to the posterior collicular boundary.

The crossed retinocollicular projection in the brain damaged hamsters did not differ markedly from that in the normal animals. A small increase in the density of the labelling in the ventral portion of the stratum griseum superficiale was visible. Very clear differences were observed in the uncrossed retinal projection to the colliculus ipsilateral to the damaged hemisphere. In the autoradiographic material, numerous labelled fibers could now be traced into the posterior one-half of the colliculus, and in many cases such fibers were visible at the caudal limit of the tectum. In sections processed for HRP, the incidence of labelled fibers in the posterior part of the colliculus was much greater than in the tissue from normal hamsters. These changes were most pronounced in the hamsters lesioned on postnatal days 3 and 6, but were clearly visible in material from all of the neonatally brain damaged animals.

In additional experiments, HRP was deposited into the colliculus ipsilateral to the damaged cortex to determine whether or not neonatal cortical ablations altered the distribution of ipsilaterally projecting retinal ganglion cells. Comparison of the resultant data with those from identically treated normal controls indicated that neonatal cortical damage did not alter the distribution of ganglion cells which contributed axons to the ipsilateral retinotectal pathway.

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132.12 ANALYSIS OF GOLDFISH OPTIC NERVE SPECIFIC PROTEINS DURING REGENERATION. W. Quitschke* and Nisson Schechter. Departments of Biochemistry, Psychiatry and The Long Island Res. Inst., SUNY-Stony Brook, New York 11794.

The process of axonal growth and synapse formation can be studied in the regenerating retinotectal pathway of goldfish. Previous analysis of proteins from optic nerve and tectum by 2D-gel electrophoresis revealed a cluster of proteins enriched in the retinotectal pathway. Furthermore, specific components of the cluster disappeared or diminished after optic nerve disconnection. Following optic nerve crush their levels are restored to normal concomitant with return of vision (Brain Res., 201, (1980) 347-360). This report is concerned with the in vitro synthesis of these proteins using ^{35}S-met. Pooled control and experimental tissues were removed and incubated in HEPES minimal salt medium in the presence of ^{35}S-met. The tissues were homogenized in 0.5% SDS and acetone extracted. The protein was solubilized in a buffer containing 0.05M Ches buffer pH 9.5, 2% SDS; 1% DTT, 10% Glycerol and 5mM PMSF. Two-dimensional gel electrophoresis was performed as described by O'Farrell. Autoradiograms were obtained by exposing the stained and dried gels to x-ray film for 7 days.

The coomassie blue stained gels of the optic nerve revealed a cluster of four well separated components. Upon optic nerve disconnection two of the four components disappear completely. The same two components are restored to control levels 60 days after optic nerve crush. The other two components remained unchanged after optic nerve disconnection. The cluster is also present in the retina, but at a level barely detectable by coomassie blue staining. The levels of the components in the retina appear unaffected by optic nerve disconnection. Autoradiograms of the optic nerve show that the two components which are unaffected by optic nerve disconnection are labeled whereas the components sensitive to disconnection remain unlabeled. Results from retinal incubations show that the two components which disappear from the nerve after crush are indeed labeled. The synthesis of these components is significantly enhanced after optic nerve crush. The interpretation of these experiments is based on the assumption that protein synthesis does not take place within axons. Consequently, the in vitro synthesis must involve non-neuronal cells of the optic nerve.

It appears that the two components which are unaffected by optic nerve crush and which are also synthesized in the optic nerve are indeed of non-neuronal origin. The components which disappear from the optic tract upon disconnection are labeled in retina, but not in optic nerve. Thus, they are likely to be synthesized by the retinal ganglion cells and localized to the optic nerve axons.

132.13 OPTIC NERVE HYPOPLASIA INDUCED BY KAINIC ACID: A CHICK EMBRYO MODEL. A. Suburo and H. Campaña*. Instituto Multidisciplinario de Biología Celular (CONICET-CIC). CC 403. 1900 La Plata. Argentina.

Optic nerve hypoplasia --as described in human beings-- is characterized by a reduced number of axons in the optic nerve of an eye showing an otherwise normal structure. The developmental mechanisms underlying this defect need further study, both for prevention of the defect and for the understanding of visual pathway morphogenesis. We report here a similar anomaly produced in chick embryos by a neuronal toxic administered at a stage when only a fraction of the total ganglion cell population has developed.

Six day-old embryos received kainic acid (Sigma, 3 mg per embryo) through a window in their shells. Their retinas were dissected after different survival times. Right retinas were flat-mounted and stained with 0.1% cresyl violet and left ones were silver impregnated (Goldberg, S., Stain Technol., 47, 65, 1971). The latter were observed with scanning electron microscopy. Controls received Hank's solution and were processed in the same fashion.

Kainic acid produced lesions in most cells lying in the layer of ganglion cells. After four hours, some cells had been completely destroyed leaving heterochromatic debris. The remaining cells showed nuclear dilatation and loss of cytoplasmic basophilia.

Kainic acid treated retinas reached the same size as control retinas, in marked contrast with the general underdevelopment of the neural tube. The ganglion cell layer of 18 day-old retinas showed a normal structural organization, i.e.: the different morphological classes were distributed in the appropiate central, nasal and temporal patterns. Cell density appeared to be the same, but the bigger ganglion cells did not attain control sizes. On the contrary, there was a marked reduction in the layer of optic fibres, its thick bundles being replaced by an incomplete layer of fibres. All the axons, however, extended in the direction of the nerve-head. The latter had the same length as in controls, but its width was reduced.

Thus, the death of a considerable number of neurons at an early period of development did not change the morphogenetic program of the retina. Besides, it seems that those cells differentiating after the administration of kainic acid were able to find the correct pathway, at least up to the nerve-head.

132.14 REAGGREGATED EMBRYONIC RETINAE DIFFERENTIATE AND FORM CONNECTIONS AFTER TRANSPLANTATION TO HOST RAT BRAIN. L. K. McLoon, R. D. Lund and S. C. McLoon. Dept. of Anatomy, Medical University of South Carolina, Charleston, S.C. 29425.

This study was undertaken to examine the role of initial cell-cell associations in the differentiation and formation of connections by central nervous system during development. Embryonic day 14 neural retinae were dissociated into single cells and reaggregated into pellets. The reaggregated tissue was cut into pieces and transplanted over the left superior colliculus (SC) of newborn rat hosts. One month later the brains of the host were examined. Host SC were injected with HRP, and transplants were examined for labeled cells. In six host rats a lesion was placed in the transplant, and degeneration methods were used to determine the distribution of connections made by the transplant with the host brain.

The reaggregated retinal transplants differentiated in the host brain. All the laminae of intact retinae were discernible and organized as rosettes or sheets. The transplants were located on the anterior surface of the cerebellum, and fiber bundles were grossly discernible coursing from the transplant over the inferior colliculus to the SC. HRP injected into the host SC retrogradely labeled a population of cells in the transplants. The labeled cells were found in the lamina corresponding to the ganglion cell layer and displayed a morphology characteristic of normal ganglion cells. Lesions of the transplants resulted in degeneration along the fiber bundles that exited the transplants. The fiber bundles were traced into the SC where degeneration was distributed throughout the superficial layers. Electron microscopic examination of the host SC confirmed the presence of degenerating synaptic terminals resembling those seen after eye removal in control rats. Degeneration was also traced into the dorsal terminal n., posterior pretectal n., olivary pretectal n. and the caudal portion of the dorsal LGN. These projections are all to nuclei that are normally retinorecipient.

It appears that the disruption of normal cell-cell associations early in retinal development and prior to transplantation did not adversely affect its subsequent ability to differentiate and form appropriate connections with the host brain. (LKM is NEI fellow EY05394. Supported by NEI grant EY03414.)

132.15 A NEW, IMPROVED QUANTITATIVE EM TECHNIQUE FOR DETERMINING THE NUMBER OF SYNAPSES PRESENT IN A VOLUME OF BRAIN TISSUE. J. J. Norden and J. A. Freeman. Dept. of Anatomy, Vanderbilt Univ. School of Medicine, Nashville, TN 37232.

Use of quantitative EM techniques is providing an increasingly definitive insight into the phenomena of synaptic formation, regulation, and plasticity. Common to such studies is the necessity of obtaining accurate counts of the number of synapses present in a given volume of brain tissue. We have compared the following quantitative EM techniques currently in use: 1) direct counting of synapses from photographic montages; 2) morphometric point-counting techniques; and 3) "on-line" counting of synapses at the electron microscope. While each of these methods has advantages, they all tend to be exceedingly time-consuming, especially if large areas or many animals must be sampled. Furthermore, the use of photographic montages for some of these methods is quite costly.

For these reasons, we have developed a new method based on statistical and stereological principles. A pseudo-random sample is made of the number of synapses present in a succession of EM viewing windows. A running tally of the standard error of the mean is computed, and in this way the minimum number of samples is determined necessary to insure a given criteria of accuracy. Additionally, we have derived correction factors to compensate for errors due to finite section thickness, grazing sections, and the overlapping of synaptic profiles in adjacent thin sections. These corrections are necessary before the number of synapses (number per volume) can be estimated from the areal density determined from counting the number of synapses in ultra-thin sections, since the average size of the synapse (as computed from serial reconstructions) is significantly larger than the thickness of the ultra-thin section. Finally, to compute the true number of synapses in a given volume from areal density requires obtaining volume information, for which we have developed a geometric method of general applicability. Volumetric density is then computed from areal density using stereological principles.

Each of the techniques described above were compared in terms of time, cost, reliability, and accuracy, using synaptic counts obtained from the optic tecta of long-term enucleated fish and amphibia. The random sampling method gave far superior results in terms of these criteria. The results of this analysis also clearly showed that without applying correction factors for errors due to finite section thickness, grazing, overlap and volume changes, none of the techniques for counting the number of synapses currently in use can be used to accurately determine the true number of synapses present.

133.1 A COMPARISON OF FUSIMOTOR EFFECTS FROM STATIC AND DYNAMIC GAMMA EFFERENTS OBSERVED WITH INTEGRATED ACTIVITY IN THE MUSCLE NERVE. K. S. K. Murthy, Lorie M. Shapiro* and Chaudhry Saleem*. Div. of Neurosurgery, Univ. of Texas Med. Sch., Houston, Tx. 77030.

Static and dynamic fusimotor actions due to γ efferents are definable with a recording of their effects on a single muscle spindle Ia afferent. However, each γ efferent is known to innervate a number of muscle spindle receptors. It may then be expected that an observation of fusimotor effects on the integrated multiple afferent activity would also help to differentiate the two types of γ efferents. The present study demonstrates a lack of correlation between the effects observed on a single afferent and that on multiple afferents in the case of dynamic γ efferents.

Experiments were performed on cats anesthetized with sodium pentobarbital. In each experiment one of the following muscles was studied - peroneus brevis (4 experiments), peroneus tertius (2 experiments), peroneus longus (2 experiments) and soleus (1 experiment) with the rest of the limb denervated. Dorsal and ventral roots L_7-S_1 were cut close to the cord. Between 4 and 14 Ia muscle spindle afferents in each experiment were isolated in dorsal root filaments and monitored for classification of fusimotor actions with single γ efferents that were isolated in ventral root filaments. Each γ efferent thus isolated and classified was investigated for effects on the multiple afferent activity recorded from the whole muscle nerve. The multiple unit activity in the muscle nerve was processed through a level detector adjusted for recording only the large action potentials from spindle afferents, rectified and integrated with a rate meter (time constant 3 msec). The level detector ensured that the smaller action potentials of the stimulated γ efferent were rejected. In each case, the effects were studied on the afferent response to a standard "ramp and hold" stretch (2mm at 10 mm /sec) and a brief, low amplitude vibration (< 100μ, lasting 20 msec).

A total of 103 static γ efferents and 11 dynamic γ efferents were studied with 91 Ia afferents. Both with large amplitude muscle stretch and the small amplitude vibration the effects of static γ stimulation on the integrated muscle nerve activity were qualitatively similar to the effects observed on a single afferent. The most remarkable difference was that with dynamic γ stimulation the effects on integrated muscle nerve record were different from that observed on a single afferent, but appeared qualitatively similar to static γ effects.

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133.2 HISTOCHEMICAL EVIDENCE FOR THE ADEQUACY OF THE PHYSIOLOGICAL TESTS FOR IDENTIFYING FAST-CONDUCTING SKELETOFUSIMOTOR (β) EFFERENTS. W.E. Cameron, W.D. Letbetter, K.S.K. Murthy and J. Petit*. Div. Neurosurgery, Univ. Texas Med. Sch., Houston, TX 77030 & Dept. Anatomy, Emory Univ. Med. Sch., Atlanta, GA 30322.

Fast-conducting skeletofusimotor (β) efferents of the cat hindlimb muscles have been identified by using a combination of four different physiological tests (Jami et al., Neurosci. Abstr. 6:395, 1980). These tests are: 1) the occurrence of "unfused oscillations" in the instantaneous frequency of the afferent when the stimulation frequency was increased beyond the level required for complete fusion of the extrafusal tension; 2) the occurrence of an abrupt increase in afferent discharge in response to a step increase in stimulation frequency of the efferent fiber without a concomitant increase in extrafusal tension; 3) the demonstration of fusimotor action of the efferent during a concurrently applied muscle stretch; and 4) the maintained increased level of afferent discharge in the absence of muscle tension due to failure at the extrafusal endplate resulting from high frequency stimulation. The present study provides histochemical evidence that these tests are more than adequate for establishing the existence of skeletofusimotor (β) innervation and, in fact, that only the first three tests are required.

Experiments were performed on adult cats anesthetized with sodium pentobarbital. Up to eight group Ia muscle spindle afferents from the peroneus tertius muscle were isolated in split dorsal root filaments. Single motor axons with conduction velocities > 90 m/sec were isolated from split ventral root filaments. Those motor axons which caused an increase in the discharge of one or more of the afferents when stimulated with a brief tetanic train were retained for further investigation. In the first experiment, all four criteria were applied to the efferent. In the subsequent three experiments, only the first three criteria were used for identification. The beta efferent from each experiment was classified according to its "fatigue index" and then subjected to a stimulus regime designed to deplete the innervated muscle fibers of their glycogen. At the end of the experiments, the muscle was frozen for histochemical processing. Alternate transverse sections were stained for myosin ATPase and glycogen content.

In each case (N=4), there was evidence of glycogen depletion of extrafusal fibers as well as intrafusal muscle fibers within a number of muscle spindles. It was concluded based on glycogen depletion results that the first three criteria mentioned above were sufficient for identification of fast-conducting skeletofusimotor (β) efferents.

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133.3 EFFECTS OF ALTERED AFFERENT SIGNALS UPON THE LOAD COMPENSATING RESPONSES AT THE HUMAN WRIST. R.J. Jaeger, G.L. Gottlieb, G.C. Agarwal, and A.J. Tahmoush. Depts. of Physiology and Neurology, Rush Medical College, Chicago IL 60612, and Bioengineering Program, Univ. of Ill., Chicago IL 60680.

A variety of electromyographic (EMG) activity has been described between the latency extremes of a tendon-tap reflex and a visual voluntary reaction time. The exact nature of these responses has been very controversial, especially with regard to their classification as reflex, triggered, or voluntary activity. One aspect of these responses that has not yet been fully clarified is their dependence on afferent input.

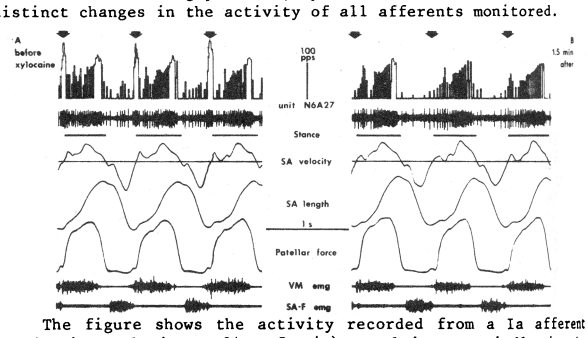
Step torque perturbations were applied to flex or extend the wrists of normal human subjects who were instructed to restore the joint to its initial position as soon as possible. The resulting EMG activity was recorded from the flexor carpi radialis and ulnaris and the extensor carpi radialis. Data were collected under four different conditions: 1) normal, 2) with vibration (50-120 hz) applied to the muscle tendon, 3) with ischemia induced in the forearm, and 4) with a local anaesthetic block of the ulnar nerve at the elbow.

The normal data was subdivided into four intervals: myotatic, late myotatic, postmyotatic, and stabilizing (voluntary) responses; the normal responses are treated in a companion abstract. Vibration reduced the myotatic response to between 10% to 60% of normal; the other responses were unchanged. Ischemia completely abolished the myotatic and late myotatic responses, with subsequent responses enhanced (up to 200% of control) or reduced (down to 25% of control). Ultimately, complete ischemic nerve block was obtained. Local anaesthetic produced varying degrees of block. During complete nerve block, all EMG activity was abolished. During mild nerve block, slight impairment of cutaneous sensations was noted. During moderate nerve block, alterations in cutaneous sensations and differential effects on the EMG were observed. EMG activity before 120 ms was attenuated much more than activity after 120 ms. Also, after complete nerve block by ischemia or local anaesthetic, a differential recovery of the responses was seen.

Evaluating these results in terms of currently accepted actions of the three procedures used and the behavior of the normal responses suggests that each of the load compensating responses has its own unique dependence on afferent information. Since individual motor units have been suggested to participate in one and only one phase of load compensation (Bawa and Tatton, Exp Br Res 37:417, 1979) it is possible that each response is a manifestation of a separate load compensating system. (This work was supported by NSF grant ENG-7608754 and NIH grants NS-00196 and NS-12877)

133.4 EFFECT OF FUSIMOTOR BLOCKADE ON DISCHARGE PATTERNS OF CAT HINDLIMB SPINDLE PRIMARIES DURING WALKING. J.A. Hoffer and G.E. Loeb. Lab. of Neural Control, NINCDS, Bethesda MD 20205.

Conduction in small-diameter nerve fibers can be selectively interrupted by local anesthetic infusion. Using implanted nerve recording cuffs fitted with catheters, we administered 2 ml of 0.3% Xylocaine to the femoral nerve of cats walking on a treadmill. In the next few minutes spindle afferent unit activity (recorded by wire microelectrodes implanted in the fifth lumbar dorsal root ganglion) reflected progressive stages of functional spindle deefferentation. Individual spindle afferents had very characteristic firing patterns; Xylocaine infusion caused marked, distinct changes in the activity of all afferents monitored.



The figure shows the activity recorded from a Ia afferent (conduction velocity = 94 ± 5 m/s) supplying a spindle in the proximal part of sartorius pars medialis (SA-F; hip and knee flexor). Normal activity during walking (A) typically included a few spikes during flexion (in spite of rapid muscle shortening), a sharp burst just prior to foot contact (arrows), and a smooth acceleration during the extension phase of gait. The sharp burst started when the muscle reached minimum length; however, its amplitude was not well correlated with velocity of subsequent stretch. Firing rates during the extension phase appeared to signal primarily muscle length. Xylocaine infusion obliterated the sharp burst (B: arrows) whereas activity during the extension phase appeared unchanged. We infer that during the flexion phase, this spindle was normally under the influence of strong static gamma bias. Dynamic gamma influence appeared slight in this unit.

Spindle afferent activity patterns have so far been too heterogeneous to summarize simply. However, this technique has allowed us to distinguish between activity generated by "passive" stretch and that caused or augmented by fusimotor input.

133.5 CORRELATION ANALYSIS OF MUSCLE SPINDLE AND TENDON ORGAN AFFERENTS IN ACTIVE MUSCLE. Connie E. Osborn and Marc D. Binder. Dept. of Physiol. and Biophys., Univ. of Washington Sch. of Med., Seattle, WA 98195.

While the ensemble behavior of a muscle's tendon organ and spindle populations closely match the parameters of whole muscle activity, individual receptors are more strongly influenced by the state of their local environment. Ia, spindle group II and Ib afferents exhibit their greatest sensitivity in the lowest range of length or tension changes and are each strongly correlated with the activity of a discrete fraction of the muscle's total motor unit populations, presumably those units in the immediate area of the receptor (Binder and Stuart, Prog. Clin. Neurophysiol., 8:72, 1980). Consistent with this view are studies by Windhorst and colleagues which demonstrated that in decerebrate cats Ia afferents innervating neighboring spindles responded to similar tension transients (as measured at the tendon) and showed strong correlations in their discharge. Afferent pairs innervating spindles widely separated in a muscle showed much less, or no correlation at all(Biol. Cyber., 31:71, 1978). We have attempted to test the generality of proximity-dependent correlations by examining the discharge of spindle group II and Ib afferents as well as Ia afferents in both decerebrate cats (N=2) and decapitate cats treated with L-Dopa and nialimide (N=10). Pairs of afferents from medial gastrocnemius were functionally isolated from small dorsal root filaments and the location of the corresponding receptors approximated by gentle probing of the muscle. Tension fluctuations in the muscle occurring either spontaneously or in response to manually or electrically evoked crossed extension, or by electrical stimulation of ventral roots were simultaneously recorded. Of the 49 afferent pairs tested (6 Ia-Ia;7 II-II;8 Ia-Ib;10 Ia-II;12 Ib-II) 14 (28%) showed significant temporal correlations in their discharge pattern. While some of the strongly correlated pairs were in closely apposed areas of muscle, several were not, and in general proximity was a poor predictor of the degree of correlation. We found that tension transients measured at the tendon were sometimes associated with specific patterns of correlated discharge, but not always. Of particular interest were 5 of the 12 Ib-II afferent pairs which displayed negative correlations, suggestive of the "dyad" receptor arrangement described by Marchand et al. (Anat Rec. 169:23, 1971), but again these correlated pairs were not always located close together in the muscle. Our results suggest that even though two receptors may appear to be in close proximity, they may not be responsive to the same set of motor units.(Supported by NINCDS Grants NS 15404 and NS00345, BRS Grant RR 05432 and GM 07108)

133.6 PROJECTIONS OF INDIVIDUAL MEDIAL GASTROCNEMIUS SPINDLE GROUP II AFFERENTS TO TYPE-IDENTIFIED MOTONEURONS. J.E. Zengel, J.B. Munson, J.W. Fleshman and G.W. Sypert. Dept. of Neuroscience, Univ. of Florida, Gainesville, Florida 32610.

Sypert et al (1) have suggested that medial gastrocnemius (MG) group Ia and spindle group II afferents may comprise one continuous functional system, organized in adherance to a size principle. Accordingly, we examined the projections of individual spindle group II afferents to type-identified MG motoneurons using the spike-triggered averaging technique. The results of this study were then compared to those previously described for Ia afferents (2).

Twelve single MG spindle afferent fibers from 9 cats were examined (2). Ninety-five motoneurons were classified into motor types based on muscle unit responses (3): fast twitch, fatigue-sensitive (FF); fast twitch, fatigue-resistant (FR); and slow twitch, fatigue-resistant (S).

Motor unit type:	FF	FR	S	Significance
Connectivity	53%	46%	52%	NS
EPSP amplitude (μV)	22	34	23	NS
Input Resistance (MΩ)	0.6	0.9	1.3	< .001

Mean afferent conduction velocity was 56 m/s. Connectivity was closely related to afferent conduction velocity: fast afferents (> 56 m/s) had high connectivity (66%) while slow afferents (< 56 m/s) had low connectivity (33%), similar to results from a previous study (1). This supports the idea that larger spindle afferents make synaptic contacts with more motoneurons than do smaller afferents. Unlike Ia afferents, the mean amplitude of single fiber EPSPs elicited by spindle group II afferents is unrelated to afferent conduction velocity (25 μV for fast vs. 23 μV for slow afferents), as previously reported (1).

Neither connectivity nor EPSP amplitude appear related to motor unit type, in contrast to Ia-motoneuron projections (2). Furthermore, there was no relation between EPSP amplitude and input resistance (R_N), suggesting an equal density of group II-motoneuron synapses over the range of motoneuron sizes. Overall there was no significant relation between R_N and connectivity; however those cells with the highest R_N (> 1.4 MΩ) had very low connectivity (3/19 = 16%).

The projections of MG Ia and spindle group II afferents to MG motoneurons share many but not all principles of organization.

References: (1) J Neurophysiol 44, 726; (2) Fleshman, et al, J Neurophysiol (in press); (3) J Physiol 234, 723.

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133.7 DO JOINT RECEPTORS AFFECT MOTONEURONE EXCITABILITY IN MAN? K. Robinson, A.J. McComas and A.Y. Belanger, Department of Neurosciences (Room 4U 7), McMaster University Medical Centre, Hamilton, Ontario L8N 3Z5.

It is known that excitability of human soleus motoneurones, as measured by H-reflex testing, is diminished if the foot is dorsiflexed. Experiments have been undertaken to determine whether this effect is due to altered discharge from receptors in the triceps surae muscle and Achilles tendon, on the one hand, or from those in the joint capsule, on the other. The principle of the method has been to compare the result of ankle dorsiflexion with that of lengthening the muscle by displacing the Achilles tendon towards the tibia, the ankle joint being fixed in mid-position. A needle was inserted into the soleus belly and allowed to tilt in front of a protractor as the Achilles tendon was depressed or as the ankle joint was dorsiflexed. The amounts of dorsiflexion and of tendon depression were adjusted to cause the same change in the inclination of the needle; the elongation of the muscle and the subsequent activation of muscle receptors must have been similar in the two situations. Since the depression of the H-reflex was also similar in the two types of manoeuvre, it would appear that any effect of the joint receptors was relatively minor. Control experiments, including the use of local anaesthesia of the skin overlying the Achilles tendon, served to exclude the possibility that cutaneous mechanoreceptors had contributed significantly to the observed results.

134.1 WALKING BEHAVIOUR OF A ROCK LOBSTER ELICITED BY A DOUBLE TREADMILL. F. Clarac* (SPON: R. R. Almon). Lab. de. Neurobiologie, 33120 Arcachon, France.

In the rock lobster, Jasus lalandii, forward or backward walking can be elicited by a treadmill with separate belts for the left and right legs respectively. Belt speed is controlled by two independent motors which can also be coupled. While walking under these conditions, the animal uses mainly its back legs (3, 4 and 5). EMG's were recorded from the basal muscles of these legs (promotor or remotor, levator or depressor).

When both belts move at the same speed, the two sides stay in absolute coordination. If the two belts move at different speeds, contralateral legs continue to walk at the same or at a slightly different step frequency (relative coordination). If the difference between the belt speeds is too great, however, the two sides of the animal become completely uncoordinated. It is also possible to evoke forward walking on one side and backward walking on the other when the belts move in opposite directions. These contralateral variabilities observed with the treadmill can also be recorded during free walking when the animal is involved in complex motor sequences such as turning or changing the direction of walking.

It can be concluded, therefore, that in the crustacean walking system, although the two sides can operate independently, there must also be functional couplings which maintain a certain degree of bilateral coordination. The main factors involved in this coordination are the durations of returnstroke and powerstroke and the amplitude of the stride.

134.2 INNERVATION OF FLIGHT MUSCLES IN THE TSETSE FLY. David G. King. Dept. of Anatomy, Sch. of Medicine, Southern Illinois University, Carbondale, IL 62901.

Among the best understood aspects of the thoracic nervous system of Muscoid flies are the motor innervation of the dorsal longitudinal muscle and the interneuronal initiation of jumping and flight. The six dorsal longitudinal muscle fibers are innervated by five motor neurons. These neurons are indirectly activated by the giant axon descending in the cervical connective from the brain. This same interneuronal giant axon also activates, by direct electrical connection, the motor neuron which innervates the jump muscle, or tergotrochanteral muscle (Tanouye & Wyman 1980, J. Neurophysiol. 44:405-421). Mutants of Drosophila are now known which affect several aspects of this system (J.B.Thomas 1980, Neurosci. Abs. 6:742). Evolutionary modification of this system in other flies may also be expected.

The similarity of this system in the flies so far studied (including Drosophila, Musca and Calliphora, representing three families) suggests a very conservative evolutionary history. However in the related tsetse fly, Glossina morsitans, previous workers have reported two notable differences: absence of the tergotrochanteral muscle (Smart 1959, Smithson. Misc. Coll. 139: 331-364) and four rather than five large axons in the nerve which innervates the dorsal longitudinal muscle (Anderson 1978, J. Morph. 155:19-34). In the present study the nerve to the dorsal longitudinal muscle was found to contain five distinctly large axons, rather than the four reported by Anderson. Thus in this respect the tsetse is in fact similar to other flies. However, the absence of the tergotrochanteral muscle was confirmed. In mutants of Drosophila which lack this muscle as adults, the muscle nonetheless develops in the pupa and then degenerates; the motor axon which innervated the degenerated muscle remains present in the adult. In contrast the tsetse shows no sign of this muscle ever forming. Furthermore, careful anatomical analysis of serial sections yielded no evidence for any component of the entire giant fiber pathway. Neither the descending cervical giant fiber, the peripherally synapsing interneuron, nor the tergotrochanteral motor axon was found. If any of these neurons is present, it does not display the characteristic defining morphology described by King & Wyman (1980, J. Neurocytol. 9:753-770).

It will be of considerable interest to observe the physiological activation of the dorsal longitudinal muscle in the tsetse fly, which apparently lacks the giant fiber pathway.

(Specimens were provided by the Tsetse Fly Research Laboratory, Langford, Bristol, England.)

134.3 IDENTIFICATION OF FLIGHT NEURONS IN THE COCKROACH PERIPLANETA AMERICANA. R.E. Ritzmann, C.R. Fourtner and A.J. Pollack.* Dept. of Biology, Case Western Reserve Univ., Cleveland, OH 44106 and Department of Biological Sciences, SUNY at Buffalo, Buffalo, N.Y.

Recent studies indicate that flight in P. americana can be reliably initiated by activity in the dorsal giant interneurons (dGIs) (Ritzmann et. al, Science, 210:443, 1980). The identifiable nature of dGIs makes this a potentially advantageous preparation for studying control of oscillatory behaviors. However, we must first increase our understanding of the neural flight apparatus that is involved.

We have begun to identify neurons in the meso and metathoracic ganglia (T_2 and T_3) that are active during flight. Neurons were impaled with glass microelectrodes filled with Lucifer Yellow. Flight was initiated by a brief wind puff or by stimulating a dGI. The flight rhythm was monitored with EMG electrodes placed in known wing muscles (Fourtner and Randall, Neurosci. 6:369, 1980). Neurons displaying depolarizations correlated with the flight rhythm were injected with Lucifer Yellow. The ganglion was then fixed and cleared for observation with a fluorescence microscope.

We have located several flight motor neurons and interneurons in both T_2 and T_3. Cell bodies of motor neurons tend to be located in the anterior region of the ganglion. In most cases axons exit the ganglion in nerves 3a, 4 and 6. Within the neuropile of T_2 and T_3 fibers of flight motor neurons tend to project medially up to but not beyond the midline. Other fibers project into the posterior region of the ganglion. We have also been able to identify the muscles innervated by some of these neurons by dissecting fine branches of motor nerves as they enter known flight muscles, and backfilling these branches with Lucifer Yellow. The resulting filled neurons can then be compared with intracellularly filled neurons. For example, two homologous flight motor neurons have been impaled in T_2 and T_3 from which activity synchronous with wing depressor muscles was recorded. They have been identified in backfills as innervating wing depressor muscles 135c (T_2) and 177c (T_3).

In addition to motor neurons, we have also located several interneurons that are active during flight. Some of these cells are restricted to one ganglion, but most have axons in interganglionic connectives. These may be involved in coordinating flight rhythms between ganglia.

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134.4 INTRACELLULAR ANALYSIS OF THE LOCUST FLIGHT SYSTEM. R.M. Robertson and K.G. Pearson, Department of Physiology, University of Alberta, Edmonton, Canada.

The numerous studies by D.M. Wilson and his colleagues during the 1960s on the flight system of the locust played an important part in the development of the concept of central programming of motor activity. More recently, however, there has been little interest in this system due to the apparent difficulty of obtaining information about the cellular events underlying the generation of the flight rhythm. In this presentation, we describe a simple preparation which readily allows intracellular recording from thoracic interneurons and motoneurons during flight, and the identification of interneurons providing some of the synaptic drive to flight motoneurons.

During flight, there are large amplitude oscillations (15 to 25 mV) in the membrane potential in the main neuropile processes of flight motoneurons, but the form of these oscillations is not identical in elevator and depressor motoneurons. The basic flight cycle consists of an elevator-depressor-pause sequence with a rapid depolarization of depressors corresponding to a rapid hyperpolarization of elevators. Depolarization of elevators is relatively slow. Elevator and depressor motoneurons also respond differently to stimuli which initiate and maintain flight (e.g., wind on the head). Elevators are strongly depolarized and most depressors are hyperpolarized.

By simultaneously recording intracellularly from interneurons and flight motoneurons, we have succeeded in identifying interneurons which make monosynaptic connections to the flight motoneurons and provide some of the tonic and phasic synaptic input to these motoneurons during sustained flight. We have also identified a large number (>20) of other interneurons whose activity is strongly modulated during flight but whose output connections remain to be determined. The striking feature of these interneurons is the heterogeneity in their structure and their electrical properties. We conclude that the patterning of locust flight activity involves the interaction of large numbers of interneurons, and is considerably more complex than suggested by all previous models of this system.

134.5 SEGMENTAL DISTRIBUTION OF VENTILATORY CONTROL MECHANISMS IN AN AQUATIC INSECT. S. C. Kinnamon*, G. K. Fitch*, K. L. Bellah* and A. E. Kammer (SPON: P. Kelly). Division of Biology, Kansas State University, Manhattan, KS 66506.

Rhythmic movements involving segmentally arranged groups of muscles are controlled by a series of central oscillators coupled by coordinating interneurons. We expect such serially homologous local oscillators to have similar properties. In Cordylalus cornutus, an insect with 7 pairs of segmentally arranged abdominal gills, this expection is not fulfilled. The abdominal ganglia differ in their ability to generate rhythmic output when isolated and in their responses to stimuli.

Selective cutting experiments show that both segments 2 and 3 possess oscillators that are active in isolation from other segments and in the absence of sensory input. These two oscillators when isolated produce rhythmic bursts of impulses in the motor root supplying the gill retractor muscle, but their frequencies often differ. Under our experimental conditions other ganglia will not generate a rhythm unless connected to ganglion 2 or 3.

Hypoxia (8% O_2) increases ventilatory frequency in an animal with an intact nervous system. This effect is readily reversible upon return to normoxia. When ganglia are isolated by cutting roots and connectives, neither ganglion 2 nor 3 respond to hypoxia. When the ventral nerve cord is cut between 1 and 2, ganglia 2 through 7 fail to respond to hypoxia, but ganglia 1 and 2 isolated together exhibit a hypoxic-mediated frequency increase from 28 to 39 beats/min, similar to that of intact preparations. These results suggest that the ventilatory response to hypoxia is mediated by receptors in ganglion 1 influencing the oscillator in ganglion 2 and perhaps 3.

Frequency modulation by sensory input was examined by stimulating a ventral nerve that contains primarily afferents from the gill. In isolated ganglion 3 or ganglion 3 plus any other ganglia, a single electrical stimulus to the sensory nerve in segment 3 advances the next motor burst in all ganglia connected to 3, and the oscillator in 3 is reset. The rhythm can be driven at a frequency greater than the unstimulated frequency by single pulses delivered rhythmically. Similar stimulation of the homologous sensory nerve in any other segment does not reset the rhythm or modulate its frequency.

Octopamine dramatically increases the ventilatory frequency of preparations with an exposed but intact ventral nerve cord. Preliminary results suggest that octopamine also has different effects on the diverse abdominal ganglia of this insect. These results suggest that various inputs act via different neural pathways and on different oscillators to modulate the ventilatory rhythm. (Supported by NSF Grant BNS 79-23096.)

134.6 INTERACTIONS OF VENTILATORY RHYTHM GENERATORS AND SENSORY MODULATION OF THEIR OUTPUTS. G. K. Fitch* and A. E. Kammer. Division of Biology, Kansas State University, Manhattan, KS 66506.

Abdominal segments 1 through 7 in the aquatic insect Corydalus cornutus have pairs of gills which are retracted in a specific metachronal sequence with a frequency which remains relatively constant over short periods of time. The ventral root on either side of an abdominal ganglion has two anatomically distinct branches, one carrying motor output to the corresponding gill and one carrying sensory information to the ganglion. It has been shown previously that each gill retraction is caused by a burst of spikes which travel to the gill retractor muscle via a single motor neuron in the motor branch of the ventral root. Extracellular recordings from this branch allow monitoring of the patterned output.

Ganglia of abdominal segments 2 and 3 are each capable of generating the ventilatory motor pattern when isolated from all other parts of the CNS. In addition, neither requires sensory input to produce the rhythm. During normal ventilation the oscillator in ganglion 3 is the principal one responsible for motor output. This result is intuitively consistent with the gill retraction sequence, which is segment 3, then 4, 5, 6, 7, 2, and 1.

Electrical stimulation of the sensory branch of ganglion 3 has dramatic effects. A single pulse delivered during the last 70% of the expected interval between motor bursts will advance the next burst. The interburst interval can be halved if the stimulus is properly timed. Stimuli delivered during the first 30% of the expected interburst interval have no effect on the timing of the next burst. The lag between a stimulus and the onset of the burst advanced by it becomes slightly less as the stimulus is delivered later in the expected interburst interval.

Analysis of successive interburst intervals suggests that such stimuli reset the oscillator rather than merely advance one burst in the motor neuron. The rhythm can be driven at frequencies greater than the unstimulated frequency by single pulses delivered in a rhythmic fashion. This effect cannot be produced if the stimulation frequency exceeds the unstimulated ventilation frequency by more than about 20%.

The gills on segments 1 and 2 sometimes miss one beat entirely. Data from this relative coordination between segments can be used to evaluate models of pattern generators, in an effort to clarify the mechanisms by which rhythmic behaviors are coordinated.

Supported by NSF grant BNS 79-23096.

134.7 PATTERNED SPIKE TRAIN ANALYSIS AND SPIKE-TRIGGERED AVERAGING OF CRAYFISH POSTURAL MOTONEURON ACTIVITY. W.G. Tatton and S.K. Wernham*, (Spon: J.K. Stevens) Playfair Neuroscience Unit, University of Toronto and Division of Medical Physiology, University of Calgary.

Existing techniques for spike train analysis or spike-triggered averaging treat each action potential in the presynaptic train as an indistinquishable event despite the fact many neurons fire in bursts or other stereotyped patterns of action potentials. We have explored patterned spike train analysis using the motoneurons in the abdominal postural system of the crayfish. Suction electrode recordings were made from the axons of the six flexor motoneurons simultaneously with intracellular recordings from single fibers of the polyinnervated slow postural muscles. Computer programs allowed specific patterns to be extracted from the motoneuron spike trains and specified spikes within the patterns to serve as point events in the analysis.

The EJP's and IJP's for each of the six motoneurons were spike-trigger averaged from the intracellular records in order to determine their magnitude and distribution to individual muscle fibers using conventional techniques. Facilitation in the EJP's generated by the F6 motoneuron was compared for naturally-occurring F6 burst patterns of two to four spikes. Facilitation ratios and the calculation of half wave areas for the EJP's revealed that patterns of four spikes with interspike intervals of 7.0 msec resulted in at least three times the increase in EJP size for the 4th spike as compared to 10.0 msec interspike intervals. The patterned analysis also provided a means of assessing the contribution of temporal summation of EJP's generated by different motoneurons to the apparent facilitation. This was shown to contribute minimally, if at all, to the increase in EJP size. The non-linear increase in EJP size with pattern length and small decreases in interspike intervals was entirely masked by conventional single spike averaging.

Intracellular recordings have established that F6 motoneurons make weak reciprocal excitatory connections with the contralateral F6 in the same ganglion. Due to the bursting patterns of the motoneurons, the firing probability alterations indicative of these connections are not detectable in cross-correlation histograms constructed for F6-F6 extracellular spike trains. Patterned analysis "extracted" the crosscorrelation peak for the F6-F6 connection and showed that the F6 firing was influenced most strongly by the 3rd and 4th spikes in the patterned bursts. These results illustrate that conventional spike train analysis and spike-triggered averaging techniques may fail to accurately reflect the "strength" or even to reveal the existence of neural connections. (Supported by MRC grant 5218)

134.8 INTRACELLULARLY OBSERVED COUPLING AMONG MOTOR NEURONS: A BASIS FOR PATTERN FORMATION. J.H. Koenig and Kazuo Ikeda, City of Hope Research Institute, Duarte, CA. 91010.

A salient characteristic of the in-flight firing pattern produced by the dorsal longitudinal flight muscle (DLM) fibers of Drosophila melanogaster is that no fiber fires immediately after any other. It has been suggested that this "exclusion" phenomenon is due to interactions between the DLM motor neurons (MN) but disagreement exists on what type of interactions might produce this effect. Wyman (1969) proposed that it is caused by mutual inhibition among the motor neurons, while Koenig and Ikeda (1980) suggested that it is due primarily to an "excitatory" effect which synchronizes the firing times of 2 MN's when they should have fired one right after another. In order to resolve this question, intracellular recordings were made from the DLM motor neurons as described below.

In the DLM, a firing pattern among the fibers which is very similar to the output pattern that occurs during flight can be induced by temperature (26°C) in the single-gene mutant, $shibire^{ts1}$ (Koenig and Ikeda, 1980). While monitoring this temperature induced firing pattern by simultaneous intracellular recordings from the DLM fibers, intracellular recordings were made from the motor neurons (which singly innervate these fibers). The motor neuron was identified by its corresponding activity to one of the DLM fibers, and by horseradish peroxidase injection after the recording.

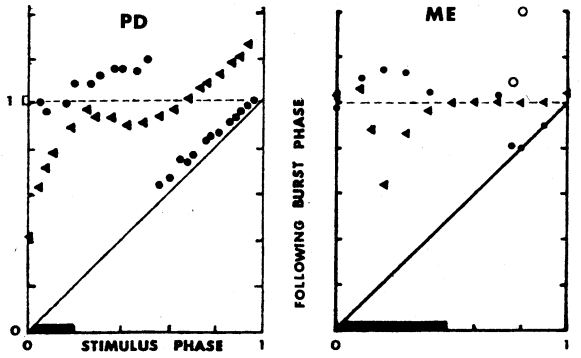
A DLM motor neuron response consisted of a slowly rising prepotential followed by a spike. The motor neuron recording revealed an apparent electrical coupling between the DLM motor neurons. Thus, a spike in another MN (observed at the muscle level) appeared as an attenuated spike in the intracellularly recorded MN. Although the coupling was weak, it was possible to strongly stimulate one DLM motor neuron and cause another DLM motor neuron to fire. This coupling was stronger between certain pairs of neurons.

The data supports the suggestion of Koenig and Ikeda (1980) that a weak electrical coupling between motor neurons whose spikes are preceded by a slowly rising prepotential, could cause the "exclusion" phenomenon observed in the DLM pattern by synchronizing the firing times of 2 MN's which would have fired one right after another. (Supported by USPHS, NIH Grant NS-07442).

134.9 PERTURBATION RESPONSES OF ENDOGENOUS AND NETWORK-DERIVED BURSTING PATTERNS. D. K. Hartline, D. V. Gassie, Jr., and C. D. Sirchia, Bekesy Lab, University of Hawaii, Honolulu, HI.

Responses to perturbations are shown for an endogenously bursting neuron (PD, lobster stomatogastric ganglion) and two computer generated network models, whose bursting is an emergent property of the network (two-cell reciprocal inhibition, RI, and 2-cell mutual excitation, ME). A continuous-output (non-spiking) model was used (Hartline, Roberts & Baker, Neurosci. Abstr. 6: 406, 1980). The figure shows timing of the burst following the perturbation for brief depolarizing (●) and hyperpolarizing (▲) pulses to the PD (left; 5 nA, 50 msec) and ME net (right: perturbation area = 1.5; ρ = 1/3; ξ = .2; γ = .7, Hartline et al. loc. cit.). Moderate depolarizing pulses at early phase (with respect to burst onset) hasten burst terminations and promote earlier occurrence of the next in both models and some PD cases. As stimulus phase increases, a delay followed by an advance is typical. Moderate hyperpolarizing pulses at early phase which "terminate" a burst cause timing advances (otherwise often a delay). In models without "rebound", midphase pulses have little effect, whereas in PD cells and models with rebound a "paradoxical" region of timing advance may intervene before the typical late-phase delay. Similarities are apparent between the two network models and the PD cells, but details vary. Models and burster share other features of a regenerative process, such as an "N"-shaped I-O curve in an "output-clamped" situation.

PD cell responses are similar to those of other endogenous bursters (Pinsker, J. Neurophysiol. 40: 527, 1977). Similarities in responses of the two network models indicate that care is needed in using such behavior to distinguish mechanisms for burst origin. Supported by NIH NS 15314.



134.10 DESCRIPTION OF IDENTIFIED SIPHON MOTONEURON ACTIVITY IN INTACT APLYSIA BY SPIKE TRAIN ANALYSIS. Lewis Eberly and Harold Pinsker, Marine Biomedical Institute, Depts. of Physiology & Psychiatry, UTMB, Galveston, Texas 77550.

We have utilized quantitative techniques to examine neuronal activity accompanying simple behavioral acts in the marine mollusc, Aplysia californica. Using a combined approach involving chronic recordings from freely behaving animals, then intracellular recordings from identified neurons, we have described the activity of identified units in the intact animal under different behavioral conditions.

The experimental approach involves simultaneously monitoring behavioral events in the siphon and neuronal activity in the siphon nerve of intact animals after implantation of a two-channel extracellular cuff electrode. After monitoring spontaneous activity and the responses to stimuli, animals are surgically reduced for intracellular identification of siphon motoneurons. A computerized spike train analysis is used to characterize the population of efferent units in the siphon nerve based on spike amplitudes and conduction velocities. Since the same unitary population can be recognized in the reduced preparation where the intracellular identifications are made, we are able to identify siphon motoneurons in samples from the intact animal.

During spontaneous background activity in the intact animal, the LBS cells typically fire at a low rate, cells LDS1, LDS2 and L7 fire more rapidly and LDS3 is usually silent. Short bursts of neuronal activity, not associated with overt siphon movements, frequently involve firing in only a single neuron. After surgical reduction for intracellular experiments, fewer individual units are spontaneously active. The remaining activity is more homogeneous and rhythmic with fewer short bursts in individual units. Spontaneously occuring Interneuron II (INT II) bursts, normally characterized by a brisk pattern of firing in the intact animal, become frequently prolonged and less intense after dissection. Furthermore, abnormally heightened postimulus activity often occurs in the reduced preparation, far outlasting the stimulus (20-30 sec).

Since the activity of identified neurons in the reduced preparation can be substantially different from that in the intact animal, a neuronal event described in the reduced preparation may not be relevant to the natural behavior in the intact animal. We are currently extending the analysis to describe other stimulus effects, including the involvement of individual neurons in response to stimuli of different intensities and the successive presentation of weak stimuli. (Supported by NSF grants 77-25584 and 80-16421 and NIH grants 16087 and 11255).

134.11 ISOLATION INDUCED CHANGES IN HELISOMA BUCCAL GANGLION NEURONAL ACTIVITY. V.J.Waks* and C.H.F.Rowell (SPON: G.Wesley Davis). Dept. of Zoology, UCB, Berkeley, Cal. 94720.

The effect of isolation on the firing pattern of selected buccal ganglion neurons was examined in the freshwater snail, Helisoma trivolvis. Representative feeding network cells, cell 4 and protractor motorneuron P19 were recorded intracellularly before and after section of the cerebrobuccal connectives (CBCs), nerve trunks connecting the buccal ganglion to both the periphery and the rest of the central nervous system. Firing patterns of the two cells were then examined for changes in interburst interval(IBI), and spike rate(the number of spikes produced/time).

CBC section consistently resulted in a statistically significant decrease in P19 IBI, accompanied by a reduction in variability of IBI values. Reported spontaneous rhythmicity in isolated Helisoma buccal mass preparations is then commensurate with increased burst rate in P19 and more rapid alternation of bursts in protractor/retractor MN pools. The induction of spontaneous rhythmic network output by nerve section suggests that the expression of bursting is regulated or inhibited by descending higher order control elements.While CBC section significantly changed the overall patterning of P19 bursts, this effect was not accompanied by the production of more spikes/time in recorded cells.

The activity of cell 4, which innervates the salivary glands, was unaffected by isolation at the levels of patterning or number of spikes produced/time. The absence of overt change in cell 4 activity does not preclude the existence of subtle, subthreshold responses and supports previous reports of a highly labile functional relationship of this cell within the feeding network.

134.12 ELECTROTONIC STRUCTURE & SYNAPTIC INTEGRATION IN IDENTIFIED NEURONS. D.H. Edwards. Dept. of Zoology, U. Calif., Davis, CA 95616.

The integrative properties of a neuron can only be understood in the context of a description of the electrotonic structure of the cell. My colleagues and I have developed a set of techniques for describing the electrotonic structure of neurons, and I have used these techniques to analyse the synaptic integrative properties of members of a set of identified neurons in the crayfish, the fast flexor motorneurons that mediate the tail flip.

The fast flexor inhibitor motor neuron has a large cell body that is connected by a thin neurite to a large, contralaterally projecting integrating segment. The integrating segment gives rise to ipsilateral and contralateral dendrites, and a large contralateral axon. Analysis of the electrotonic structure of this neuron suggests that the large cell body and the large axon act as current sinks at either end of the integrating segment. The analysis predicts that inputs on dendrites ipsilateral to the soma will evoke large, fast-rising EPSPs in the soma and smaller, more slowly rising EPSPs near the axon. EPSPs arising from inputs on contralateral dendrites will have the opposite relation at the two sites. This prediction has been verified for the responses to identified presynaptic neurons. Another prediction, that the contralateral inputs (which evoke the smaller EPSPs at the soma) will more readily fire the cell, is currently being tested.

A similar analysis for a fast flexor excitor motor neuron predicts that the same input addressed to different dendrites will evoke the same pattern of EPSPs at the soma, integrating segment and axon. In this cell, ipsilateral and contralateral dendrites converge on the same region of the integrating segment. The analysis suggests that synaptic currents will be modified in similar ways during their passage inward along different dendrites, and will thereby give rise to a similar pattern of potentials following convergence at the integrating segment. This prediction is currently being tested.

This work is supported by USPHS Grant NS12295 and NSF Grant BNS 78-10516.

134.13 EVIDENCE FOR NON-MODULATORY OCTOPAMINERGIC TRANSMISSION MEDIATING LUMINESCENCE IN LARVAL FIREFLIES. T. A. Christensen and A. D. Carlson*. Department of Neurobiology & Behavior, S.U.N.Y., Stony Brook, New York 11794.

The terminal abdominal ganglion (A_8) of larval Photuris versicolor fireflies contains a consistently identifiable cluster of four dorso-medial somata, each approximately 25 μm in diameter. A primary neurite arises from each soma and bifurcates only once within the neuropil sending an axon through both the right and left ventro-lateral nerve roots. The four axon pairs travel within their respective roots to the periphery where they terminate within the photogenic tissue of the paired larval lanterns, located on the lateral margins of the eighth abdominal sternite. The neurons from which these four symmetrical pairs of axons arise therefore fit the description of true dorsal unpaired median (DUM) neurons.

Antidromic stimulation of the DUM neurons through either nerve root causes the lantern innervated by the contralateral root to glow. Moreover, both lanterns glow synchronously in response to intracellular stimulation of a single DUM neuron. In either stimulating condition, a weak single shock stimulus is sufficient to elicit a detectable glow response. However, a brightening of the glow response is always associated with increased firing frequency as recorded from the DUM neurons.

The effect of DUM neuron stimulation is mimicked by superfusion of octopamine (OA) over the lanterns at a threshold concentration between 10^{-5} and 10^{-6} M. Furthermore, the receptors mediating the glow response are optimally sensitive to OA and its N-methylated derivative, synephrine. The receptors resemble those of the α-adrenergic class in that the glow response is blocked by chlorpromazine but not by β-adrenergic antagonists.

Treatment with d-amphetamine, reserpine or denervation of the lanterns two days prior to OA application does not diminish the glow response. Furthermore, high concentrations of the putative insect neurotransmitters glutamate, ACh, 5-HT and GABA are unable to induce glowing.

In a series of preliminary experiments performed in collaboration with R. E. McCaman, whole terminal abdominal ganglia as well as isolated and pooled DUM somata were assayed for their OA content. Between 0.1 and 0.2 pmol of OA was found within each ganglion and at least part of this OA was localized within the DUM somata.

Here we report the identification of four OA-containing DUM neurons that bilaterally innervate the two larval lanterns. This is the first report of octopaminergic DUM neurons in Coleopteran insects, and demonstrates a function for DUM neurons in the direct activation of firefly photogenic tissue.

134.14 GENETIC DISSECTION OF THE DROSOPHILA GIANT AXON PATHWAY. Gadi Benshalom* and Daniel Dagan*. (Spon: Chien-Ping Ko). Section on Functional Neuroanatomy, NINCDS, NIH, Bethesda, MD 20205 and Faculty of Medicine, Technion, Haifa, Israel.

A genetic dissection of the Drosophila giant axon pathway was done to clarify some obscure features of this neural pathway, through which electrical stimulation of the cervical connective evokes electrogenic responses of the thoracic, indirect flight muscles. Gynandromorph flies mosaic for the $para^{ts}$ mutation ($+ w^{vc} +/y\ w\ para^{ts}//y\ w\ para^{ts}/0$), with an external y w mutant phenotype involving only their entire thorax, or a mirror image half of their head, or thorax, or both head and thorax, were selected for study. The recessive, x-linked, temperature-sensitive paralytic mutation, $para^{ts}$ causes paralysis of mutant flies at elevated temperature. A neural conduction block, upon elevation of temperature, was expected only in the neural components of these gynandromorph flies, which originate from mutant, paralytic regions. Intracellular recordings of the neurally-evoked electrical responses of the thoracic indirect flight muscles revealed a functional coupling between the left and right giant axon pathways of the Drosphila: all ipsilateral and contralateral muscle fibers of the dorsolongitudinal muscles (DLMs) of any wild type or $para^{ts}$ fly responded at room temperature to cervical stimulation with a common, sharply-defined excitation threshold; in $para^{ts}$ mutant flies, all the neurally evoked DLM fiber responses were always blocked simultaneously at elevated temperature and recovered simultaneously upon cooling. This functional coupling between the giant axon pathways could not be abolished at restricted temperatures in any of the bilateral gynandromorph flies. On the contrary, the DLM fibers on both sides of each gynandromorph fly always responded uniformly, mostly as in normal but sometimes as in $para^{ts}$ flies. Picrotoxin (10^{-4} M), induces spontaneous activity of neural origin in both DLMs and dorsoventral muscles (DVMs). In addition to the normal response to cervical stimulation, a delayed evoked response is recorded coincidently from fibers of both muscle systems. Analysis of the interaction between these spontaneous and evoked electrogenic responses in wild type flies, supports the notion that both DVMs and DLMs share a common activating pathway. Applying picrotoxin to the gynandromorph flies exposed the existence of additional neuronal elements, that can activate flight muscles in response to cervical stimulation. Intracellular recordings from gynandromorph flies of the $para^{ts}$ mutation exhibited a poor correlation between the mosaic external phenotype of these flies and the genotype of certain neural pathways. Nevertheless, these mosaic flies were found useful to confirm the presence of at least one interneuron in the giant axon pathway activating the DVMs.

135.1 THE ORGANIZATION OF LIMBIC CORTICAL EFFERENTS IN THE ALVEUS-FIMBRIA-FORNIX COMPLEX OF THE MONKEY. Steven Demeter and Gary W. Van Hoesen. Department of Neurology, Division of Behavioral Neurology, and Anatomy, University of Iowa College of Medicine, Iowa City, IA 52242.

Tritium labeled amino acids (leucine, lysine, proline) were injected into the hippocampus, the entorhinal (Brodmann area 28) and posterior parahippocampal (Bonin and Bailey areas TF and TH) cortices and other limbic areas in 17 monkeys (Macaca mulatta). After seven day survivals, the brains were processed in the standard manner for autoradiographic tracing.

Labeled axons arising from allocortical regions coursed through the fimbria to reach the splenium. In contrast, axons from the proisocortical posterior parahippocampal gyrus coursed through the alveus to reach the splenium, mostly without traversing the fimbria. Periallocortical fibers from the entorhinal cortex took an intermediate course, overlapping with adjacent fibers. This relationship was maintained in the splenial portion of the alveus-fimbria-fornix complex. Allocortical fibers from the fimbria assumed the most lateral position. Axons from proisocortical regions coursed most medially. Periallocortical axons maintained their intermediate position.

Surprisingly, few fibers from allocortical and periallocortical regions decussated in the hippocampal commissure. Instead, these fibers crossed the midline throughout the body of fornix. Decussating as well as ipsilateral fibers maintained their relative positions throughout their course in the fornix. The hippocampal commissure was observed to be primarily composed of proisocortical posterior parahippocampal axons, rendering this designation inaccurate.

135.2 FRONTAL LOBE AFFERENT INPUT TO AREA 6 IN THE RHESUS MONKEY. H. Barbas and D.N. Pandya. Harvard Neurol. Unit, Beth Israel Hospital, Boston, and V.A. Medical Center, Bedford, MA.

There are at least two cytoarchitectonically distinguishable subregions within area 6 in the rhesus monkey. A dorsal region lies above the spur of the arcuate sulcus and extends medially to the cingulate sulcus. This region lacks a granular layer IV, and its medium and large size pyramidal cells in layers III and V form a central band. The cells show a columnar arrangement throughout the cortical layers. A ventral area 6 region lies below the spur of the arcuate sulcus. Unlike the dorsal area 6, ventral 6 has incipient layers II and IV, small cells in layer III, only medium size pyramidal cells in layer V, and lacks a columnar organization.

Analysis of the sources of ipsilateral frontal lobe afferents to area 6, studied with horseradish peroxidase (HRP), revealed that the two subregions of area 6 have different afferent connections. Thus ventral 6 receives projections from both cytoarchitectonic subregions of area 6, the ventral half of the precentral gyrus (area 4), the prefrontal cortex (areas 8, 12, 46, 10), the depths of the cingulate sulcus (area 24d), and the frontal operculum. In contrast, the sources of input to dorsal area 6 are more restricted and are confined primarily to dorsal parts of areas 6 and 4. To demonstrate further these differences, the number of HRP labeled cells within a specific frontal cortical region was expressed as a percentage of the total number of labeled cortical cells in the frontal lobe (opercular, motor, premotor, and prefrontal cortices). Of all the neurons in the frontal lobe projecting to ventral area 6, 53% were in ventral, medial, and dorsal parts of area 6, and 17% were in area 4. The remaining HRP labeled neurons were in the prefrontal cortex, in the depths of the cingulate sulcus, and in the frontal operculum. In contrast, 91% of the cells projecting to dorsal area 6 were in adjoining regions of dorsal and medial area 6, and 5% were in area 4. A small percentage (4%) of HRP labeled neurons was in ventral area 6, and in areas 9 and 8.

The results suggest that the two cytoarchitectonic subregions of area 6 also receive input from divergent sources: Dorsal area 6, which lacks a granular layer IV, receives input mostly from neighboring agranular premotor (area 6) and motor cortices. On the other hand, ventral area 6, having incipient layer IV, receives input not only from motor and premotor regions, but also from prefrontal and opercular granular regions.

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135.3 THALAMIC PROJECTIONS OF AREA 6 IN THE DOG. S.T. Sakai, D. Tanaka Jr., G.B. Stanton & O.I. Weeks*. Dept. of Anatomy, Michigan State University, East Lansing, Michigan 48824 & Dept. of Anatomy, Howard University, Washington, D.C. 20059.

Based on electrophysiological findings, the medial one-third of the anterior sigmoid gyrus (ASG) in the dog has been identified as the premotor area while the region located immediately lateral to it has been identified as the supplementary motor cortex (MII) (Gorska, 1974). The purpose of this study was to investigate the cytoarchitecture and thalamic connectivity of these two cortical areas. The cytoarchitecture was defined by Nissl stains and thalamic projections were traced with the horseradish peroxidase (HRP) method. Thalamic subdivisions were defined both by Nissl stains and by acetylthiocholinesterase histochemistry.

Based on cytoarchitectonic analysis, two cortical areas were identified on the ASG corresponding to the functional subdivisions defined by Gorska. The cortical region occupying the medial one-third of the ASG most notably contained a wide layer III composed of small and medium pyramidal cells. Further the demarcation between layer IIIc and layer Va was indistinct. This region corresponds cytoarchitectonically to area 6aα of Hassler and Muhs-Clement (1964) in the cat. Following HRP injections confined to this area, clusters of labelled cells were found in a dorsoventral band in the ventral anterior (VA), ventral lateral (VL), ventral medial (VM) and lateral part of the mediodorsal (MD) nuclei. In addition, HRP positive neurons were seen in the central lateral (CL), paracentral (Pc) and central medial (CeM) nuclei. In contrast, the cortical region identified as MII by Gorska was characterized cytoarchitectonically by a clear strip between layers Vb and VI and the presence of large, medium and small pyramidal cells in layer V. Layer III contained medium and small cells loosely arranged in clusters. This area corresponds cytoarchitectonically to area 6a β of Hassler and Muhs-Clement in the cat. After HRP injections into this area, labelled thalamic cells were also distributed in a dorsoventral band but were located more sparsely through VA, VL, VM and lateral MD. It appeared that this band was partially coincident with the HRP labelled band seen following the medial ASG injection. The intralaminar nuclei, CL, Pc and CeM, also contained HRP positive neurons. In summary, the two functional and cytoarchitectonic subdivisions of area 6 in the dog appear to receive afferents arising from similar thalamic nuclei.

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135.4 THE FASTIGIO-VENTROMEDIAL THALAMIC-CORTICAL PROJECTION. J.P. Roy*, J. Hada* and M. Steriade, Lab. Neurophysiol., Dept. Physiol., Fac. Med., Laval Univ., Québec, Canada.

The cerebellar inputs and cortical projections of thalamic neurons were investigated in cats acutely prepared under ketamine anesthesia, by recording extracellularly single units in the lateral part of ventralis anterior-ventralis lateralis (VA-VL), medial paralaminar part of the VL (VLpl), centralis lateralis-paracentralis (CL-Pc) and ventralis medialis (VM) nuclei. Out of 137 elements activated orthodromically from one of the three cerebellar nuclei, 86 neurons could also be antidromically invaded from cortical areas 4, 6, 8 & 5. A first component of the cerebello-cortical system originates in the interpositus cerebellar nucleus and is relayed by neurons mainly located in the VL that project to areas 4 and 6. A second component consists of afferents from the dentate nucleus; they activate neurons in both VA-VL and CL-Pc (and, to a lesser degree, VLpl cells) that project to areas 6, 4 & 5. Finally, the majority of fastigio-thalamic axons excite VM cells that project to medial parts of areas 6 and 8.

In addition to the already known cerebello-cortical projections relayed in the VA-VL complex, these data provide evidence at a unitary level of cerebellar inputs to cortically projecting CL-Pc and VM nuclei. In particular, the fastigial → VM → areas 6 & 8 projection seems distinct from the interpositus → VA-VL & CL-Pc → areas 4 & 6 projection: the slower conduction velocity in the VM → cortical path (≃7 m/sec) matches the longer latency of fastigial-evoked discharges in VM neurons (median 2.4 msec) compared to that of interpositus-evoked discharges in VA-VL and CL-Pc neurons (median: 1.7 msec and 1.8 msec, respectively). These differences revealed by electrophysiological analyses are further stressed by the predominant or even exclusive projections from the VM to the cortical layer I of cat, as indicated by our electrophysiological and HRP anterograde labeling experiments (Glenn et al., this volume). The cerebello-cortical operations in motor control and the fastigial diffuse influence on the electrical activity of the neocortex should be viewed by taking into account this superficial fastigio-VM-cortical projection.

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135.5 RECIPROCAL PROJECTIONS BETWEEN THE VENTROMEDIAL THALAMUS AND PRECRUCIATE AREAS 6 AND 8 IN CAT. L.L. Glenn, J. Hada*, J.P. Roy, M. Deschênes*, and M. Steriade, Lab. Neurophysiol., Dept. Physiol., Fac. Med., Laval Univ., Québec, Canada.

Autoradiographic experiments have disclosed that the nucleus ventralis medialis (VM) of the thalamus projects to layer I of almost the entire neocortex in the rat (Herkenham, J. comp. Neurol. 183:487, 1979). We investigated the laminar distribution and spatial extent of the cortical projections of VM in the cat, since we are interested in structures with widespread influences on cortical electrical activity. Horseradish peroxidase (50%, 100 nl) was injected into the VM; the tetramethyl benzidine method was used to reveal transported enzyme 48 h. later. Furthermore, the distribution of field potentials evoked by focal VM stimulation was determined in most of the neocortex.

After HRP injections, an intense retrograde labeling was seen in structures known to provide afference to VM, such as the substantia nigra and fastigial cerebellar nucleus. The anterograde label was situated almost exclusively in a 60-70 μm band in the superficial third of layer I of the ipsilateral anterior neocortex. The highest grain density was in the anterior sygmoid and proreus gyri, which included cytoarchitectonic areas 8, 6, & 4. When present, the layer I label in the postcruciate part of area 4 consisted of only sparse grains, much less dense than that found in the precruciate areas. No projection could be detected posterior to area 4. Retrogradely labeled neurons were found in cortical layer VI and the deep part of layer V. The spatial extent of this band paralleled the anterograde projection to layer I strikingly.

Clear VM-evoked responses could be recorded only in the medial part of precruciate areas 6 & 8. They consisted of a purely surface-negative wave in area 8, but in area 6 this component was preceded by two distinct surface-positive deflections of much smaller amplitude. The surface-negative wave reversed superficially (0.2 - 0.4 mm), indicating a termination in layer I. The initial surface-positive waves reversed more deeply. The superficial nature of the surface-negative wave was tested by superfusing the surface with warmed normal Ringer solution containing either manganese (2mM) to reversibly block synaptic transmission or calcium (2mM) as a control. Manganese selectively reduced or abolished the VM-evoked surface-negative, depth-positive wave.

The anterograde transport and depth profile findings confirm that VM projects almost exclusively to layer I, but indicate that the projection is confined to precruciate areas in the cat.

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135.6 DISTRIBUTION AND MORPHOLOGY OF PYRAMIDAL TRACT-CELLS VISUALIZED WITH TWO HORSERADISH PEROXIDASE METHODS. M. A. Biedenbach and J. L. DeVito, Dept. of Physiol., Univ. of Texas Health Science Center, San Antonio, Tx. 78284 and Primate Research Center, Univ. of Washington, Seattle, Wash. 98195.

In anesthetized cats horseradish peroxidase (HRP) was applied to all axons in the pyramidal tract (PT), that had been sectioned at the medullary level. The retrogradely transported HRP product in PT cells was visualized with both DAB and TMB histochemistry. All labelled cells in a sample of the brain sections were plotted to establish extent of PT-cortex and PT-cell density in subregions within. Quantitative morphological information was obtained from camera Lucida drawings of labelled PT-cells which were re-traced with a graphic digitizer. The output of the digitizer and alphanumeric data were stored in files of a Minc-11 computer for later analysis. Electron micrographs were taken from thin sections through the medullary pyramid. The outlines of the axons were also traced with the digitizer to determine their size and number.

Results of the TMB method, compared with those of the DAB method, revealed a roughly similar extent of PT-cortex but much greater density of labelled cells visualizing virtually all PT-cells (estimated from the number of axons in the medullary pyramid). With both methods, PT-cells were highly concentrated in the two banks of the cruciate sulcus and in a band of cortex continuing over the anterior sigmoid gyrus into the hidden lateral bank of the presylvian sulcus. PT-cells were less concentrated in surface cortex extending caudally from cruciate sulcus and laterally down to the orbital sulcus; here cell concentration was sparse with DAB, but moderate with TMB.

The TMB-visualized cell population contained a larger fraction of small cells. For each cortical subregion in PT-cortex, frequency distribution histograms of soma diameter and cross-sectional area will be presented. They will be compared with similar histograms obtained from the axons of the medullary pyramid. Most labelled cells were basically pyramidal-shaped with a prominent apical dendrite oriented towards the cortical surface. Other soma shapes occurred among labelled cells throughout PT-cortex but more often in areas where section plane was not perpendicular to the cortical cell layers.

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135.7 SURVIVAL TIME PROFOUNDLY AFFECTS THE LAMINAR DISTRIBUTION OF HRP LABELED NEURONS IN CEREBRAL CORTEX. C. L. Pappas and P. L. Strick. Dept. of Internal Medicine. U. South Florida Medical School, Tampa, FL., and Depts. of Physiology and Neurosurgery, Upstate Medical Center, and VA Med. Ctr., Syracuse, NY.

Recently, we reported the results of a study in which the retrograde transport of horseradish peroxidase (HRP) was used to examine patterns of connectivity in the callosal system of cat motor cortex. During that study, we noted that the survival time of the animal following HRP injection seemed to influence the laminar distribution of subsequently labeled neurons. In the present study, we have performed experiments examining this influence in both the cat and primate motor cortex callosal systems.

In 7 cats, survival time following multiple HRP injections into one motor cortex was varied from 26 to 50 hours. Following histochemical processing with TMB, the numbers of labeled neurons in superficial (I-III) and deep (V-VI) layers of homologous regions in the contralateral motor cortex were counted. We sought to normalize the slight variations in staining from animal to animal by converting these numbers into a superficial-to-deep (S/D) ratio. The S/D ratio decreased with increasing survival times. Similar observations were made in the primate.

A decrease in the S/D ratio could be accomplished by either an increase in the number of deep neurons labeled, a decrease in the number of superficial neurons labeled, or both. We found that while the absolute number of deep neurons labeled was rather constant, the number of superficial neurons labeled decreases with increasing survival time.

We found no difference in the size of labeled neurons when the two groups (short vs. long survival) of either superficial or deep neurons were compared.

One interpretation of these results is that neurons in deep and superficial layers either metabolize and/or transport HRP at different rates. It is clear from these results that carefully varied and controlled survival times must be employed in studies using HRP histochemistry in the cerebral cortex.

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135.8 THALAMOCORTICAL CONNECTIONS TO THE ROSTRAL FORELIMB AREA OF THE RAT MOTOR CORTEX. E.L. Bold* and E.J. Neafsey. Dept. Anatomy, Loyola Univ. Med. Center, Maywood, IL 60153

Two separate forelimb motor areas have recently been described in rat frontal cortex (Neafsey, Neurosci. Abstr. 6:156, 1980). The present study investigated the thalamocortical projections to the rostral forelimb region (RFL) by mapping retrogradely labeled thalamic neurons following injections of HRP or HRP conjugated with wheat germ agglutin (HRP-WGA) into the RFL. The rats were anesthetized with ketamine HCl (100 mg/kg,IP) and placed in a stereotaxic frame. The cisterna magna was opened to prevent cortical swelling and a small piece of bone (5x2mm) was removed just rostral to bregma on one side. The RFL was identified by locating the region where intracortical stimulation (.25 msec pulses, 100 μamps or less, 300 msec train @ 300 Hz) evoked digit or wrist movements rostral to an area where neck movements were elicited. Then .01 to .02 μl of either a 30% solution of HRP (Sigma VI) or a 1% solution of HRP-WGA (Polysciences) in physiological saline was injected into RFL using a 1 μl Hamilton syringe fitted with a 50 μ diameter pipette tip (average diameter of injection=1.5mm). After survival periods of 1-2½ days, the animals were reanesthetized, transcardially perfused with 1.25% glutaraldehyde and 1% paraformaldehyde, and the tissue was processed for HRP histochemistry according to the TMB procedure of Mesulam (J. Histochem. Cytochem. 26: 106-117,1978).

Retrogradely labeled neurons were found in several thalamic nuclei, including the ventromedial (VM), ventrolateral (VL), intralaminar, dorsomedial (DM), and the lateral posterior/posterior (LP/Po) nuclei. A prominent longitudinal column of labeled cells was found throughout VM, except in its most rostral portion where only a few scattered cells were labeled. In VL, labeled cells were found only in the rostral one-third and were situated dorsally, just lateral to the intralaminar nuclei. Within the intralaminar nuclei, labeling seemed to move from ventral to dorsal as one moved caudally, with the heaviest labeling found in paracentral and central lateral nuclei. Sparse labeling was also seen in the lateral portion of DM, where it bordered the intralaminar nuclei. This labeling was restricted to the middle third of DM. LP demonstrated positively labeled cells throughout its extent, again forming a longitudinal column of cells running rostral to caudal and merging with a similar column of cells in the anterior two-thirds of Po.

Thalamocortical projections in the monkey have been shown to arise from longitudinal columns or strips that may cross nuclear boundaries (Kievit and Kuypers, Exp.Brain Res.29, 1977). The present findings suggest such a pattern exists in the rat also.

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135.9 LIGHT AND ELECTRON MICROSCOPICAL FEATURES OF TWO TYPES OF NEURONS IN THE CAT'S VENTRAL MEDIAL THALAMIC NUCLEUS AS REVEALED WITH HRP INJECTIONS IN THE MOTOR CORTEX. K. Kultas-Ilinsky, S. Warton* and I. Ilinsky. Depts. of Anatomy and Surgery, St. Louis Univ. Sch. of Med., St. Louis, MO 63104.

Retrograde HRP transport was used to study the relationships between thalamo-cortical projection neurons (PN) and local circuit neurons (LCN) in the ventral medial nucleus (VM). Injections of 35-50% HRP in 2% DMSO were made in the medial part of the precruciate gyrus in three cats. The animals were sacrificed in 24 hrs by perfusion with 2.0% glutaraldehyde and 0.5% paraformaldehyde in 320 mOsm phosphate buffer. Serial 100 um thick sections were cut on a vibrotome and alternate sections were reacted for HRP according to one of three procedures: Adams (1977), Malmgrem and Olsson (1978), or Itoh et al. (1979). After incubation, disks of tissue 1 mm in diameter were punched out from the VM and processed for EM. The disks embedded in Epon were either sectioned serially at 1 μm for light microscopic analysis and cell counting or cut on an ultramicrotome for EM analysis.

Large numbers of cells were found to contain retrogradely transported HRP granules throughout the anterior-posterior extent of the VM. LM analysis showed that HRP positive cells belonged to a distinct neuronal population which had morphological characteristics quite different from those of unlabelled cells. The former were relatively large (20-30 μm dia.) with angular cell bodies, numerous dendritic processes and large amounts of cytoplasm. The unlabelled cells were small (10-16 μm dia.), with oval or elongated cell bodies, a few dendrites and a larger nuclear-cytoplasmic ratio. None of these small cells were observed to contain HRP. This allowed us to classify the large neurons as being PN and the small cells as LCN.

The ratio of the two types of cells was estimated from maps of complete series of 1 μm plastic sections (from four different tissue disks) prepared using an x-y plotter. The ratio of PN to LCN varied in different parts of the VM. PN tended to be in groups, while LCN were scattered without any recognizable pattern. At the ultrastructural level HRP granules could be traced quite far within the PN dendrites up to the smallest branches. In no case were HRP granules seen within vesicle-containing dendrites providing further evidence for our earlier suggestion that vesicle-containing dendrites belong to LCN. Another feature was that axosomatic synaptic contacts which are usually very rare in the VM are found almost exclusively on the perikarya of the PN and not on the LCN. Furthermore, no synaptic contacts were observed on the axon hillock or initial segment areas of the PN which were easily identifiable due to the presence of HRP granules. Supported by a grant from American Parkinson Disease Association.

135.10 ANTEROGRADE HRP AND AUTORADIOGRAPHIC EVIDENCE FOR COLUMNAR ORGANIZATION IN IPSILATERAL CORTICAL AND CALLOSAL PROJECTIONS OF RAT FRONTAL CORTEX. A. Isseroff, M.L. Schwartz, J.J. Dekker and P.S. Goldman-Rakic, Sect. of Neuroanatomy, Yale Univ. Sch. of Med., New Haven, CT. 06510.

Although columnar organization in the ipsilateral and callosal projections of various cortical areas has been observed in primates, cats and other species, its presence in rats has not been clearly established. Patchy terminations, suggestive of columns, have been noted in the callosal projections of rat somatosensory and posterior neocortex (Cippoloni and Peters, Brain Res. 176:33, 1979; Wise and Jones, J. Comp. Neurol. 168: 313, 1976). However, Akers and Killackey (J. Comp. Neurol. 181: 513, 1978) reported that the pattern of anterograde degeneration of ipsilateral projections from rat somatosensory cortex was homogeneous. Thus, discontinuities in callosal terminations could be attributed solely to intercalation of areas receiving callosal input with cytoarchitectonically and functionally distinct acallosal regions.

Using autoradiographic and HRP tracing methods, we have obtained evidence that the termination of both ipsilateral and callosal projections from rat frontal cortex have a columnar organization similar to that found previously in the rhesus monkey (Goldman and Nauta, Brain Res. 122: 393, 1977). ^{3}H-leucine was injected and acrylamide-bis gel HRP pellets implanted in the motor, premotor and medial prefrontal cortex of adult albino rats. Placement of tracer was verified by observations of labeling in thalamic nuclei. Anterograde labeling of ipsilateral terminations in frontal and somatosensory cortex as well as that from callosal projections to homotopic areas took the form of distinct columns, most of which were 250-500 μ wide in the deeper layers and somewhat wider and more prominent in superficial strata, similar to columns demonstrated in the frontal cortex of rhesus monkeys. Thus, some of the label-free areas in callosal terminations may be cytoarchitectonically distinct acallosal regions; however, columns in ipsilateral projections and within contralateral terminal fields are apparently indicative of the kind of modular parcellation of afferents observed in primate frontal cortex.

A previous study (Bugbee and Goldman, Neurosci. Abs. 6: 822, 1980) demonstrated that the frontal cortico-cortical projections of both rhesus and squirrel monkeys terminate in columns 250-500μ wide. Present data provide further evidence that columnar organization is a pervasive characteristic of mammalian neocortex, and that the vast differences in cortical volume in different species apparently result in differences in the number, but not the width, of the columns. (Supported by NIH Grant NS16666 and NIH Fellowship Awards NS06158, MH08308 and MH 00298).

135.11 HORSERADISH PEROXIDASE DETERMINATION OF POSTERIOR LATERAL HYPOTHALAMIC INNERVATION. S. L. Scharoun, F. C. Barone, M. J. Wayner, R. Guevara-Aguilar and H. U. Aguilar-Baturoni. Brain Research Lab., Syracuse Univ., 601 University Ave., Syracuse, NY 13210 and Depto de Fisiologia, Facultad de Medicine, U.N.A.M., Mexico.

Horseradish peroxidase (HRP, Sigma Type VI, 13%) was injected into the posterior portion of the lateral hypothalamus-medial forebrain bundle (LH-MFB) of male hooded rats. After 20-24 hr the animals were perfused intracardially with a phosphate buffer plus sucrose wash followed by glutaraldehyde and paraformaldehyde fixative. The whole brain was removed and sectioned at 50 μm. Alternate sections were processed with DAB and counterstained with cresyl violet for the brown reaction and with TMB and counterstained with neutral red for the blue reaction. Sequential sections were examined and positively labeled neurons were identified and photographed through a microscope with both light and dark fields. Labeled neurons and axons rostral to the LH were identified in the zona incerta, internal capsule, and radiatio thalami intermedia. Axons were also identified in the caudate putamen, nucleus ventralis thalami, nucleus reticularis thalami and nucleus dorsomedialis (hypothalami) par ventralis. Soma rostral to the LH were identified in the globus pallidus. Labeled cells and axons in the reticular formation, zona incerta, substantia nigra zona compacta and zona reticulata were also observed. Additional posterior axons were identified in Forel's Field H_1, H_2, nucleus ventralis thalami, nucleus parafascicularis and the nucleus posterior thalami. These results based on the retrograde labeling of neurons following HRP ejection in the posterior LH-MFB indicate a considerable degree of synaptic input to this region from the brain stem, especially from the reticular formation and other MFB associated structures. (Supported by NIH Grant NINCDS USPHS No. 13543.)

135.12 THE DORSAL NUCLEUS PRAEEMINENTIALIS OF A GYMNOTIFORM FISH. A CORRELATIVE GOLGI AND NISSL STUDY. E. Sas and L. Maler. Univ. of Ottawa, Sch. of Med., Ottawa, Ont. K1N 9A9. (SPON: W. Hendelman).

This study was prompted by the lack of information on the cytoarchitecture of this important nucleus in the feedback loop of the electrosensory pathway of the weakly electric fish Eigenmania Viriscens.

The n. praeeminentialis (N.PRAE) is an isthmic structure located dorsolateral to the lateral lemniscus, and anterior to the eminentia granularis - a subdivision of the archicerebellum of fish.

It can be divided into a large dorsal portion, concerned with electroreception and a small ventral portion involved solely with mechanoreception (this is also the case in mormyrids).

In this work the structure of the N.PRAE is examined using both Nissl and Golgi material, and an attempt is made to correlate this information with data from H.R.P. studies (Maler-Sas, unpublished observations) on the afferent and efferent connections of this nucleus.

The N.PRAE consists of three parts: a pars medialis (p.M.), a large pars principalis (p.P.) and a narrow pars lateralis (p.L.). The p.P. presents three zones: a dorsal (Dz), a central (Cz) and a ventral zone (Vz), which are reciprocally and topographically connected with the 3 zones of the posterior lateral line lobe (PLLL); medial PLLL with ventral zone, central PLLL with central zone and lateral PLLL with dorsal zone.

Several types of projection cells are present in the N.PRAE: a) neurons that show preferential orientation of their long dendrites in relation to the afferent fibre systems, b) cells with wide dendritic fields radiating in all directions, and c) cells with small well oriented dendritic fields.

Two small interneurons are also identified, showing different axonal ramifications. A detailed representation of these cells is shown in Golgi as well as diagrams of the afferent fibre systems to the N.PRAE from PLLL, caudal lobe, and torus semicircularis.

The complex processing within this nucleus is reminiscent to the feedback loops in the phylogenetically related auditory system.

135.13 A "NEW" HISTOLOGICAL PROCEDURE FOR USE IN DEVELOPMENTAL STUDIES: THIONIN COUNTERSTAINED-REDUCED SILVER. G.H.Gross* (SPON: J.D. Greenspan). Neurobiol. Prog., Univ. of N.Carolina,Chapel Hill,NC 27514 and Neuroembryol. Lab.,Dorothea Dix Hosp.,Raleigh,NC 27611.

Avian and mammalian embryos and amphibian larvae stained with a Cajal-DeCastro reduced silver technique (Levi-Montalcini,R., J. Comp.Neurol. 91:209-241,1949) were counterstained for Nissl substance with the basic aniline dye, thionin. This simple modification produces highly differentiated histological material at all embryonic ages studied in these species. Early in development the background is pale green, young neurons and glia are blue, cartilage is purple, and fibers, which are relatively unaffected by counterstaining,range from black to gold.As development proceeds, some neurons develop a remarkable burgundy coloration of the soma and dendrites, which is startling against the deepening green background. This coloration may reflect the development of argyrophilia in these neurons, and may be useful as a rapid visual index of maturation.

Thionin Nissl stain reveals cell perikarya several developmental stages before the cytoplasm of young neurons has developed sufficient argyrophilia to be visible in reduced silver preparations. However, use of the combination of stains permits study of relations between young neurons and nerve fibers from early stages when such critical events as cell death occur, up to the later stages of development and synaptogenesis. In addition, since the combination stain shows fibers, nucleoli, and perikarya clearly, it is possible to obtain accurate cell counts during the early development of cell groups which require a fiber stain for consistent delineation of nuclear boundaries in the adult,e.g. avian vestibular complex. Comparisons of cell counts in thionin-, reduced silver-, and combination-stained material suggest that argyrophilia may develop at different rates, and to different degrees even within the boundaries of a conventionally-defined "homogeneous" nuclear group. In counterstained material it is clear that an argyrophilic neuron, evident in reduced silver, may be surrounded by neurons of generally comparable size and morphology which stain only for Nissl substance, and hence could not be detected by reduced silver technique. Consequently, cell counts based on classic reduced silver could be misleading.

The following examples will be given to illustrate the technique: 1.Development of the spoon ending between a vestibular colossal fiber and a n.tangentialis principal cell in chick,pigeon, and finch. 2. Development of primary vs. secondary motoneurons in Rana catesbeiana. 3.Development of dorsal root ganglia. 4.Development of vestibular sensory epithelia.

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135.14 CALLOSAL AND ASSOCIATIONAL AXON COLLATERALS OF PREFRONTAL CORTICAL NEURONS IN THE RHESUS MONKEY DEMONSTRATED BY RETROGRADE FLUORESCENT TRACERS. M.L. Schwartz and P.S.Goldman-Rakic. Sect. of Neuroanatomy, Yale Univ. School of Med., New Haven, CT. 06510.

The recent development of double labeling techniques using retrogradely transported fluorescent tracers has made it possible to identify neurons which issue axon collaterals to divergent targets (Kuypers et al., Exp. Brain Res., 40: 383, 1980).In the present study we have used this method to determine the extent to which individual callosal neurons may also have associational projections within the same hemisphere. In order to answer this question, the fluorescent dye Fast Blue (FB) was injected into the posterior bank of the intraparietal sulcus (Brodmann's area 7) of one hemisphere: 8-10 days later, a second dye, Nuclear Yellow (NY) was injected into the principal sulcus (Brodmann's area 9) in the other hemisphere. FB selectively labels the cytoplasm of a cell, while NY labels the cell nucleus. Double labeled neurons display a blue fluorescent cytoplasm and a whitish fluorescent nucleus.

The types and distribution of fluorescing neurons were analyzed in the principal sulcus of the hemisphere ipsilateral to the FB injection and contralateral to the NY injection. This region contained large numbers of retrogradely labeled neurons. Most were single labeled with either Fast Blue or Nuclear Yellow. However, a very small fraction, perhaps less than 1%, were double labeled. The single labeled neurons were present in greatest density within layer 3, where they were frequently intermixed in both the deep and superficial portions of this layer. The small population of double labeled neurons was randomly interspersed among the single-labeled neurons. Double labeled neurons were not confined to the cortex of the principal sulcus but were also observed, for example, in the cingulate cortex. Again, the neurons containing NY + FB were few in number and were scattered among numerous single labeled neurons containing one or the other dye.

The present study indicates that a small fraction of callosal neurons in the primate brain have widely diverging axon collaterals, some of which even project to heterotopic regions across the callosum. Thus, a neuron in the cingulate cortex may project to the principal sulcus in the frontal lobe of one hemisphere and to the parietal cortex of the other. The scarcity of double labeled cells may reflect their rarity in the adult cortex or alternatively a greater number of callosal neurons may have such divergent collaterals but project to areas that were not injected in the present study.

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135.15 SOME FLUORESCENT COUNTERSTAINS FOR NEUROANATOMICAL STUDIES. L.C. Schmued*, L.W. Swanson and P.E. Sawchenko* (SPON: W.M. Cowan). The Salk Institute, La Jolla, California 92037.

An increasing number of methods in neurobiology rely on the detection of fluorescent markers in tissue sections or in culture; these include fluorescence methods for monoamines, immunohistochemical (IHC) methods for the localization of specific antigens, and pathway tracing methods based on the retrograde transport of fluorescent markers. Small amounts of bright fluorochromes can be detected against a dark background, but the dark field is a disadvantage when trying to determine the precise location of labeled cells within specific structures. We have, therefore, screened some 40 fluorescent compounds to determine those best suited for counterstaining 1) IHC labeled cells in tissue sections and in culture, 2) retrogradely transported fluorochromes, and 3) normal material.

There are several requirements for adequate counterstaining of immunofluorescence material. The counterstain should not mask FITC-labeling, it should fluoresce a different color, it should be nearly invisible at wavelengths used to excite fluorescein, it should be effective at neutral or basic pHs, and it should provide a crisp Nissl stain. The compound that meets these criteria best is ethidium bromide. At dilute concentrations (.00001%), it produces a moderately bright red Nissl stain (green excitation), which provides good contrast to the green FITC label (blue excitation). Ethidium bromide may be used with either histological sections or with cells in tissue culture.

We have also found counterstains suitable for localizing cells that are labeled with retrogradely transported fluorescent tracers. Since these markers fluoresce with ultraviolet excitation, a counterstain that is visible only when excited with longer wavelengths is desirable. The acridines (acridine orange and quinacrine) are suitable for this application since they are visible when excited with blue light, but not with ultra violet. Other effective dyes for this purpose include ethidium bromide, neutral red, and astrazone red.

When immunofluorescence and retrograde transport methods are combined to characterize biochemically the cells that project to a particular terminal field, a counterstain is needed that does not mask either FITC or retrograde markers. Ethidium bromide best meets these requirements. It is quite bright when illuminated with green light, moderately visible when examining fluorecein conjugated label with blue light, and barely visible when examining retrogradely transported fluorochromes with ultraviolet light.

Bisbenzimide and nuclear yellow are particularly effective counterstains for normal material at low concentrations. At pH 7.2 both dyes produce a nuclear stain, while at pH 2.0 they produce a brilliant Nissl stain. Neutral red, safranin-0, and astrazone red differentially stain cell bodies and myelineated fibers, producing a fluorescent "Kluver-Barrera" stain.

135.16 COMPARISON OF THE DISTRIBUTION OF MECHANOSENSORY TARGET (MERKEL) CELLS IN MAMMALIAN AND AMPHIBIAN EPIDERMIS USING QUINACRINE FLUORESCENCE AS A MARKER. C.A. Nurse, K.M. Mearow, B. Visheau, M. Holmes and J. Diamond. Dept. of Neurosciences, McMaster University, Hamilton, Ontario L8N 3Z5.

The Merkel cells are specialized sensory cells located at mechanosensitive spots in the vertebrate epidermis. Following a report that these cells bind the fluorescent dye, quinacrine, (Crowe and Whitear, Cell Tiss. Res. 190: 273-283, 1978) we have adapted the technique to aid visualization of the Merkel cell distribution within whole mounts of epidermis known to contain these cells from electronmicroscopic (E.M.) studies. The tissues (Xenopus tadpole tentacles, salamander skin, rat touch domes and vibrissae) were removed 3 hrs-4 days following injection of 5-15 mg/kg quinacrine dihydrochloride into the tail musculature of the tadpole or intraperitoneally in rat and salamander. The distribution of the quinacrine fluorescent cells (QFC) was viewed under dark field illumination after the tissues were mounted in liquid paraffin and exposed to U.V. light. In the tadpole, many epidermal QFC were arranged 25-40 μm apart in rows along the length (up to ca. 2 cm) of the tentacle. In salamander epidermis, separated mechanically from the dermis following 1 hr incubation in BSS containing 1 mg/ml collagenase, individual QFC were scattered 40-100 μm apart; in a few tests, touch-sensitive spots mapped physiologically on the skin (over an area of about 0.16 mm^2) correlated closely with the QFC-distribution in that skin. Rat touch domes from trunk skin contained scores of QFC at the basal epidermis. Frequently, the QFC were arranged as a flat elliptical annulus surrounding an eccentric tylotrich hair; the QFC tended to concentrate caudal or caudo-lateral to the exit-point of the hair. We are interested in the possibility that this array may be the morphological basis for the directional sensitivity to tylotrich hair displacement reported for the rat touch dome (Smith, J. Comp. Neurol., 1967, 131: 459). The mechanically isolated outer root sheath of the neonatal rat vibrissa contained several hundred QFC periodically arranged in a cylindrical sleeve adjacent to the basement membrane. In all cases, the location, size and shape of the QFC corresponded to that of the Merkel cells as revealed by other light and E.M. studies. We have also enzymically dissociated cells from the quinacrine-labelled outer root sheath for use in other studies in vitro; about 6% of isolated cells were clearly fluorescent within a larger non-fluorescent cell population. We are now confirming directly the Merkel cell identity of the QFC.

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135.17 A TECHNIQUE ENABLING THE ELECTRON MICROSCOPIC EXAMINATION OF LUCIFER YELLOW- INJECTED NEURONS. A.R. Maranto. The Biological Laboratories, Harvard Univ., Cambridge, MA 02138.

Because of the ease of microinjection and quick diffusibility of the dye Lucifer Yellow it was of interest to develop a procedure to make cells filled with this dye visible for electron microscopy. The technique reported here is based on the ability of irradiated Lucifer Yellow to sensitize the photo-oxidation of 3,3'- diaminobenzidine (DAB) to an insoluble osmophilic polymer.

To demonstrate the staining obtainable with this technique the S interneuron of the leech was used because of its easily identifiable large axon. S cells were filled with Lucifer Yellow CH by pressure injection or iontophoresis. Ganglia were then allowed to incubate for 20 minutes in a solution of DAB (1mg/ml) in normal leech Ringer's adjusted to pH 7.4. The photooxidation step was performed next with the intense blue light from a fluorescence microscope equipped with a mercury arc lamp and suitable excitation filters. When the dye fluorescence faded below visibility the ganglia were washed in Ringer's, fixed in glutaraldehyde, and post-fixed in OsO_4. After embedding in Spurr's medium, the reaction product could be identified in 1 micron sections by its reddish-brown appearance. In ultra-thin sections the S cell axon was marked by dense granular deposits throughout the cytoplasm and especially along the inner surface of the plasmalemma.

The compatibility of this technique with other intracellular electron dense markers such as metal precipitates and the horseradish peroxidase reaction product suggests its possible value in double marker experiments to establish interactions between neurons at the ultrastructural level.

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135.18 A COMPUTER SYSTEM FOR NEUROANATOMICAL DATA ACQUISITION, ANALYSIS AND DISPLAY. W.K. Smith, D.S. Schlusselberg and D.J. Woodward, Dept. Cell Biology, Univ. Texas Health Science Ctr., Dallas, TX 75235.

Ongoing anatomical studies in this laboratory on cell topography and connectivity in brain stem nuclei have generated a need for new techniques in anatomical data acquisition and display. In this report we describe progress in development of a general computer-based system for acquiring and viewing neuroanatomical data.

An initial program, MACRO, employs a light microscope drawing tube, an X/Y stepper motor stage and digitizing tablet. After an initial calibration procedure, the operator may digitize fiducial marks, lines to note structural boundaries and points for cell locations. Independent designation of different anatomic components is performed through the use of a 12-character label which may be referenced at later stages of analysis. Convenience features include on-line graphical output of data obtained at any magnification, scaled and transformed from a common file system to a relevant biological coordinate system.

REFINE, the primary data manipulation program, employs a disk-based virtual memory array with 24-bit mantissa floating point elements so that numerical accuracy and memory size are not limiting. Objects for display can be constructed by combining and aligning labeled serial section data from different experiments. Available output devices include a Tektronix graphics terminal, a Versatec matrix printer/plotter and an Ikonas color video frame buffer with microprogrammable processor.

Graphics software includes display of stick frames, contours, labeled cell distributions and surface reconstruction with hidden object removal. Sequential contours are processed by a minimum surface area algorithm which generates an optimum set of triangles spanning the contours. Smooth shaded images in 3-space are constructed after defining an eye position and light source to compute the proper view in perspective. A flexible file system has been developed consisting of strings of four numbers - X, Y and Z coordinates plus a real number CODE. Files are organized as tree structures with a system of pointers from a root to define classes of structures, segments, strips and complex objects. The system is being employed to quantitate and display gross brain surfaces, cytoarchitectural boundaries of brain stem nuclei, cell distributions and objects reconstructed from serial electron microscopy. (Support from NSF BNS77-01174 and the Biological Humanics Foundation.)

135.19 COMPUTER GENERATED THREE DIMENSIONAL RECONSTRUCTION OF SERIALLY SECTIONED TISSUE.

David P. Baker* and Jeanine R. Carithers. Department of Veterinary Anatomy, Iowa State University, College of Veterinary Medicine, Ames, Iowa 50011.

Computer generated 3-dimensional modeling created from 2-dimensional images has been utilized by several investigators in recent years for analysis of neurons and neuronal systems. This technique has required the collaborative efforts of a neuroscientist, a computer scientist, and a digital electronics technician. The results of these efforts have led to a clearer understanding of the gross and microscopic architecture of the nervous system. However, the equipment needed has been prohibitive in cost. Furthermore, a variety of computer systems were used in the early studies, and attempts to replicate these systems have been hampered by hardware and software incompatibilities between devices from different manufacturers. Additional incompatibilities are introduced when system dependent interfacing is required for the input and output of data.

The techniques presented here utilize an Apple II+ computer system, with programs written in BASIC and 6502 machine language, and a Houston Instrument Hi Pad graphics tablet for data entry. Tissue sectioned for light microscopy can be digitized from 2x2 Kodachromes, photographs, or a camera lucida. Data from electron microscopic preparations can be entered from the viewing port of the microscope using an optical accessory, from negatives, or micrographs. The data is entered as a series of x, y coordinates and the z data is relative to the thickness of the tissue sections.

This system provides quantification of neuronal structure and simultaneous morphometric analysis of cytoarchitecture using a small, inexpensive microcomputer instead of a large mainframe computer. Different pathways or structures can be highlighted in up to 16 colors with complete 360 degree rotation. The method presented here is limited only by the magnification limitations available from the input devices. It could be used equally well to reconstruct images of organs from gross anatomical material or computer assisted tomography images.

136.1 MORPHOMETRIC DETERMINATION OF AXON DIAMETER AND MYELIN SHEATH THICKNESS IN NEURONS MYELINATED BY BOTH SCHWANN CELLS AND OLIGODENDROCYTES. J.K. Donnelly and J.P.H. Wyse, Dept. Anat., Faculty of Medicine, Univ. of Calgary, Calgary, Alberta, Canada T2N 1N4.

Myelin sheath thickness (m) increases concomitantly with axon diameter (d) in both central (CNS) (Hildebrand & Hahn, J. Neurol. Sci. 38: 421, 1978) and peripheral (PNS) (Williams & Wendell-Smith, J. Anat. 109: 505, 1971) nervous systems. The relationship between myelin sheath thickness and axon diameter is frequently described as the g-ratio (i.e. the ratio of axon diameter to total fiber diameter). The purpose of the present study was to examine the relationship between fiber thickness and axon diameter in a population of CNS axons myelinated by ectopic Schwann cells and to determine if the relationship is similar to that present in either peripheral nerves or central tracts. The model system used in this study was the Bmn-wys (BW) strain of rat. These animals are affected by several coinherited neurological defects including a dopaminergic abnormality which is manifest in the retina, hypothalamus and nigrostriatal system as well as an unusual Schwann cell myelination of axons in the retinal nerve fiber layer (Wyse, J. Neurocytol. 9: 107, 1980). Since the latter axons are sequentially myelinated first by Schwann cells in the retina and then by oligodendrocytes in the optic nerve, this model system provides the opportunity to determine whether myelin sheath thickness differs for the two populations of myelinating cells when the neuronal population is constant. Measurements of axon diameter and sheath thickness were made from electron micrographs of myelinated axons in retinae, optic nerves, and sciatic nerves of one year old BW rats as well as in sciatic nerves of control animals. Myelinated axons in retinae and optic nerves ranged up to 4 μm in diameter. Sheath thickness increased significantly ($p < 0.01$) in retina, optic nerve and sciatic nerve samples as axons increased from 1 to 2 and from 2 to 3 μm in diameter. In all samples, values of g ($g = d/D$; $D = d + 2m$) increased rapidly over the smaller range of axon diameters with transition to a more linear relationship as axon diameter continued to increase. Average g-values ($\bar{X} \pm SD$) for the largest ensheathed axons ($d = 2.5$ to 4 μm) in both the retina (0.83 ± 0.02) and optic nerve (0.81 ± 0.06) were significantly greater than the g ratios of axons of comparable diameter in both BW (0.71 ± 0.05) and control (0.71 ± 0.05) sciatic nerves. These data suggest either that g-ratios are higher in CNS than in PNS or that special sensory neurons have higher g-values than do motor neurons of comparable diameter. Furthermore, since the maximal values of g in the retina were the same as those in optic nerves, it may be inferred that myelin sheath thickness is more dependent upon the neuron than on the type of ensheathing cell.

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136.2 QUANTITATIVE MORPHOLOGICAL ANALYSES OF MOTONEURONS. M. D. Egger and L. D. Egger*. Department of Anatomy, CMDNJ-Rutgers Medical School, Piscataway, New Jersey 08854.

Horseradish peroxidase (HRP) was injected intracellularly into motoneurons responding to cutaneous stimulation of the central foot pad of the hind limb in cats (Egger et al., J. Physiol. (Lond.), 306: 349-363 (1980)). Three motoneurons were selected for further analysis: two excited by foot pad stimulation, sectioned transversely (ET) and horizontally (EH). The third, sectioned transversely, was inhibited postsynaptically by foot pad stimulation (IT). Dendritic lengths and areal measurements should be considered lower limits: dendritic segments broken from their main stems were excluded, and some segments may not have filled completely with HRP. Cell bodies of EH and IT were about 0.4 mm from the lateral border of the ventral horn in S1 segment; that of ET, also in S1, was within 50 μm of the dorsolateral ventral horn border. Major and minor diameters of cell bodies were 96.4 X 66.7 μm (ET), 73.0 X 61.2 μm (EH), 59.4 X 43.9 μm (IT). The overall lengths of the dendritic trees were 15.8 mm (ET), 15.2 mm (EH) and 20.4 mm (IT). Total surface areas were 0.185 mm^2 (ET), 0.161 mm^2 (EH), 0.173 mm^2 (IT). The highest order of branching for ET and EH was VIth; for IT, it was VIIth. ET had 9 primary dendrites of 69 dendritic branches; for EH, it was 8 of 80; and for IT, 14 of 136. ET had 39 terminal dendrites, 31% > 0.8 mm from soma to tip; 4 > 1.4 mm. EH had 44 terminal dendrites, 20% > 0.8 mm; 4 > 1.0 mm. IT had 76 terminal dendrites, 12% > 0.8 mm; 1 > 1.0 mm. Using published values for R_m and R_a, and assuming uniformity throughout the dendritic tree, electrotonic lengths of terminal dendrites were estimated. The means were 1.3±1.1 (ET), 28% > 2.0; 1.3±0.8 (EH), 18% > 2.0; 1.2±0.7 (IT), 13% > 2.0. 75.2% of the total dendritic length of ET was accounted for by branches of 3 of its 9 primary dendrites; for EH, 69.1% by 3 of 8; and for IT, 47.9% by 3 of 14. However, for each motoneuron, when the lengths of dendrites were analyzed by order of branching, dendritic branches of orders II-V each included > 15% of overall dendritic lengths. Four patterns of dendritic branching were observed: 1) one daughter ≤ 50% of its sister's diameter; 2) the sum of the diameters of the daughters ≤ the diameter of the parent, & one daughter > 50% of its larger sister; 3) diameters of parent and two daughters about equal; 4) all others. The means of the quantity $\{(d_{d1}^{3/2} + d_{d2}^{3/2}) / d_p^{3/2}\}$ were calculated: 1.3±0.5 (ET); 1.1±0.9 (EH); 1.4±0.6 (IT). Means for ET and IT were significantly different from 1.0 ($P < 0.01$). However, the means of this quantity for branches of category "4)" were close to 1.0: 1.1±0.1 (ET); 0.9±0.2 (EH); 1.1±0.2 (IT).

136.3 The hypothalamic suprachiasmatic nucleus of rat: quantitative stereological assessment of neuropil and perikarya. Anthony N. van den Pol, Richard Bucholz*, and Rania Baik*. Section Neurosurgery, Yale Univ. Sch. Med., New Haven, Ct. 06510.

To further examine the organization of the suprachiasmatic nucleus (SCN) (JCN 191: 661-702), we have undertaken a stereological analysis of ultrastructural components. Four inbred male adult Fischer rats were perfused with a cacodylate-buffered 2.5% glutaraldehyde, 2.5% paraformaldehyde fixative. Silver thin sections from the SCN were saved on 300 mesh grids. Photomicrographs with a final magnification of 17,080, 37,950, and 75,900 were studied with a Bit Pad Digitizer interfaced with a Horizon microprocessor, and with the dot superimposition and line intersection methods described by Weibel (1973). Over four hundred 11 by 14 inch micrographs were quantitatively studied.

The synapse analysis included measurement of the area, synaptic membrane specialization, size of postsynaptic structure, number of vesicles, and size and number of mitochondria in 790 synapsing boutons from four different areas of the nucleus (anterior, ventrolateral, dorsomedial, and posterior). The mean bouton area in thin sections was 0.65 um^2 ± 0.41 um^2 (SD)(range: <0.1 to 2.7 um^2). The mean length of the synaptic membrane specialization was 0.43 um ± 0.18 um (SD) (range: <0.1 to 1.8 um). The mean number of vesicles per bouton was 48 ± 30 (range: 4 to 283). These data are based on two-dimensional data and are therefore underestimates of the true size. Statistically reliable differences were found between some of the characteristics of the synapses of the four SCN loci studied.

By examining a total of 21,818 regularly spaced points superimposed over photomicrographs magnified 17,080X, the volume compartments of different stuctures within the SCN could be determined. In the ventrolateral SCN, which receives afferent input from the retina, ventral lateral geniculate, and raphe, neuropil accounted for 76%, neuron somata 14%, glial cell bodies 2%, and cellular nuclei for 8% of the total SCN volume. Relative membrane lengths were determined by their intersections with 10,829 two cm regularly spaced straight line segments. Neuronal perikaryal membrane accounted for 903 intersections, glia soma for 129, and nuclear membrane for 615 intersections.

These and additional data will be examined relative to metabolism, structure, and intercellular communication in the suprachiasmatic nucleus. Some of the data support the emerging picture that regional morphological differences exist with the SCN.

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136.4 THREE-DIMENSIONAL SCANNING ELECTRON MICROSCOPY OF HIPPOCAMPUS. T.J. Teyler, J. Gilliam* and D. Ayers*. N.E. Ohio College of Medicine, Rootstown, OH 44272.

Scanning electron microscopy (SEM) permits the visualization of relatively large areas of neural tissue (as compared to transmission electron microscopy) at greater resolution than is possible with light microscopy. This permits a consideration of neural geometry not readily achieved by other approaches. When combined with the ability of SEM to provide three-dimensional, stereoscopic images, the result provides an informative and beautiful view of central nervous system architecture.

The hippocampus of rat was employed for these pictures. To enhance the surface imaging capabilities of the SEM, we dissected free the fixed hippocampus and mechanically split it along the lamellar plane and at various other orientations. We found that the fixative employed contributed greatly to the quality and usefulness of the imaged specimen. Best results were obtained with standard formalin fixation of hippocampus. The amount of time in formalin fixative determined the degree of rigidity of the tissue and the degree to which it could split to yeild an interesting surface. Unlike freeze-fracture techniques, formalin-split tissue largely preserves cell and process integrity. Following fixation and splitting, specimens were critical point dried and sputter-coated with gold.

The images produced by SEM of formalin-split hippocampal tissue, clearly indicate such pronounced features as the cell body layers, dentritic tree orientations and the lamellar organization of the tissue. Higher resolution, three-dimensional images show the proximity of neighboring somata, the fiber terminations in the dentritic tree and details of the synaptic contacts in the hippocampal neuropil.

(Supported by research grants from NSF and NIH)

136.5 COMPLEX RAMIFICATIONS OF HIPPOCAMPAL CA1 PYRAMIDAL CELL AXONS DEMONSTRATED BY LUCIFER YELLOW *IN VITRO*. W. Douglas Knowles and Philip A. Schwartzkroin. Dept. of Neurological Surgery, University of Washington, Seattle, WA 98195.

Intracellular injections of Lucifer Yellow into CA1 pyramidal cells of the *in vitro* guinea pig hippocampal slice enabled us to examine in detail the morphology of the axons of these neurons. We found that many of these axons bifurcate in the alveus with the major branch projecting caudally towards the subiculum and the second, thinner branch projecting rostrally towards the fimbria. Either axon may further bifurcate to produce several axon branches which follow parallel paths in the alveus. These axons also have local collaterals which project into strata oriens and pyramidale. In addition, a very fine plexus of axonal processes was observed in stratum oriens located largely within the basal dendritic field of the parent cell. The local axonal elaborations may be involved in recurrent pathways mediating feedback inhibition and/or excitation. It is possible that the synchronization of neural activity seen during epileptiform activity may be mediated by these axonal processes.

136.6 MORPHOLOGY OF VISUAL PRETECTAL NUCLEI IN THE CAT: A GOLGI STUDY. K.M. Gregory. Dept. of Biology, CSU Long Beach, CA 90815.

The pretectal complex in the cat was studied using the Golgi-Cox impregnation procedure. The olivary pretectal nucleus (PO) is characterized by cells with a fusiform or crescent shaped body with 1 or 2 dendrites emanating from each end. The dendrites branch repeatedly to form a bushy plexus of spiny dendrites which are confined to the central area of the nucleus. The nucleus of the optic tract (NTO) contains large and medium sized multipolar cells with moderately spiny robust branching dendrites which extend out for 300-500 μm and which are predominately oriented in a plane perpendicular to the path of the brachium. Neurons of the anterior pretactal nucleus (PA) have 5-6 main dendrites which branch into 2-3 secondary dendrites at varying distances from the cell body. Cells of the posterior pretectal nucleus (PP) possess 5-6 long (300-500 μm) thin dendrites of which 1 or 2 may branch about midway. The neurons of the PA and PP do not appear to have any specific dendritic orientation in transverse sections. Except for the PO, there is a lack of distinct nuclear boundaries due to dentritic overlap. The PA and PP are especially difficult to demarcate from each other due to the similarily of neuron morphology. There are no distinct ventral boundaries to the PA or PP nuclei.

136.7 EFFERENT PROJECTIONS FROM THE ENTORHINAL AREA TO THE BASAL FOREBRAIN AND FRONTAL CORTEX ORIGINATE IN LAYER IV. Ch. Köhler*and L. Eriksson(SPON:R.S.Schmidt) ASTRA Research Laboratories,Dept. of Neuropharmacology,Södertälje,Sweden.

Microinjections of 3H-leucine/proline(5uCi;50nl) into the rat entorhinal area(EA)resulted in anterograde axonal transport to several forebrain structures in addition to the perforant path projection: the septum(SEPT),the nucleus accumbens(AC),the piriform(PIR) and the frontal cortex(FC).In order to determine the exact localization of the cells that give rise to these extrahippocampal projections of the EA,small injections of either HRP(40%;100nl) or granular blue(GB,5%;100 nl) were made into each brain area of individual rats.After survival periods ranging between 2-5 days retrogradely labelled cells were found almost exclusively in layer IV of the medial and lateral EA.Comparisons between the HRP and fluorochrome methods indicated that GB was a more sensitive marker of retrograde transport than HRP in the present system.A small population of retrogradely labelled cells were always found in layer III of the most ventral parts of the lateral EA and in layer IV of the contralateral EA after all injections.Analysis of serial horzontal sections through the entire EA revealed that cells projecting to the various brain regions of the forebrain occupies different but partly overlapping levels of the EA.Thus,cells which project to the SEPT and AC are found at all levels of the EA,while cells innervating the FC and the PIR are concentrated predominantly at middle and ventral levels, respectively.Studies using double retrograde fluorescent tracing where propidium iodide(3%;100 nl) was injected into the PIR and GB into the SEPT,AC and FC,respectively,showed that some cells in layer IV of the EA have branching projections to the PIR and the SEPT or AC.Evidence for such simultaneous innervation by these cells of the PIR and the FC could not be obtained.The present results link the EA closely to several forebrain structures and suggest that the EA may directly influence the neural activity not only in the hippocampus via cells in layers II and III but also in several regions of the basal forebrain through cells in layer IV.

136.8 AN ELECTRON MICROSCOPIC STUDY OF HORSERADISH PEROXIDASE LABELLED NEURONS IN THE PHRENIC NUCLEUS OF THE ADULT ALBINO RAT. H. G. Goshgarian and J. A. Rafols. Department of Anatomy, Wayne State University School of Medicine, Detroit, Michigan 48201.

Phrenic motor neurons in the rat spinal cord were labelled by the retrograde transport of horseradish peroxidase (HRP) after the enzyme was applied to the central stump of the transected phrenic nerve in the neck. Forth-eight hours after the application of the enzyme the animals were perfused transcardially using conventional methods for electron microscopy. One hundred micron thick frontal and sagittal sections from the C3-C5 levels of the spinal cord were obtained with a tissue chopper. The sections were reacted for peroxidase activity using diaminobenzidine as the substrate. Labelled phrenic neurons were identified under the light microscope, trimmed from their surrounding tissue, osmicated and embedded in Araldite blocks. One μm sections were cut from the blocks and stained with toludine blue for light microscopy. Ultrathin sections were obtained from those blocks which yielded heavily labelled neurons in the 1 μm thick sections. In order to confirm the presence of HRP electron dense granules, unstained ultrathin sections as well as sections stained with lead citrate and uranyl acetate were studied with the EM. Cell body and dendritic profiles of phrenic neurons contained HRP reaction granules in membrane bound vesicles, cisternae of smooth endoplasmic reticulum, Golgi apparatus, lysosomes, and multivesicular bodies. Phrenic perikarya were characterized by the presence of coarse Nissl granules, numerous cisternae of rough endoplasmic reticulum, ribosomal rosettes and other organelles. The labelled somata and proximal dendrites were contacted by four different types of presynaptic endings. Type I endings contained flattened synaptic vesicles, had an electron lucent matrix and formed symmetrical synapses. Type II endings contained round synaptic vesicles, also had an electron lucent matrix and had some microtubules and neurofilaments. The endings formed asymetric synapses and were often associated with wide extracellular clefts which were filled with a granular or fibrillar material. Type III endings had an electron dense matrix, contained flattened, plieomorphic synaptic vesicles, microtubules, neurofilaments, and numerous mitochondria. These endings formed symmetrical synapses. Type IV endings were similar to type III endings except that they contained round synaptic vesicles. Axoaxonic and serial synapses were also observed in association with labelled phrenic profiles. In the neuropil immediately adjacent to labelled profiles, dendrites were commonly separated by astroglial processes. Direct apposition of dendritic membranes seldomly occurred.

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136.9 SENSORY CELLS AROUND THE MOUTH OF HYDRA: ULTRASTRUCTURAL CORRELATES OF MOUTH OPENING BEHAVIOR. J. C. Kinnamon and J. A. Westfall. Department of Anatomy and Physiology, Kansas State University, Manhattan, Kansas 66506.

Previous work in our laboratory has established that sensory cells are concentrated in the top 120 μm of the hypostome of Hydra littoralis. Using transmission electron microscopy of 0.1 μm thick serial sections of Epon-Araldite embedded specimens of Hydra we have determined the patterns of synaptic connectivity of sensory cells in the circumoral region.

Neurons at the apex of the hypostome are arranged in two layers, with sensory cells in the superficial region of the epidermis. Their sensory cell papillae protrude through the cuticle into the environment. Below the sensory cells, ganglion cells form a second layer of neurons. In a previous study on the tentacle, we found an average of only one sensory cell per epitheliomuscular cell. In the present study, however, we found sensory cells to be abundant at the apex of the hypostome, especially in the rosette of epitheliomuscular cells surrounding the mouth. In this region up to four sensory cells are enveloped by a single epitheliomuscular cell, and the basal portions of these sensory cells are in direct contact with each other. They form sensory cell-sensory cell synapses, including reciprocal synapses between two sensory cells. Sensory cells around the mouth also have synapses with ganglion cells and epitheliomuscular cells similar to those previously observed in the tentacle and the hypostome-tentacle junction.

Ultrastructural characteristics and patterns of synaptic connectivity indicate that these cells are sensory in nature; their circumoral location suggests a function associated with feeding behavior. We propose that adequate stimuli (mechanical and/or chemical) to oral sensory cells can initiate direct responses and also indirectly affect adjacent sensory cells and ganglion cells. Reciprocal synapses may modulate the threshold levels between sensory cells and sensory cell-neuro-epitheliomuscular cell junctions might initiate local contractions of the oral musculature. Sensory cell-ganglion cell synaptic pathways may be used to transmit impulses to epitheliomuscular cells throughout the oral region. The end result of this activity would be opening of the mouth, swallowing, and associated feeding movements involving oral musculature. We have provided ultrastructural evidence for the neuronal substrates underlying one of the more complicated behaviors coordinated by this simple nervous system.
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136.10 LAMINAR DIFFERENCES IN THE MORPHOLOGY OF LATERAL GENICULATE NUCLEUS CELLS IN GALAGO. E. Birecree* and V.A. Casagrande. Depts. of Anat. & Psych., Vanderbilt Univ., Nashville, TN. 37232

The lateral geniculate nucleus (LGN)of Galago consists of six layers which can be divided into three cell size pairs. The first pair (1&2) contain magnocellular cells, the second pair (3&6) contain medium size cells equivalent to the parvocellular layers of monkeys, and the third pair contain small size cells (Casagrande & Joseph,'80). Analysis of receptive field properties has shown that different LGN cell size pairs contain functionally different neuronal classes. Thus, medium and large size layer pairs have X-like and Y-like units, while the layers with small cells have a mixture of unit types some of which have W-like properties (Norton & Casagrande, '80; '81). In the present study we used Golgi preparations to determine if the physiologic and cell size differences seen in LGN laminae of Galago could be correlated with differences in cell morphology. Our material reveals four basic cell types, three of which correlate with cell size layer of origin. In the magnocellular layers, the majority of cells have large somata (400-600um^2), 4-8 thick primary dendrites that tend to branch repeatedly forming a complex network extending in a radially symmetric pattern around the soma. The latter closely resemble the Class 1 cells described by Guillery ('66) in cat LGN. In contrast, cells in layers 3 & 6 have medium size somata (200-400um^2) with asymmetrically grouped dendrites that are elongated either perpendicular or parallel to the plane of the layers. These cells have from 1-4 main dendritic groups but in all cases at least one group is oriented with its main axis perpendicular to the layers. Cells in the small cell layers can be distinguished from the other two types by their soma area (100-200um^2) and very simple dendritic organization consisting of only one or two thin primary dendrites with few branches. In each of the above three cell classes, subtypes can be distinguished according to the distribution, shape, and arrangement of dendritic appendages and the tendency of dendrites to cross laminar borders. The latter features do not, however, appear to correlate with cell size. In addition to the three classes described, a fourth category of very small cells (50-70um^2) exists that resembles the smallest squirrel monkey LGN cells described by Wong-Riley ('72). These stellate cells have very small thin dendrites that project in a radially symmetric pattern for a short distance around the cell body and are not confined to particular LGN laminae. From the above results we conclude that several morphologic features of Galago LGN cells can be correlated with cell-size layer origin and thus function. In several respects these distinguishing features resemble those described for X, Y, and W cells by Friedlander et.al. ('81) for the cat. Supported by EY01778, 1K07-EY00-061, BRSG-RR-05424-17.

136.11 ULTRASTRUCTURAL LOCALIZATION OF PHOTORECEPTOR ANTIGENS IN RAT. D. M. Fekete* and C. J. Barnstable (SPON: R. H. Masland). Depts. of Anatomy and Neurobiology, Harvard Med. Sch., Boston, MA 02115.

A number of monoclonal antibodies have been produced which react with vertebrate photoreceptor cells (Barnstable, C.J., Nature, 286: 231, 1980). Initial localization of antigens was carried out at the light microscopic level using indirect immunofluorescent labeling of fixed tissue sections. Antibody RET-P1 labeled the cell bodies, inner segments and outer segments of rods, but not cones. Antibody RET-P2 labeled only the photoreceptor outer segments. An antibody against the photopigment rhodopsin (a gift of M. Applebury) labeled the photoreceptor outer segments strongly and the inner segments weakly.

An electron microscopic study was undertaken to determine with greater resolution the distribution of the photoreceptor antigens. All antibodies in these experiments were diluted in a phosphate-buffered saline solution containing 1% bovine serum albumin, 1% goat serum and 0.05% Triton X-100. Vibratome sections of fixed rat retinas (Long Evans strain) were incubated overnight in the monoclonal antibodies. Control tissue was incubated in the buffer solution alone and subsequently processed exactly as the experimental tissue. The following day, one group of sections was placed into a ferritin-conjugated goat anti-mouse IgG antibody solution. A second group was incubated in goat anti-mouse IgG antibody followed by a mouse peroxidase-anti-peroxidase incubation. The sections were then reacted with diaminobenzidine. Tissue from both groups was then osmicated and processed for electron microscopy.

RET-P1 antigen was shown to be associated with the plasma membrane of both the inner and outer segments. The ferritin labeling technique showed that RET-P1 was distributed on both the outside face of the plasma membrane and the inside face of the discs. In suitable sections, RET-P1 could be localized within the membrane folds at the base of the outer segment, the area from which discs are assumed to be formed. It was not possible to localize RET-P1 on the photoreceptor cell bodies, as was seen with fluorescence, presumably because diffusion was limited by the tightly packed arrangement of cells in the outer nuclear layer. The labeling pattern for RET-P2 will be compared with that for RET-P1.

The particular anti-rhodopsin antibody used was shown to react with that portion of the molecule on the exterior of the plasma membrane. Rhodopsin was also found on the inner segment plasma membrane; this tends to support the suggestion that outer segment membrane is first inserted in the inner segment rather than flowing up through the connecting cilium and then being inserted. (Supported by grants NS13126, NS17309 and EY03735).

136.12 THE TRIDIMENSIONAL ASPECT OF THE "STATUS SPONGIOSUS" IN A CASE OF JACOB-CREUTZFELDT DISEASE, AS IS SHOWN BY THE GOLGI METHOD. J.P. Machado-Salas.Div. Neurocien cias, Neuromorfología, ENEP, Iztacala, UNAM, y Div. de Patología, Depto.Inv.Cient.CMN IMSS.México, D.F. MEX.

In this communication it is shown, by the first time, some interesting morphological changes that were observed in dendrites and axones from the frontal cere bral cortex of a young adult, who was clinically and histológically diagnosed as a case of Jacob-Creutzfeldt.Long time ago,it was learned that the nervous tissue from these cases, showed some "holes" or perforations, which,in some time, were regarded as artifacts. More recently, they were demonstrated with the E/M, and thereafter considered as a substantial feature of this neurological disease.It is the purpose of this presentation to provide further structural knowledge obtained in a cortical biopsy impregnated with the Golgi method.

The overview of the Golgi-stained material, showed a generalized decrement in the size of neurons, as well as in the number of spines and dendrites.Nevertheless, the remaining dendrites and axones showed a rather normal aspect, even though some appeared thinner.When these structures were studied at higher magnification, it became clear the presence of spherules along the neuronal branches.In general, each process bears 2 or 3 of them (dendrites).Ocassionally they were seen at the level of the neuronal somata.Those in the axones were more numerous.On the other hand,those of dendritic origin were less spherical.It is important to emphasize, that the Golgi-impregnated material did not show any "holes" or perforations, as it was expected to do.

These findings allow to state that in the spongiform status, there is a segmentary and well defined process of dilation in dendrites and axones, which apparently never ends with rupture of membranes, but it parallels the decline of neural function.

136.13 ULTRASTRUCTURE OF CULTURED OLIGODENDROCYTES. R.L.Wollmann and S.Szuchet:Dept. of Neurology, University of Chicago,950 E. 59th St.,Chicago, Illinois 60637:

The presence of junctional complexes between oligodendrocytes in vivo has been documented.(Mugnaini,E.&Walberg,F.Rev.Anat. Embrol.&Cell Biol.37,194,1964;Dermietzel et al.,Cell&Tissue Res. 193,61(1978). Here we report that oligodendrocytes maintained in vitro also develop specialized junctions. Oligodendrocytes were isolated from ovine white matter and were maintained in Dulbecco's modified MEM supplemented with 20% horse serum and 20mM glutamine(Szuchet et al.,Brain Res.200:151,1980). We have followed the ultrastructural changes accompanying the initial seeding and further development of oligodendrocytes in culture, using transmission electron microscopy. Fourteen hours after being plated on plastic culture dishes most cells have formed floating aggregates; these take several days to attach. The ultrastructure of clustered cells, unlike the cells fixed immediately after isolation, revealed an electron lucent cytoplasm, no clumping of nuclear chromatin and abundance of intracellular organelles. These cells resembled the light oligodendrocytes described by Mori and Leblond(J.Comp.Neurol;139,1,1970). The cells in clusters were in close apposition to each other. The apposed plasma membranes exhibited intense osmiophilia which at high magnification appeared as septilamellar structures approximately 21nm in thickness. In some sections, the two inner lamellae were seen to be bridged in a regular fashion by filaments or tubules. These junctional complexes were reminiscent of gap junctions. After 4 to 5 days in culture, most cells had attached to the culture dishes but a certain proportion remained as floating clusters. Examination of the latter showed the disappearance of the "gap"junctions but now short complexes resembling zonula adherens were found. The cells that adhered to the culture plates did not differ significantly in their cytoplasmic constituents from the floating cells. Attached cells extended long membranous processes which contained large numbers of microtubules,filaments,polyribosomes, and an occasional mitochondrion. In established cultures the membranous sheets became highly complex and organelles such as cisternae of endoplasmic reticulum,membrane bound vesicles, lipid droplets and mitochondria were in abundance. Oligodendrocytes isolated from young lambs are able to express in vitro, properties associated with these cells in the intact organism. Hence, cultured oligodendrocytes should prove useful for studying interactions between oligodendrocytes and between these cells, neurons and astrocytes, and thus, enhance our understanding of myelin synthesis and maintenance.

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136.14 DEVELOPMENT OF TANYCYTES IN THE RAT THORACIC SPINAL CORD FROM DAY 14 OF GESTATION TO ADULTHOOD-A LIGHT MICROSCOPIC STUDY.
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Ependymal tanycytes lining the cerebro-ventricular system and the central canal of the spinal cord have been implicated in a number of processes including: 1) neuroendocrine regulation, 2) the absorption and transport of CSF-borne substances, 3) neural migration and 4) axonal guidance. Using a modified Golgi-Cox stain this study was undertaken to further explore the ontogeny of spinal cord tanycytes in the rat thoracic spinal cord from day 14 of gestation to adulthood.

At day 14 of gestation the length of the somata, which either border the central canal or are located within the deep layers of the ependyma, ranged between 15 and 40µm. The somata often gave rise to one or more cilia-like processes that projected 2-8µm into the central canal. The necks of the cell bodies ranged from 10-30µm in length and often had mound-like varicose enlargements giving rise to long (4-12µm) filopodial-like processes. The tanycyte shafts, extending to the pial surface, also had similar intermittent mound-like varicosities from which filopodial-like (often bifid) processes 6-10µm in length protruded into the neuropil. These mound-like enlargements were most numerous on the distal one-third of the shafts. The proximal one-third of the shafts had scattered barbed spines 1-3µm in length while the middle one-third of the shafts was usually quite smooth, but did have either an occasional short spine or mound-like varicosity with a filopodial-like process. The shafts often branched and contacted cerebral vasculature and neural elements as they coursed through the neuropil prior to terminating in small (4-8µm long) swollen end-feet on the pial surface of the spinal cord.

At birth the tanycyte somata and necks remained similar to those described at day 14 of gestation. However, by birth the shafts had bead-like enlargements from which an occasional short (.5-1µm) spine protruded. Otherwise, the necks and shafts were virtually spine-free. The pial end-feet continued to enlarge (20-40µm in length), and there was an increase in the number of long (6-10µm) filopodial-like process arising from the end-feet.

By adulthood (60 days of age) the tanycytes arising from the lateral aspects of the central canal had stubby necks and very short (20-40µm) shafts that exhibited occasional short (.5-2µm) spines. The pial end-feet had continued to enlarge (40-80µm in length) with most of the filopodial processes having been replaced by longer and thicker dendrite-like processes. These "differentiated" pial end-feet resembled sub-pial astrocytes.

136.15 SCANNING ELECTRON MICROSCOPY (SEM) OF THE MIDLINE FLOOR OF THE FOURTH VENTRICLE OF THE RABBIT. B.T. Burnett*, V.H. Gattone*, B.A. Connors*, A.P. Evan*, and D.L. Felten. Department of Anatomy, Indiana University School of Medicine, 1100 W. Michigan St., Indianapolis, IN 46223.

Recently we have shown tanycytes on the floor of the fourth ventricle in the rabbit brain with shafts entering raphe dendrite bundles (J. Comp. Neur., 183:1-24; Neurosci. Lett., 16:275-280). In order to assess the possible functional roles of these cells, we examined their surface ultrastructure with SEM. The midline floor of the fourth ventricle contains clusters of tanycytes adjacent to nuclei raphe obscurus and dorsalis, demonstrating tight junctions between the lateral tanycyte surfaces. With SEM, differences were noted in the pattern and density of cilia, microvilli, and supraependymal elements and fibers on the midline (25-75 um) floor, including scant clusters of cilia with preterminal and terminal bullae, prominent single cilia, a dense and relatively uniform mat of clavate and filiform microvilli, and occasional areas of smooth ependymal surface apparently devoid of microvilli and cilia. A threadlike reticular network of fibers was found on nearly all of the midline ventricular surface, consisting of fine (0.3 um or less) strands which intertwined and possessed bullous and fusiform varicosities approximately 1.0 um long at 2-4 um intervals. This reticular network often converged in a radiating pattern in areas relatively devoid of microvilli which possessed multiple globoid 2-4 um swellings. Portions of the reticular network coursed through the microvillous cover of the surface, disappearing into the clusters of cilia. Supraependymal smooth spherical elements (0.3-0.6 um) occurred singly, in clusters of 50-80, or intermixed with clusters of cilia at their base. Some single elevated projections (0.5-1.5 um) were covered with microvilli. Two cell-like supraependymal structures were noted in the midline. Small (4 um dia.) ovoid cells possessed a single bifurcating process near the soma, which disappeared into the ependymal surface. Large (15-20 um dia.) tear-drop shaped unipolar cells sent a tapering, spiraling process across the ependymal surface, where it disappeared into the ependyma within 40 um of the soma. We suggest that the prominent microvillous cover serves an absorptive function for tanycytes. The smooth globoid structures and areas of cilia may serve a secretory function. The supraependymal cell-like structures and the reticular fibrous network may represent a supraependymal neuronal network of connections. The present SEM findings are consistent with an active absorptive and secretory role for tanycytes on the midline floor of the fourth ventricle.
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136.16 ORTHOGONAL ARRAYS IN GLIA OF THE MEDIAN EMINENCE. James D. Hatton and Mark H. Ellisman, Department of Neurosciences, U. Calif. at San Diego, La Jolla, CA 92093

The distribution of orthogonal arrays of particles and their relationships to gap and tight junctions has been studied in the glia of the freeze-fractured rat median eminence (ME). These rectilinear clusters of intramembrane particles are thought to represent trans-membrane channels for ions or metabolites. Analysis of the cellular distribution of these structures may aid in understanding their function. These orthogonal arrays were found to be densely packed on the membranous laminations of the pial-glial limitans forming the ventral border of the ME. Arrays were found to be present on all of the perivascular glial end-feet examined, surrounding either fenestrated or non-fenestrated capillaries. Two classes of end-feet were distinguished by their relative densities of orthogonal arrays. End-feet displaying low densities of arrays occurred more frequently in the internal zone, while end-feet displaying high densities occurred more often in the external zone. Similar distinctions based on orthogonal array density could be made in membranes from other regions of the cell as well. Cross-fractures revealing the cytoplasm underlying these array-poor membranes often exposed lipid inclusion bodies, suggesting that membranes containing few arrays belong to tanycytes or to "astrocyte-like" tanycytes. Such inclusion bodies were not found where cross-fractures exposed cytoplasm beneath array-replete membranes. The distribution of arrays appeared to be unrelated to the distribution of gap junctions in the membranes of astrocytes, tanycytes, and "astrocyte-like" tanycytes of the ME, appearing near to and far from gap junctions with approximately equal frequency. Orthogonal arrays were absent from glial membranes near synaptic profiles in the ME. Arrays were also absent from the microvillous membranes of the apical surfaces of ependymal cells, from the cytoplasmic protrusions into the CSF of tanycytes, and from the vicinity of the tight and complex junctions linking the tanycyte and ependymal cell lateral membranes near their apical poles. These results suggest that there is a gradient of array density for most glia of the ME, increasing from ventricular to pial surface. Furthermore, it is apparent that orthogonal arrays are present on glial end-feet surrounding capillaries in an organ lacking the traditional blood-brain barrier, suggesting that arrays are not functionally involved therein.

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136.17 LIGHT- AND EM ANALYSIS OF THE PROLIFERATING GLIAL CELLS IN A PARTIALLY DENERVATED NEUROPIL AT EARLY POSTLESION STAGES. C.Avendaño. Dept. Morfología, Fac. Medicina, U. Autónoma, Madrid 34, SPAIN.

Substantial controversy exists about the nature of the non-neuronal cells that react in the nervous tissue in response to an insult. The kind of cellular response appears to depend largely on the degree of inflammation produced by the lesion (Vaughn & Pease, '70): stab wounds or inflammatory diseases clearly elicit an invasion of the lesioned area by circulating phagocytes, while the participation of these cells in wallerian or retrograde degeneration processes, if any, is controverted. In the latter processes some agreement exists that the cells that proliferate and turn into phagocytes resemble morphologically either microglia or undifferentiated "stem cells". Moreover, well differentiated oligodendrocytes, perivascular elements and astrocytes have been described to proliferate in some cases. While there is a good deal of information on the proliferative glial response to wallerian degeneration in myelinated fiber tracts, little is known of this response in the gray matter, perticularly in areas in which no retrograde degeneration of neurons is involved. The rat dentate gyrus (FD) is a well-suited model for studying the glial reaction to denervations of controlled intensity. Avendaño & Cowan ('79) have shown that the cells that proliferate after interrupting the commissural connections of FD (which presumably elicits no noticeable inflammation in FD) do not exhibit typical features of microglia at LM. Here, using the same ^{3}H-thymidine-treated, plastic-embedded material, a direct correlation was made between the LM and EM appearance of the cells labeled at the peak of the proliferative period (6 h after an injection of ^{3}H-thymidine given 30-36 h after lesion). The technique used was similar to Kaplan & Hinds' ('77) procedure of re-embedding and thin-cutting 2 µm-thick sections which had been previously coated with photographic emulsion, exposed and revealed for autoradiography. At LM the labeled nuclei appeared to be fairly large (6-8 µm larger diameter), ovoid or elongated, sometimes markedly indented, with chromatin clumps standing out against a relatively light nucleoplasm. The cytoplasm, scanty, weakly stained and usually devoid of dense inclusions, was sometimes seen to accumulate or to send a broad process at one side of the nucleus. The most relevant features at EM were: well-developed Golgi complex, abundant polisomes, few and short cisternae of granular ER with a moderately electron dense content, multivesicular bodies, absence of filaments and scarcity of microtubules and inclusion bodies. The surrounding extracellular space appeared sometimes moderately enlarged. These findings suggest that most of these proliferating cells correspond to poorly-differentiated microglia-like cells in a reactive state.

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136.18 ALTERATIONS OF ASTROCYTIC PLASMA MEMBRANES AFTER EXPOSURE TO HIGH CO_2 IN VITRO. J. J. Anders and M. W. Brightman. (Spon. David I. Rubin). NINCDS/LNNS, National Institutes of Health, Bethesda, MD 20205.

Distinct aggregates (assemblies) of small (6-7 nm) particles characterize the P-face of frozen, cleaved and replicated astrocytic membranes. In mammals, assemblies increase with the age and reactivity of the astrocytes. Astrocytes in primary cultures of dissociated cerebral cortex from 7 day old rats have assemblies, but they are fewer than in in vivo astrocytes of a comparable age. The longer astrocytes remain in vitro, the fewer the assemblies within their membranes. For this reason, astrocytes 14 days in vitro were used in all experiments. We have reported that assemblies are affected by the protein inhibitor cycloheximide (10^{-4}M, 3 hrs) and protein denaturants, guanidine HCl (2M, 30, 60 min) and urea (5M). Distribution of assemblies in astrocytic membranes is also differentially altered by cytochalasin B (5×10^{-5}M, 60 min) and colchicine (10^{-4}M, 30, 60 min). With cytochalasin B, intramembranous particles, including assemblies, clump, while with colchicine, the assemblies selectively form "cap-like" aggregates. These changes suggest that the distribution of assemblies is associated with cytoplasmic contractile proteins. Since assembly/disassembly of cytoplasmic proteins is related to pH, CO_2 was chosen to alter the intracellular pH of the astrocytes. Primary cultures were subjected to 100% CO_2 at 37°C until the medium was saturated (pH of medium, 5.5). The cultures were then fixed with 5% glutaraldehyde after 5, 15 and 30 minutes. The cells were frozen, fractured and replicated by standard procedures. By 15 minutes, some astrocytic membranes had clumped intramembranous particles resembling the distribution seen after cytochalasin B treatment. After 30 minutes, the number of assemblies increased in the majority of astrocytes, while in others, the assemblies were clumped. Most of these assemblies were small, consisting of only a few particles, and resembled those in astrocytic membranes of fetal and newborn rats where assemblies are added. The cap-like formations caused by colchicine were not seen after CO_2 treatment. Cultures exposed to hydrochloric acid at low pH (2,4,6) had no effect on the membranes. We are currently determining whether the increase in assemblies is due purely to anoxia or acidification of the cytoplasm. Evidence is increasing which suggests that intracellular pH is an important factor in cytoskeletal regulation. Thus, decreases in pH which occur in the brain during seizures and ischemia may cause a change in intracellular pH affecting not only the astrocytic cytoskeleton but also its membrane morphology.

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137.1 ^{3}H-RAUWOLSCINE BINDING TO α_2-ADRENERGIC RECEPTORS IN BOVINE BRAIN
B. D. Perry* and D. C. U'Prichard (SPON:E. Silinsky). Dept. of Pharmacology, Northwestern Univ. Med. Sch., Chicago, Ill. 60611

α_2-Adrenergic receptors (R) have been labeled in central and peripheral tissues with ^{3}H-catecholamine and ^{3}H-imidazoline agonist ligands. ^{3}H-Agonists label brain α_2-R in the high affinity (agonist binding) state. It is unclear whether ^{3}H-agonist sites represent the total brain α_2-R population. This question may be addressed by examining the binding characteristics of a selective α_2-antagonist ligand. ^{3}H-Yohimbine (YOH) has been used to characterize α_2-R in platelets and neuroblastoma X glioma cells; in brain, however, the mixed α-adrenergic R population and the moderate α_2-selectivity of YOH complicate characterization. Recently it has been established that rauwolscine (RAUW, α-yohimbine) is a more potent and selective antagonist than YOH at central α_2-R (Hedler et al., Eur.J.Pharm., 70:43-52. 1981), indicating that ^{3}H-RAUW may be a more suitable ligand for examining α_2-R in tissues containing a mixed population of adrenergic R. ^{3}H-RAUW (80-90 Ci/mmol) binding was examined in bovine cortex membranes. Norepinephrine (NE)-displaceable ^{3}H-RAUW (1.0 nM) binding was 75-80 % of total binding. Binding was optimal in 50 mM Na-K phosphate buffer (pH=7.4) at 4^{o}C. Association and dissociation kinetics revealed a single order of sites with k_1/k_{+1} = 1.2 nM. ^{3}H-RAUW binding was saturable, and again monophasic, with an apparent K_D of 1.0-3.0 nM, in good agreement with the kinetic K_D. Agonists and antagonists inhibited ^{3}H-RAUW binding in a manner suggesting α_2-R interactions: K_i values for (-)-epinephrine (EPI), (-)-α-Me-NE, (-)-NE, (+)-NE, (-)-isoproterenol, p-aminoclonidine, and oxymetazoline were 150; 200; 500; 4600; 40,000; 2.0 and 2.0 nM, respectively. Agonist competition curves were shallow (n_H = 0.6-0.8). For antagonists, yohimbine (4 nM) was 1000 times more potent than prazosin (4 μM). Antagonists had high (0.9-1.0) n_H values. In contrast, prazosin inhibited cortex ^{3}H-YOH binding with greater potency and in a heterogeneous manner. (-)-EPI K_i values, in 4^{o}C conditions, were increased 5-fold by 100 μM GTP alone and 2-fold by GTP in combination with 60 mM Na^+. GTP increased agonist n_H values to about 1.0. In contrast, Na^+ increased (-)-EPI K_i values 4-fold but did not increase the n_H values. Regional distribution of ^{3}H-RAUW sites (30 bovine brain regions) was consistent with prior studies with ^{3}H-α_2-agonists. The areas of highest ^{3}H-RAUW binding included frontal cortex, anterior hypothalamus, and cerebellum. ^{3}H-RAUW saturation in 5 brain areas revealed similar K_D values. The results indicate that ^{3}H-RAUW binds with high affinity and selectivity to a single population of brain α_2-adrenergic R. ^{3}H-RAUW should prove a useful implement to further analysis of α_2-R function. Supported by USPHS grant NS 15595.

137.2 VIPOXIN: A SNAKE VENOM PROTEIN LIGAND FOR BIOGENIC AMINE RECEPTORS.
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Vipoxin (VPX) is a small, highly basic protein, molecular weight 13,000, which we have purified to apparent homogeneity from the venom of Russell's viper (Vipera russelli). VPX inhibits in a dose-dependent manner the binding of ^{3}H-ligands to biogenic amine receptors, with apparent Ki values of 3 nM at alpha$_1$-adrenergic receptors, 5 nM at alpha$_2$-adrenergic receptors, 15 nM at dopamine receptors, and 32 nM at serotonin receptors. VPX up to 1 μM is inactive at beta-adrenergic, histamine, nicotinic cholinergic, muscarinic cholinergic, adenosine, GABA, benzodiazepine, and opiate receptors. The inhibition of alpha-adrenergic receptor binding is essentially irreversible over a 20-hour period, but can be protected against by 100 μM norepinephrine. VPX also possesses phospholipase A_2 activity, which appears not to account for its actions because VPX is active in 1 mM $CoSO_4$, which completely and selectively inhibits the phospholipase activity.

We have studied the physiological actions of VPX at alpha-adrenergic receptors in the rat vas deferens. At alpha$_2$-adrenergic receptors, VPX is an agonist. Its response is blocked by yohimbine and piperoxan selectively, and is of slow onset and extremely slow recovery. VPX is over an order of magnitude more potent than norepinephrine itself. VPX is neither a simple agonist nor an antagonist at alpha$_1$-adrenergic receptors, but potently and selectively potentiates the actions of norepinephrine. It is unexpected for a venom protein to have agonist-like actions, and it is particularly interesting that an agonist should have phospholipase activity.

We have recently prepared ^{125}I-VPX to a specific radioactivity of 4000 Ci/mmole, with at least partial retention of pharmacologic activity. We are beginning binding studies in brain and peripheral tissues, and expect VPX to be a useful ligand for receptor binding, as well as a valuable tool for the purification and biochemical characterization of receptors.

137.3 COMPETITION BY ESTROGENS FOR CATECHOLAMINE RECEPTOR BINDING IN VITRO.
C. M. Paden, L. Snyder*, V. DeGroff* and B. S. McEwen. The Rockefeller University, New York, New York 10021.

Estrogens with hydroxyl groups on either the 2 or 4 carbon atoms of the A ring are referred to as catechol estrogens, and these compounds can be formed by hydroxylation of estrogens in neural tissue. Catechol estrogens have been shown to inhibit catecholaminergic enzymes and to displace ^{3}H-spiperone from anterior pituitary dopamine receptors. These reports have led us to investigate the interactions of catechol estrogens as well as other steroid hormones with a variety of catecholaminergic receptor sites in the rat brain.

Female Sprague-Dawley rats were ovariectomized at least one week prior to use. Membrane binding assays were performed using published procedures with 28,000 x g pellets centrifuged and resuspended twice in tris buffers. Incubations were performed at 25^o C for 30 min. and bound ligand separated by rapid filtration over Whatman GF/B filters. Dopamine receptors in striatum and pituitary were assayed using ^{3}H-spiperone; specific binding was that suppressed by 1 μM d-butaclamol. Beta noradrenergic receptors in cortex were assayed using ^{3}H-dihydroalprenolol; specific binding was that suppressed by 1 μM propranolol. Alpha noradrenergic receptors were assayed using 2,6-dimethoxy ethyl amino methylbenzo-1,4-dioxan (^{3}H-WB4101) or ^{3}H-prazosin (α_1) and ^{3}H-yohimbine (α_2); specific binding was that suppressed by 1 μM phentolamine. The α_2 agonist ^{3}H-clonidine was also used, and specific binding was that suppressed by 100 μM norepinephrine.

An initial survey utilized six steroids added to incubations at a final concentration of 100 μM; 2-hydroxy estradiol (2OHE$_2$), estradiol-17β (E$_2\beta$), estradiol-17α (E$_2\alpha$), progesterone, testosterone and corticosterone. Only the 17β estrogens, 2OHE$_2$ and E$_2\beta$, were effective and only binding of ^{3}H-spiperone in striatum and pituitary and ^{3}H-WB4101 and ^{3}H-prazosin in cortex were reduced. Thus only putative dopaminergic and α_1 noradrenergic sites appear to recognize estrogens. Further experiments varying steriod and ^{3}H-ligand concentrations independently revealed that estrogens were competitive inhibitors of ^{3}H-prazosin binding. The IC_{50} of the most potent estrogen, 2OHE$_2$, was 32 μM, comparable to that of norepinephrine (13 μM). In both striatum and pituitary, 2OHE$_2$ was again the most potent estrogen, but it was far less effective than dopamine itself in displacing ^{3}H-spiperone, with IC_{50}'s of 160 μM and 1 mM in striatum and pituitary, respectively, compared to 12 μM and 5 μM for dopamine. These results show that there is specificity of steroid interactions with catecholamine receptors, both in terms of steroid structure and receptor type. However, the physiological relevance of these effects remains to be established.

137.4 ADRENERGIC RECEPTOR SUBTYPES IN AUTONOMIC TISSUES: RADIOLIGAND BINDING TO RABBIT IRIS-CILIARY BODY MEMBRANES.
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Receptor subclasses in iris-ciliary body were determined by direct binding of radioactive dihydroalprenolol (DHA), yohimbine (YOH), WB-4101 (WB) and prazosin (PRZ), classified respectively as β_1 + β_2, α_2, α_1, and α_1 subtype selective ligands (based on binding to brain adrenergic receptors). Rabbit iris-ciliary body membrane fragments in 50 mM phosphate buffer, pH 7.4, 5 mM $MgCl_2$, 0.1 mM GTP and ascorbic acid (0.5 -.025%) were used in glass fiber filter binding assays. Specific binding was defined by competition with appropriate unlabelled agonist and antagonist in each case. Binding data was analysed by use of library programs of the PROPHET computer system.

Binding parameters for the labelled ligands were determined from 4 or more complete binding isotherms as follows: K_d in nM, B_{max} in fmoles/mg protein; DHA = 0.675 $\pm$ .024, 132 $\pm$ 4.9. YOH = 12.48 $\pm$ 1.8, 256 $\pm$ 27.8. WB = 1.33 $\pm$ 0.45, 152 $\pm$ 9.6. PRZ = 0.552 $\pm$ .174, 51.8 $\pm$ 5.9. The Hill coeffs. were close to unity in all cases.

Subclass specificity was also determined by binding competition of the labelled ligand (at a 3-10 fold K_d concentration) with increasing concentrations of cold agonist or antagonist. Cold ligand binding parameters were obtained from Dixon and Scatchard plots. Hill coeffs. for agonists were in the range 0.7 - 0.9, and for antagonists 0.8 - 1.1, except as noted.

For β receptors (determined against DHA) K_d for norepinephrine = 8.54 μM and K_d for epinephrine = 1.07 μM. The ratio of affinities (8 - 15) classify the DHA binding receptors as >80% β_2 subtype.

For β receptors subtype specificity of binding ligands could not be defined. Clonidine (classified as α_2 specific) had similar K_d for PRZ, WB and YOH (0.47, 0.38, 0.50 μM respectively). The K_d's (nM) for cold competing antagonists were determined as follows: Against labelled PRZ, cold WB = >1000, cold PRZ = 0.73; against labelled WB, cold WB = 1.16, cold PRZ = >1000, cold YOH = 50.4 (Hill coeff. 0.5); against labelled YOH, cold WB = 58.7 (Hill coeff. 0.5), cold YOH = 7.1.

Adrenergic receptor density of iris-ciliary body is $\pm$ 600 fmoles/mg protein of which 20-25% are primarily β_2 receptors. Three distinct subpopulations of α-receptors, 10%, 25% and 40-45%, bind PRZ, WB and YOH respectively, each with high specificity for its corresponding ligand but with 10-1000 fold lower specificity for the other two ligands. (Supported by Grant EY-02619 from the National Institutes of Health).

137.5 THE EFFECTS OF MICROINJECTION OF 6-HYDROXYDOPAMINE INTO THE LOCUS COERULEUS OR THE LATERAL RETICULAR NUCLEUS ON ALPHA$_1$-RECEPTORS AND NOREPINEPHRINE CONCENTRATIONS WITHIN THE VENTRAL HORN OF CAT CERVICAL SPINAL CORD. C.H. Park*, J.P.Suyderhoud*, and V.J.Massard Dept. Pharmacol., Howard U., Col. of Med., Washington, DC 20059.

Experiments are in progress to determine the quantitative contribution of two "bulbospinal" noradrenergic (NE) nuclei to measured levels of NE within individual lamina of each segment of the spinal cord. It was also of interest to determine the normal distribution of alpha$_1$ adrenergic receptors in specific laminae of the spinal cord, and to investigate if these receptors could be manipulated pharmacologically. Two groups of cats received a bilateral sterotaxic injection of the NE neurotoxin 6-hydroxydopamine (4μG/μL)into either the locus coeruleus (A$_6$)or the lateral reticular nucleus (A$_1$). A control group was operated upon, but not injected. All animals were sacrificed 10 days after surgery and the entire central nervous system was rapidly removed and frozen on dry ice. Correct lesion placement was verified histologically. The spinal cord was cut in a cryostat alternately into 660μ slabs and 60μ sections. The thin sections were stained and used as a guide to the microdissection. NE concentration was determined in Lamina IX for each segment of the cervical enlargement of the spinal cord i.e.,C$_6$,C$_7$,C$_8$,and T$_1$. Alpha$_1$ receptor binding was measured in a pooled aliquot from the same tissues. Alpha$_1$ receptor binding was estimated using the specific alpha$_1$ receptor antagonist Prazosin-H^3 as a ligand. In control cats,NE concentration in Lamina IX did not vary significantly from segment to segment in the cervical enlargement. A$_6$ lesions caused a uniform and statistically significant 62±5% (X̄±S.E.M.)reduction in NE in all these segments. However A$_1$ lesions caused a significant 56±8% reduction of NE only in segment C$_7$. A$_1$ lesions did not effect ^{3}H-Prazosin binding to alpha$_1$ receptors. A$_6$ lesions, however, caused a 20% increase in binding which just missed statistical significance ($p<.06$). These data show that the quantitative contribution of the A$_1$ nucleus to measured levels of NE in discrete laminar areas of the spinal cord varies from segment to segment. Previous data suggested that A$_1$ projects only to the dorsal horn of the spinal cord. The present results indicate that A$_1$ also has a NE terminal projection to Lamina IX of the ventral horn in segment C$_7$. Within this segment, it also appears that almost all the NE terminal input may come from the A$_1$ and A$_6$ neuclei Lesions of A$_1$ caused relatively little decline in NE levels in Lamina IX of the entire cervical enlargement. Correspondingly,no effect was seen on alpha$_1$ receptor binding. On the other hand A$_6$ lesions, which caused a substantial decrease in NE throughout this area,caused a slight increase in Prazosin binding,which may reflect the onset of denervation supersensitivity.

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137.7 REGULATION OF α_2-ADRENERGIC RECEPTOR SITES IN NEUROBLASTOMA X GLIOMA HYBRID CELLS. D.J.Kahn and D.C.U'Prichard. Department of Pharmacology, Northwestern University Medical School, Chicago, IL., 60611.

We have examined the regulation of rodent neuroblastoma x glioma, NG 108-15, α_2-receptors (R) using the antagonist radioligand, ^{3}H-yohimbine (YOH, mean K_D 8 nM), the partial agonist, ^{3}H-p-aminoclonidine (PAC, K_D 1.8 nM) and the full agonist ^{3}H-epinephrine (EPI, K_D 11 nM), along with the coupled biochemical response of a GTP-dependent inhibition of adenylate cyclase(AC). We have previously shown that agonists preferentially label a high-affinity form of the α_2-R in NG 108-15 cells, α_2(H), while ^{3}H-YOH recognizes α_2(H) and a low affinity form, α_2(L), with equal affinity. In order to examine regulation of α_2-R, NG 108-15 cells were grown for 1-16 hours in the presence of (-)-EPI. Results indicate that in NG 108-15 cell α_2-R down-regulation, uncoupling precedes receptor loss. That is, after 1-4 hours of EPI incubation there was no change in the total receptor number labeled by any of the radioligands, but the number of high-affinity sites labeled by ^{3}H-EPI or ^{3}H-PAC was increased. This corresponded to an observed decrease in the potency of EPI for AC inhibition, while the affinity of YOH for reversing AC inhibition or the maximal EPI response were unchanged. Longer incubation times increased basal and stimulated AC, but there was no observed tolerance to the α_2-R AC response. However, following 8-16 hours of EPI incubation there was a progressive loss of receptor sites. By 16 hours the B_{max} of ^{3}H-YOH was decreased by 60% with no change in K_D, while ^{3}H-EPI and ^{3}H-PAC binding were similarly reduced in extent (60-70%) with an accompanying increase in K_D for both agonists (EPI K_D from 8 to 17 nM, PAC K_D from 1.9 to 6.6 nM). The results indicate that NG 108-15 α_2-R are able to be regulated and that down-regulation involves two phases. An earlier phase may be uncoupling of the α_2-R from AC, represented by a shift from low to high affinity states but no receptor loss, and a decrease in EPI potency for AC inhibition. A second phase observed after longer agonist incubation periods is represented by significant loss of α_2-R from NG 108-15 cell membranes, along with a steadily increasing basal AC activity. Agonist and antagonist radioligand binding data suggest that high affinity (α_2(H)) states are preferentially lost in the second phase of α_2-R down-regulation. Studies are in progress to examine agonist-induced loss of different α_2-R states in more detail, and to correlate regulation of binding sites and the adenylate cyclase response.

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137.6 REGULATION OF ADRENERGIC RECEPTORS ON RAT CORTEX NEURONS BY CONGENITAL NORADRENERGIC HYPERINNERVATION AND SUBSEQUENT DENERVATION C. H. Wang and D. C. U'Prichard. Dept. of Pharmacology, Northwestern Univ. Med. Sch., Chicago, IL 60611.

Bilateral lesion of noradrenergic (NA) bundles, or intracerebroventricular (i.c.v.) 6-hydroxydopamine (6-OHDA) treatment, causes increases in rat cortical β-, α_1- and α_2-adrenergic receptors (Adr-R)(U'Prichard et al. 1979, 1980). It is not known however if different cortical Adr-R subtypes can be down-regulated to the same extent by increasing the amount of presynaptic NA inputs. In this study, we examined pharmacologically-induced fetal NA hyperinnervation, and subsequent NA lesion with 6-OHDA, as a means of modulating presynaptic NA input to rat cortical neurons, and we measured subsequent alterations in different Adr-R subtypes labeled with both agonist and antagonist ligands. Pregnant female rats injected with the antimitotic agent methylazoxymethanol acetate (MAM) on gestation day 15 gave birth to offspring with microencephaly and cortical NA hyperinnervation. Previously Johnston and Coyle (Brain Res. 170:135,1979) reported MAM-induced reductions in β-receptor binding per mg cortex prot. We found significant decreases in B_{max} (fmole/mg prot.) for all cortical Adr-R subtypes in 10-16 wk old male or female progeny of MAM-treated rats:β-R (-28%, ^{3}H-dihydroalprenolol, DHA);α_1-R (-19%, ^{3}H-prazosin);α_2-R (-40%, ^{3}H-yohimbine, YOH; -27%, ^{3}H-p-aminoclonidine,PAC) Radioligand K_D values were not significantly altered. In control cortex, catecholamine agonist competition curves at β- and α_2-R labeled with antagonist radioligands are shallow, indicating multiple R affinity states ((-)-isoproterenol vs. DHA: IC_{50}=41 nM, n_H=0.72); (-)-epinephrine vs. YOH: IC_{50}=130 nM, n_H=0.79).Residual β- and α_2-R after MAM treatment were further characterized by examining agonist competition profiles. No changes were observed in either IC_{50} or n_H values for (-)-isoproterenol vs. DHA or (-)-epinephrine vs. YOH, between MAM and control groups. Thus there is no preferential loss of high-affinity states of β- or α_2-R as a result of MAM treatment. Bilateral i.c.v. 6-OHDA treatment of 16 wk old MAM rats increased B_{max} values 80% for cortex PAC binding, but only 18% for YOH binding. No change was seen in ligand K_D values. These increases in α_2-R densities were significantly greater than increases observed after i.c.v. 6-OHDA in control rats. The results indicate that different cortical Adr-R subtypes can be reduced in total number by increasing presynaptic NA input, with no change in other R characteristics. Differential effects of 6-OHDA lesions in control and MAM rats indicate that denervation-induced increases in Adr-R number on cortical neurons may depend on the density of NA input prior to denervation.

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137.8 IDENTIFICATION OF β-ADRENERGIC RECEPTOR (β-AR) SUBTYPE ON RAT ASTROGLIA AND OLIGODENDROGLIA. K.D. McCarthy and T.K. Harden (Spon: R.B. Mailman) Dept. Pharmacology, Univ. North Carolina School of Medicine, Chapel Hill, NC 27514.

Membrane preparations from rat cerebral cortex and cerebellum express very different ratios of β_1-AR to β_2-AR (81:19 in cerebral cortex, 15:85 in cerebellum; Minneman et al, Mol. Pharmacol. 16:34, 1979). The cellular localization of these receptor subtypes is not known. However, indirect studies using drug-induced alterations in noradrenergic input suggest that it is the β_1-subtype that receives endogenous innervation by norepinephrine-containing neurons (Minneman et al, Science 204:866, 1979). The present study was undertaken to identify the β-AR subtype that exists on glial cells. Astroglia and oligodendroglia were purified from rat cerebral cortical cultures. Astroglia were also purified from rat cerebellum cultures. ^{125}I-hydroxybenzylpindolol (^{125}IHYP) was utilized to identify β-AR in membrane preparations obtained from these cells. ^{125}IHYP bound with a similar Kd (20-35 pM) to receptors of cultured astroglia, oligodendroglia, C6BD12 glioma, and 132INI astrocytoma cells. Similar affinities for ^{125}IHYP were also obtained using membranes from rat heart, lung, and cerebral cortex. Competition binding curves were generated with the β_2-AR selective drugs, zinterol and ICI 118,551, and the β_1-AR selective drug, practolol. The following K_i values (nM) were obtained (n = 2-6):

Tissue	Zinterol	ICI 118,551	Practolol
heart	364±25	48±13	669±116
lung	42±3	2±0.4	13,214±3004
cerebellum astroglia	309±77	67±15	1004±178
cerebral cortex astroglia	800±203	101±32	780±70
cerebral cortex oligodendroglia	375	118	
C6 BD12 glioma	480±50	42±2	
132INI astrocytoma	47±7	2±1	

Hofstee plots of competition binding curves with these three drugs using membranes from cultured astroglia and oligodendroglia as well as membranes from C6 BD12 glioma and 132INI astrocytoma cells were fit by a single line suggesting that ^{125}IHYP interacts with a single receptor subtype. In contrast, Hofstee analysis of competition curves with membranes from adult rat cerebral cortex and heart were nonlinear suggesting the presence of two binding components. Our data are consistent with the conclusion that a single subtype of β-adrenergic receptor exists on astroglia from rat cerebral cortex and cerebellum and oligodendroglia from rat cerebral cortex. This receptor is of the β_1-subtype. Supported by NS 16992.

137.9 REMOVAL OF CHAOTROPIC AGENTS ELIMINATES THE ENHANCEMENT OF BICUCULLINE INHIBITION OF HIGH AFFINITY GABA BINDING IN BOVINE RETINAL MEMBRANES. Lynn Churchill and Dianna A. Redburn, Dept. of Neurobiol. & Anat., Univ. of Texas Med. Sch., Houston, TX 77025.

The chaotropic agent, ammonium thiocyanate, enhanced bicuculline inhibition of high affinity binding of γ-aminobutyric acid (GABA) to rat forebrain membranes (Enna and Snyder (1977) Mol. Pharmacol. 13, 442) and the chaotropic agent, sodium perchlorate, was used to assay both agonist (GABA) and antagonist (bicuculline) binding under the same conditions in rat cerebellum (Mohler (1979) In: GABA-Biochemistry and CNS Functions (Mandel and DeFeudis, eds) Plenum Press, N.Y., p. 355). We have examined bicuculline inhibition of high affinity (10 nM) GABA binding in synaptosomal fractions from bovine retina and found a similar enhancement of bicuculline inhibition in the presence of both ammonium thiocyanate and Tris perchlorate. The 50% displacement (IC_{50}) of high affinity GABA binding with bicuculline was 0.2 μM in the presence of 100 mM ammonium thiocyanate in comparison with 2 μM for the same tissue in the presence of 100 mM ammonium chloride as a control. Likewise, the IC_{50} in the presence of 100 mM Tris perchlorate was 0.5 μM compared with 6 μM for Tris chloride as the control. Since solubilization of a component blocking the receptor binding site or a regulatory component has been proposed as an explanation for the action of chaotropic agents, we tested this hypothesis by removing the chaotropic agent and testing to determine if the enhancement of bicuculline inhibition is still present after removal. After removing the chaotropic agents by centrifugation through 0.32 M sucrose, the IC_{50} for bicuculline was 1-3 μM for all of the membranes whether treated with chaotropic agents or control solutions prior to the centrifugation. The additional washing decreased the IC_{50} for the controls and removal of the chaotropic agents increased the IC_{50} values to the same values as the control. This data indicates that the presence of the chaotropic agent is necessary for the enhancement of bicuculline inhibition and strongly suggests that solubilization and removal of a component blocking or regulating the receptor site is not a reasonable explanation for enhancement of bicuculline inhibition.

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137.10 SOLUBILIZATION AND CHARACTERIZATION OF NEUROLEPTIC-RECEPTOR BINDING SITES FROM RAT STRIATAL MEMBRANES. J.Y. Lew*, J.C. Fong* and Menek Goldstein. Neurochemistry Lab., New York University Medical Center, Dept. of Psychiatry, New York, N.Y. 10016.

To determine the molecular properties of dopamine (DA) receptors with high affinity for DA antagonists (neuroleptic receptors), we have solubilized and characterized the receptor from rat striatal membranes. Among a number of tested detergents, only the recently described one, CHAPS (zwitterionic acid of cholic acid) (L.M. Hjelmeland, Proc. Natl. Acad. Sci., 77, 6368, 1980) was found to have the ability to solubilize active neuroleptic receptors. The neuroleptic receptors were solubilized by incubation of membranes in 0.05M Tris-HCl buffer, pH 7.4, containing 2mM dithiothreitol (Tris buffer) and 10mM CHAPS for 20 min at 0°C. At the end of the incubation period, the mixture was centrifuged at 100.000xg for 30 min. and aliquots of the supernatant were used for assay. The receptor assay mixture contained 0.2 ml of supernatant solution (solubilized proteins from 10 mg striatum), 0.05 ml of ^{3}H-Spi (Sp. act. 35.9 Ci/mmol) and 0.7 ml of Tris buffer. Haloperidol and (+) butaclamol, but not (-) butaclamol, displaces ^{3}H-Spi from the soluble receptor at nanomolar concentrations. Apomorphine (Apo) and DA displace ^{3}H-Spi only at μmolar concentrations. The displacement of ^{3}H-Spi by Apo is not affected by GTP, indicating that the soluble receptor is not coupled to the GTP sensitive component. Scatchard plot analysis revealed that ^{3}H-Spi binds to a low affinity site (K_D = 0.16nM, B_{Max} = 2.5 pmole/g tissue) and to a low affinity site (K_D = 1.25nM, B_{Max} = 11.2 pmoles/g tissue). Thus, the binding characteristics of ^{3}H-Spi for the soluble receptor are similar to those reported for the membrane bound receptors. In some experiments, the detergent was removed after centrifugation by passing the protein solution through a Bio-Beads SM-2, 20-50 mesh (Bio-Rad) column. The neuroleptic receptor remained active even after removal of the detergent. The molecular weight of the soluble receptor complex is now being determined by gel filtration on a calibrated Sepharose 6-B column. This study was supported by NINDS Grant 06801 and NIMH Grant 02717.

137.11 IN VITRO AND IN VIVO INTERACTION OF NORHARMAN (BETA CARBOLINE) WITH THE BENZODIAZEPINE RECEPTOR. A.M. Morin*, I.A. Tanaka*, C.G. Wasterlain* (SPON. A.S. Kling) Neurology and Epilepsy Res. Labs., V.A. Medical Center, Sepulveda, CA 91343 and Dept. Neurology, U.C.L.A., Los Angles, CA 90024.

Norharman (β-carboline) has been described as an in vitro competitive inhibitor of [^{3}H]-diazepam binding in mouse forebrain (Morin and Wasterlain, Life Sci. 28(20)2257-2263, 1981; Trans. Amer. Soc. Neurochem 12(1)128, 1981). This unsubstituted β-carboline along with other structurally related β-carbolines appears to antagonize the action of diazepam (Skolnick et al. Eur. J. Pharmacol. 69:525-527, 1981; Braestrup et al., PNAS 77 (4)2288-2292, 1980; Nielsen et al., J. Neurochem. 36(1):276-285, 1981). Binding data in mouse brain areas indicate linear Scatchard plots unlike the curvilinear Scatchard plot obtained with β-carboline-3-carboxylate in rat brain (Braestrup et al., PNAS 77(4):2288-2292, 1980). EC_{50} values are in the 3-6μM range. The profile of this compound along with other β-carbolines indicate that in vitro norharman binds to the same site as benzodiazepines and in a similar manner.

The in vivo administration of norharman produces profound behavioral effects. Mice become ataxic within 5 minutes of receiving an i.p. dose (50 mg/kg). Animals remain sedate and lose the ability to right themselves when placed on their sides. They exhibit tremors and a stiffness in limbs. Diazepam (3.5 mg) prolongs the sedation, reduces the stiffness and stops tremors. Unlike the benzodiazepines, norharman has no anti-convulsant activity. Given prior to pentylenetetrazol (70 mg/kg) or bicuculline (6 mg/kg), norharman (50 mg/kg) reduced the latency time (50% to 75%) to seizures (P<.05). In vivo brain concentrations of norharman peak within 5-10 min. of an i.p. dose (50-100 mg/kg). Determination of in vivo concentrations in various brain areas (cortex, hippocampus, striatum and cerebellum) indicate that behavioral responses to norharman occur at brain concentrations in the range of 0.20 μM. Levels of inhibition of in vitro [^{3}H]-diazepam binding measured in whole homogenate in these areas showed regional differences with 29.3±3.4% in cortex; 47.4±5.4% in cerebellum; 16.2±3.5% in hippocampus and 18.9±5.0% in striatum. It seems likely that if norharman exerts some effects in vivo through the benzodiazepine receptor, it does so by binding with a higher affinity than that observed in vitro and perhaps through greater binding to receptors in a specific area (i.e. cerebellum).

137.12 TEMPERATURE-DEPENDENT BINDING TO THE BENZODIAZEPINE RECEPTOR. R.L. Kochman and J.D. Hirsch. Dept. Biol. Res., G.D. Searle & Co., Chicago, IL 60680.

Recently, several non-benzodiazepines have been found to antagonize some of the effects of diazepam (DZ). In an attempt to find a biochemical means of distinguishing these compounds from benzodiazepine-like drugs, we investigated the temperature dependence of their in vitro binding to the benzodiazepine receptor.

Approximately 0.5mg of protein from a 1000xg supernatant prepared from whole rat brain homogenate was preincubated with various concentrations of test compounds and 50mM Na-K phosphate buffer containing 0.2M NaCl for 15 minutes at 0°,10°,20°, or 30°C. Binding was initiated by adding ^{3}H-DZ (final concentration 4nM). Incubation was continued at the appropriate temperature for 30 minutes, then samples were filtered under vacuum pressure through Whatman GF/C papers and rinsed with 8mls of 0.15M NaCl. The IC50 value for each compound was determined by log-logit analysis of the displacement of ^{3}H-DZ binding by the compound, and the related K_I value obtained using the Cheng-Prusoff equation. Saturation binding of ^{3}H-DZ and ^{3}H-β-carboline-3-carboxylic acid propyl ester (βCPE) were analysed by Woolf plots to obtain affinity constants (K_D). The K_I and K_D values were then used to calculate the thermodynamic parameters of binding for each compound (Weiland et al., Nature 281:114, 1979).

The apparent K_Is of DZ, βCPE, β-carboline-3-carboxylic acid ethyl ester (βCEE), flunitrazepam, chlordiazepoxide, alprazolam (A), zopiclone (Z), and irazepine all decreased with increasing temperature. To the contrary, the K_Is of triazolam (T) and SC-35195 (SC), a pyridobenzothiazinol which also appears to antagonize DZ, were enhanced with increasing temperature. Thermodynamic analysis indicated that for all compounds binding was an entropy-driven process, with entropy values (ΔS^o) ranging from 5 entropy units (e.u.) for A to 72 e.u. for T. Enthalpy values (ΔH^o) ranged from -6kcal/mol for Z to -8kcal/mol for DZ, while T and SC binding were endothermic (approx. 6kcal/mol).

Preliminary results using a washed P_2 homogenate and 50mM Tris·HCl indicate ΔH^o and ΔS^o values similar to those above. However, whereas DZ binding is stimulated by both GABA and $NiCl_2$, βCPE, βCEE, and SC are differentially affected. Also, GABA stimulation increases, while stimulation by $NiCl_2$ decreases, with increasing temperature.

137.13 FLUORESCENT PROBE STUDIES OF MOUSE BRAIN BENZODIAZEPINE RECEPTORS. James D. Hirsch, Dept. Biol. Res., G.D. Searle and Co., Chicago, IL 60680.

The membrane events associated with and subsequent to ligand binding to the brain benzodiazepine receptor (BDZR) are largely unknown. In order to go beyond measuring only ligand-BDZR recognition and to begin examining these events in more detail, the anionic fluorescent dye 8-anilino-1-naphthalene sulfonic acid (ANS) was used to probe various loci in mouse brain membranes. Upon excitation at 380nm, ANS fluorescence in membranes was greatly enhanced and blue-shifted with an emission maximum at 490nm. This baseline fluorescence was enhanced further by Triton X-100 (EC_{50} 0.03% v/v), ethanol, halogen anions (Cl > Br > F > I), monovalent cations (Li > Na > K > NH_4), divalent cations (Hg, Cu, Mn, Ni, Mg, Ca, Zn), and Al^{+3}. Fe^{+2} and DMSO decreased fluorescence. DMSO caused a red shift in emission as well. ANS binding measured by fluorescence was saturable and the K_m (45-55 µM) was similar in membranes from whole brain, cerebellum, frontal cortex and olfactory bulbs. However, cerebellum membranes contained 25% more ANS sites than other regions. In all brain regions, 100mM NaCl enhanced ANS fluorescence by increasing the B_{max} by about 70% with no change in K_m. Two binding sites for Cl^- and I^- ions were detected. Pretreatment of membranes for 30 min at 30° with 100 µM diisothiocyanostilbene disulfonic acid (DIDS), an irreversible anion channel blocker, decreased the number of Cl^- sites with no change in K_m. Tetrodotoxin (100nM) obliterated a high affinity Na^+ site (K_m=25mM) but had no effect on a low affinity site (K_m=200mM). The H_2O-soluble BDZ's chlordiazepoxide, flurazepam, and chlorazepate (5-10000nM) decreased ANS fluorescence emission at 490nm in a concentration-dependent fashion. Irreversible occupation of the BDZR by irazepine (200nM, 30 min, 4°) and avermectin B1a (10µM, 60 min, 4°) caused a 25% decrease in ANS fluorescence due solely to a decrease in dye K_m. Photoaffinity inactivation of the BDZR with UV light and flunitrazepam (20nM, 4 hr. U.V., 4°) had the same effect. Administration of 50 mg/kg chlordiazepoxide i.p. to mice followed 30 min later by fluorescence measurements in brain membranes from treated animals also resulted in decreases in ANS fluorescence emission. These data suggest that ANS binds to the Cl^- and Na^+ channels and to the brain BDZR. Occupation of the receptor by BDZ's and other ligands in vitro and in vivo quenches receptor-bound probe fluorescence. This may be due to ligand-induced conformational changes in the receptor that lead to decreased hydrophobicity in the probe's microenvironment. Ligand-induced perturbation of the BDZR structure might be the first step in the ligand-receptor-signal transmission process.

137.14 BRAIN MONOAMINE RECEPTOR CHANGES ASSOCIATED WITH CATECHOLAMINE DEPLETION AND HYPOTENSION DURING CONTINUOUS α-METHYLDOPA INFUSION D.C.U'Prichard, C.H.Wang and C.R.Freed. Dept. of Pharmacology, Northwestern Univ.Med.Sch.,Chicago,IL 60611, and Depts. of Medicine and Pharmacology, Univ.Colorado Med.Ctr., Denver, CO 80262.

The antihypertensive drug α-methylDOPA (α-MeDOPA) has been shown to deplete central stores of norepinephrine (NE) and dopamine (DA). The hypotensive effect of α-MeDOPA may be due to interaction of its metabolites α-MeNE and α-MeDA with presynaptic catecholamine (CA), especially α_2-adrenergic, receptors (R); however it is unclear which metabolite is more important in mediating hypertension, and whether other R may also be secondary sites of action. In studies designed to compare effects of α-MeDOPA and other antihypertensive α_2-agonists, we examined central monoamine R changes after continuous i.v. αMeDOPA infusion and correlated R changes with depletion of endogenous CA and steady-state levels of α-MeDOPA metabolites. Conscious male Sprague Dawley rats (200-300 g) under mild restraint were infused via jugular vein cannula with α-MeDOPA (0,5 or 20 mg/kg/hr) plus the decarboxylase inhibitor Carbidopa (2.5 mg/kg/hr) for 72 hr. Blood pressure (BP) was measured before and after 72 hr infusion via an indwelling aortic catheter below the level of the renal arteries. After infusion, brains were hemisected and one half used to measure CA and α-MeDOPA metabolite levels by HPLC electrochemical detection. In the other half, monoamine R levels in cerebral cortex,corpus striatum and rest of brain were assayed as follows (R, ligand, blank): β-R ^{3}H-dihydroalprenolol (DHA), 0.2 µM (-)-propranolol; α_1-R, ^{3}H-prazosin (PRAZ), 100 µM NE; α_2-R, ^{3}H-p-aminoclonidine (PAC),10 µM NE and ^{3}H-rauwolscine (RAUW), 100 µM NE; DA-R, ^{3}H-spiroperidol (SPIRO), 1.0 µM (+)-butaclamol; 5HT1-R, ^{3}H-5HT, 1.0 µM 5HT;5HT2-R SPIRO, 1.0 µM cinanserin. 0,5 and 20 mg/kg/hr α-MeDOPA infusion caused dose-dependent decreases in BP (-1,-2,-17 mm Hg), decreases in hypothalamic DA (2.5, 1.7, 0.5 nmole/g) and increases in α-MeDA (0, 3.8, 11.5 nmole/g). Depletion of hypothalamic NE and appearance of α-MeNE was not dose-dependent; maximal effects were observed at 5 mg/kg/hr α-MeDOPA. 72 hr α-MeDOPA infusion caused dose-dependent reductions in cortex α_2-R and increases in cortex α_1-R and striatal DA-R: PAC (max. -54%), RAUW (max. -33%), PRAZ (max. +19%), SPIRO (max. +58%). Cortex β-R and 5HT-R were not changed. α_1-R and α_2-R changes in rest of brain were similar but less extensive. The results show that α-MeDOPA metabolites directly down-regulate brain α_2-R, and also up-regulate α_1-R and DA-R, either by CA depletion or reducing CA release via presynaptic R interactions A strong correlation was observed between DA depletion, α-MeDA appearance and R changes, suggesting that R changes are mediated primarily by α-MeDA, which achieves a brain concentration sufficient to interact at α_2-R.

137.15 ADENOSINE RECEPTOR HETEROGENEITY. Kenneth M.M. Murphy and Solomon H. Snyder., Johns Hopkins University, School of Medicine, Dept. of Neuroscience, Pharmacology and Psychiatry, Baltimore, MD 21205.

The existence of multiple receptors for individual neurotransmitters has implications for developing drugs with therapeutic selectivity. Adenosine's actions upon neuronal and non-neuronal systems involve at least two distinct receptors. Reduction of adenylate cyclase activity involves A_1 receptors which respond to nanomolar adenosine while micromolar adenosine is required to stimulate adenylate cyclase at A_2 receptors. ^{3}H-N^6-cyclohexyladenosine (^{3}H-CHA) at 25°C labels A_1 adenosine receptors in membranes of bovine, guinea pig, rat, rabbit and human brains. The xanthine antagonist ^{3}H-1, 3-diethyl-8-phenyl-xanthine (^{3}H-DPX) at 0°C labels typical A_1 adenosine receptors in bovine, rat and rabbit brain. In guniea pig membranes, ^{3}H-DPX at 0°C labels sites which have micromolar affinities for adenosine analogs and other properties suggesting interactions with A_2 receptors (Bruns, Daly, Snyder, Proc. Natl. Acad. Sci., USA, 77:5547-5551, 1980). Thermodynamics of adenosine receptor interactions vary considerably among species and differ for agonists and antogonists. Agonists become less potent with reduced temperature at A_1 receptors, while antagonists generally become more potent. Between 25°C and 0°C, agonist affinities fall 5-10 fold in bovine brain, 10-20 fold in rat and rabbit, while a much greater decline in agonist affinities occurs for guinea pig brain. Accordingly, the weak competition by agonists for ^{3}H-DPX binding to guinea pig brain at 0°C, resembling A_2 receptors, may reflect one end of a continuum of temperature-dependent agonist affinities at A_1 receptors. Besides these temperature variations, drug potencies vary markedly at ^{3}H-CHA binding sites in various species. Thus, the K_i for inhibition at 25°C of ^{3}H-CHA binding by DPX is 10 nM, 100 nM, 150 nM and 500 nM in bovine, rat, rabbit and guinea pig brain membranes, respectively. These data suggest a considerable heterogeneity of adenosine receptors. For a substance which influences a wide range of physiological processes, such as adenosine, clarification of receptor heterogeneity may facilitate the design of tissue specific drugs.

137.16 SOLUBILIZATION, PHOTOAFFINITY-LABELLING AND INITIAL PURIFICATION OF THE GLYCINE RECEPTOR IN RAT SPINAL CORD. H. Betz*, D. Graham* and F. Pfeiffer*. (SPON: R. Nowakowski). Max-Planck-Institute for Psychiatry, Dept. of Neurochemistry, D-8033 Martinsried, Federal Republic of Germany.

Glycine is a major inhibitory neurotransmitter in mammalian spinal cord. The hyperpolarizing action of this amino acid can be selectively antagonized by the alkaloid strychnine, the latter being a highly specific ligand for the postsynaptic glycine receptor. Here we report the solubilisation, photoaffinity-labelling and purification of the glycine receptor from membrane preparations of rat spinal cord.

High-affinity binding sites for ^{3}H-strychnine were solubilized from rat spinal cord membranes by using Triton X 100, and were subsequently detected by a polyethylene glycol precipitation assay. Scatchard analysis of ^{3}H-strychnine binding data showed a single class of binding sites with dissociation constants of 11 nM and 20 nM, for membrane-bound and solubilized receptor, respectively. For both preparations, similar concencrations of glycine, ß-alanine and taurine were needed to produce half-maximal inhibition of ^{3}H-strychnine binding. The solubilized receptor exhibited a stokes radius of 73 Å upon Sepharose 6B gel exclusion chromatography and gave a sedimentation coefficient of 8.5 S on sucrose density gradients.

Incubation of the membrane fractions with ^{3}H-strychnine in the presence of UV light led to an irreversible binding of the alkaloid to a membrane protein which exhibited a molecular weight of 48,000 ± 2,800 (n=3) on 10 % SDS polyacrylamide gels. The labelling of this protein was specifically inhibited by the addition of either glycine or unlabelled strychnine to the incubation mixture.

The solubilized receptor was retained on concanavalin A - and wheat germ agglutinin - sepharose, and thus is a glycoprotein. Affinity chromatography on strychnine-derivatised agarose beads gave approx. a 500-fold purification of the glycine receptor.

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137.17 GLUCOCORTICOID BINDING BY SOLUBLE FRACTIONS FROM RAT SUPERIOR CERVICAL GANGLIA. A. C. Towle* and P. Y. Sze (SPON: S. C. Maxson). Dept. of Biobehavioral Sciences, Univ. of Connecticut, Storrs, CT 06268.

Several investigators have suggested that the sympathetic nervous system may be a target organ for adrenal glucocorticoids. We have investigated glucocorticoid binding capacity in the soluble fractions (100,000 g supernatants) of the adult rat superior cervical ganglia. A remarkably high level of ^{3}H-corticosterone binding (2150 femtomoles/mg protein) was found in the ganglion preparations. However, there was no detectable binding for ^{3}H-dexamethasone. The ^{3}H-corticosterone binding protein was characterized as a transcortin-like protein by various biochemical criteria, including isoelectric focusing and chromatograph on DEAE-cellulose and DNA-cellulose. The transcortin-like protein found in the ganglia was not from contaminating blood, as shown by the complete clearance of intravenously injected ^{14}C-inulin following extensive perfusion of the animal. When the sheath was removed from the whole ganglion, 20% of the ^{3}H-corticosterone binding capacity remained in the desheathed ganglion; the other 80% was found in the interstitial fluid. Our data indicate that a binding protein with glucocorticoid receptor-like characteristics is not present in the ganglia. This raises a question about the mechanism of action of dexamethasone, which has been commonly used to elicit biochemical effects in the ganglia.

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137.18 REGULATION OF ^{3}H-DOPAMINE BINDING TO RAT STRIATAL MEMBRANES BY GUANINE NUCLEOTIDES AND CATIONS. Mark W. Hamblin, Stuart E. Leff and Ian Creese, Dept. Neurosciences, Univ. Calif. San Diego, La Jolla, CA 92093.

Both guanine nucleotides and physiological cations are known to regulate radioligand binding to a variety of different neurotransmitter receptors. Guanine nucleotides have previously been shown to lower the Kd for the binding of the dopaminergic agonist ligand ^{3}H-apomorphine without affecting the Bmax, and to lower agonist affinity for dopamine (DA) receptors labeled by ^{3}H-spiroperidol. Conversely, divalent cations have been shown to enhance binding of ^{3}H-apomorphine. No such studies have previously been made with ^{3}H-dopamine itself, nor has the interaction of guanine nucleotides and ions in the regulation of dopaminergic ^{3}H-agonist binding been described. We have now characterized these effects using fresh rat striatal membranes.

Little specific ^{3}H-DA binding to well washed membranes can be detected in the absence of added metal cations or chelating agents. Addition of Ca^{++} or Mg^{++} produces a dose dependent increase in Bmax to maximum values of approximately 20 pm/g tissue, with no change in Kd (2 nM). EC50's for both ions are approximately 100 µM. EDTA (0.01 mM to 10 mM), as reported previously, also increases the Bmax, although only to a level half that obtainable with Ca^{++} or Mg^{++}. Maximal effects of the divalent cations or of the ions and EDTA are not additive. This high affinity ^{3}H-DA specific binding is partially eliminated by nigral 6-OHDA lesions, consistent with both pre- and postsynaptic locations for these sites.

Na^{+} (10-300 mM) and GTP (300 µM) have no effect on specific binding in the absence of other added metal cations or EDTA. Na^{+}, GTP, GDP and the stable GTP analogue GppNHp, however, reverse the effects of divalent cations or EDTA on Bmax, again without affecting Kd. GMP and ATP are ineffective. 300 mM Na^{+} or 300 µM GTP reduce specific binding in the presence of 1 mM Ca^{++} or 1 mM EDTA to control levels.

These results may reflect the existence of two interconverting states of the ^{3}H-dopamine binding site. The presence of Ca^{++} or Mg^{++} appears to favor the formation of the high affinity state, whereas Na^{+} and GTP favor the formation of a low affinity state not labeled by ^{3}H-DA in filtration assays. These results also indicate that the comparison of absolute numbers of various dopamine receptor subtypes labeled by different ^{3}H-ligands is invalid unless optimum ionic conditions are employed. (Supported by GM07198 and MH32990)

138.1 EFFECTS OF SOMATOSTATIN ON CORTICAL NEURONS IN CULTURE: MEMBRANE EFFECTS WITH UNUSUAL DOSE-RESPONSE CHARACTERISTICS. John R. Delfs and Marc A. Dichter (SPON: Howard W. Blume). Dept. of Neurology, Children's Hospital Medical Center, Boston, MA 02115.

Previous reports from our laboratory have described that somatostatin causes increased synaptic activity when applied to rat cortical neurons in cell culture. This effect was blocked by simultaneous perfusion with tetrodotoxin, suggesting an unobserved direct membrane effect.

We have now applied known concentrations of somatostatin by local miniperfusion to over 150 neurons during intracellular recordings. The following additional points can be made about the effects of somatostatin on rat cortical neurons in culture:

1. Somatostatin depolarizes a subpopulation of the neurons. This depolarization is usually small (6 +/- 4 mV), but is often associated with an increased generation of action potentials (APs) by the depolarized neuron. The effect of somatostatin to increase the frequency of PSPs is likely due to depolarization and excitation of one or more neurons in a particular neuronal circuit.

2 The observed effect of somatostatin, whether to increase PSP frequency or to depolarize, is greatest with the initial application of somatostatin. A rapid decrement in the amplitude of the response was seen with repeated applications.

3. An especially remarkable finding was a dose-response relationship in which lower concentrations of somatostatin (high pM or low nM) caused larger or more predictable responses than did higher concentrations (high nM or low uM). Application of a higher concentration often resulted in no observable response, while the application of a lower concentration to the same neuron would often result in a significant depolarization or an increase in synaptic activity. These dose-response characteristics suggest similarities with other agents known to have biphasic dose-response characteristics and have major implications for an understanding of the physiologic role of somatostatin in the central nervous system.

4. In some neurons a low concentration of somatostatin caused an increase in the frequency of spontaneous or stimulus-evoked APs, while a higher concentration inhibited action potential generation in the same neuron. This may suggest the existence of at least two receptor sites of different affinities for somatostatin on cortical neurons, one mediating an excitatory and the other an inhibitory response.

138.2 SOMATOSTATIN HAS EXCITATORY ACTIONS ON MURINE SPINAL CORD NEURONS IN PRIMARY DISSOCIATED CELL CULTURE. R.L. Macdonald and L.M. Nowak. Department of Neurology, The University of Michigan, Ann Arbor, MI 48109.

Somatostatin (Somatotropin release inhibiting factor; SRIF) is a putative neurotransmitter of small diameter primary afferents. We have investigated SRIF actions in cell culture and report that this tetradecapeptide depolarized some multipolar spinal cord neurons and decreased membrane potassium conductance (g_K).

Spinal cords and attached dorsal root ganglia (DRGs) were dissected from 12-13.5 day old fetal mice. Cells were mechanically dissociated, plated on 35 mm collagen-coated dishes and grown in culture at 35° for 4-10 weeks prior to electrophysiological investigation. Intracelllar recordings were made with high impedance (30-40 MΩ) 4M KAc-filled or 3M KCl-filled micropipettes from neurons bathed in buffered saline on the modified, heated (35-36°) stage of an inverted phase contrast microscope. Single electrode voltage-clamp recordings (3 KHz switching frequency; 50% duty cycle) were made with low impedance (10-20 MΩ) KCl-filled micropipettes which were shielded (to 1-2 mm of tips) with conductive paint and insulated from the recording medium with nail enamel. SRIF (Sigma, St. Louis; Bachem, Torrence; Peninsula, Belmont) were dissolved in 0.02 M ammonium acetate-acetic acid buffer (pH 4-4.8) with 0.1% BSA, split into aliquots and stored at -27°C. Peptides were diluted in recording medium and applied directly to the neuronal surface by pressure pulses (1 sec at .25-3.0 psi) from blunt glass micropipettes (2-10 μm tips).

SRIF produce dose-dependent (3-300 nM), slow (4-8 sec to peak), reversible depolarizations and decreased membrane conductance. SRIF-responses (current or voltage) increased when membrane potential was held at depolarized potentials and decreased at hyperpolarized holding potentials. Extrapolated reversal potentials were more negative than resting membrane potential. SRIF evoked inward current at resting membrane potential, suggesting that it decreased either g_K or g_{Cl}.

Thus, we have demonstrated that SRIF, a putative primary afferent neurotransmitter, had excitatory actions in primary dissociated cell culture and produced membrane depolarization by decreasing membrane conductance.

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138.3 PEPTIDES FROM NEURONS R3-R14 AFFECT FIRING PATTERNS OF OTHER NEURONS IN APLYSIA. D.G. Gibson*, C.Y. Lin*, and D.J. McAdoo. Marine Biomedical Inst., Univ. Texas Medical Branch, Galveston, TX, 77550.

We are investigating the possibility that the peptides synthesized by cells R3-R14 in the Aplysia parietovisceral ganglion (PVG) have hormonal effects.

Our test preparations were the buccal ganglion of Aplysia, PVG parabolic burster R15, and PVG left upper quadrant cells L3 to L6. All have previously been shown to respond to peptides secreted by the bag cells of the PVG, and none are synaptically connected to R3-R14. We utilized A. californica and A. brasiliana.

R3-R14 cell bodies and processes were dissected in sucrose-PMSF solution to inhibit proteolysis. Whole-cell homogenates were made in physiological solution and tested immediately; homogenates were also fractionated based on molecular weight on a Sephadex G-50 column. Dosages were equivalent to 10% to 100% of the material from one animal. Most tests were carried out in artificial seawater with elevated magnesium (4.5X normal) and zero calcium to eliminate synaptic components from the recorded responses.

Whole-cell homogenates evoked pacemaking discharges in previously quiescent third buccal nerves of both species, an effect similar to results obtained with egg-laying hormone (ELH) (Stuart and Strumwasser, 1980. J. Neurophysiol. 43:499-519). Onset of effect required up to 10 minutes, and did not persist as long as the effect of ELH.

The fraction of cell body material weighing from 2000 to 4000 Daltons, presumably peptides, increased burst duration and decreased interburst intervals in the PVG burster R15. Intracellular records indicate that pattern changes were mediated by baseline depolarizations of 5 to 10 mV. These effects differ from those of ELH in that burst intensity (max. spike rate) is not increased, bursts occur more frequently, and the effect is washed out more readily. Left upper quadrant neurons (L3 through L6), reportedly inhibited by bag cell activity (Mayeri et al., 1979. J. Neurophysiol. 35:202-219), showed no consistent response to R3-R14 products.

The response of R15 and the buccal ganglion to the test materials supports the idea that cells R3-R14 may release both peptides and glycine (Sawada et al., 1981. Br. Res. 207:486-490) as neurochemical messengers.

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138.4 VASOACTIVE INTESTINAL POLYPEPTIDE EXCITES DORSAL HORN INTERNEURONS IN THE CAT SPINAL CORD. M. Randić and S. Jeftinija* (Spon: K. Sikora-VanMeter). Dept. of Vet. Physiol. Pharmacol., Iowa State University, Ames, IA 50011.

Vasoactive intestinal polypeptide (VIP) has been reported to be present in small sensory ganglion cells and in high concentration in the superficial parts of the spinal dorsal horn, the area where primary afferent fibers are known to terminate. Since the functional role of VIP in the spinal cord is at present unknown, it was of interest to study the central effects of synthetic VIP (1.5 mM, pH 4.7) by applying it iontophoretically onto functionally identified dorsal horn neurons.

We found that VIP caused a strong but slow excitation of a majority of the tested dorsal horn interneurons in laminae I-V. Excitation was observed as initiation of firing in a previously quiescent unit, or as an increase in the rate of spontaneous and/or evoked firing. In general, excitation began at a latency of 10-15 sec following the onset of VIP ejection and outlasted application for several minutes. VIP appeared to possess an excitatory action in all categories of neurons recognized in spinal preparations of cats in this area on the basis of their excitability to different kinds of afferent input.

These results indicate that VIP should also be added to the ever growing list of peptides having possible functional roles as transmitters or modulators in the dorsal horn of the cat spinal cord. (Supported by NSF grant BNS 23871 and the U.S. Dept. of Agriculture).

138.5 VASOPRESSIN ENHANCES SPONTANEOUS NEURAL ACTIVITY IN HIPPOCAMPAL SLICES. M. Mühlethaler* and J.J. Dreifuss. Dept of Physiol., Univ. of Geneva Med. Sch., Geneva, 1211 Switzerland.

Vasopressin administered intracranially has been shown to affect memory storage and retrieval (De Wied, Proc.R.Soc. B210:183, 1980) To investigate a possible mechanism for this effect, we have applied vasopressin and analogs to hippocampal slices obtained from adult rats while monitoring the spontaneous firing activity of neurones located in the pyramidal layer of the CA1 region. Since the posterior hippocampus is known to receive axon terminals which react with vasopressin antisera (Buijs, J.Histochem.Cytochem. 28:357, 1980), posterior hippocampal slices were prepared conventionally and placed on a nylon grid at the medium-oxygen interface at 37°C. Substances to be tested were dissolved in Yamamoto medium containing 0.8-1.0 mM $CaCl_2$ and bath-applied at a rate of 2.0 ml/min.

Stable extracellular recordings were obtained from cells which displayed short, intermittent bursts of activity. Arginine-vasopressin (AVP) and lysine-vasopressin (LVP) produced a dose-dependent (10^{-8}-10^{-5} M), sustained but reversible increase in the mean firing of 16/19 cells in 12 slices and prolonged the burst duration. The antidiuretic analog DDAVP had a much weaker action. Inhibitory effects were not observed. The vasopressor antagonist d(CH_2)$_5$Tyr(Me)AVP (Kruszynski et al., J.Med.Chem. 23:364, 1980) applied at 10^{-6}M in 5 slices had no effect per se on neuronal firing. However in 4/4 cells it totally and reversibly blocked the stimulatory effect of 10^{-6} M AVP. In slices trimmed to contain only the CA1 region, AVP was as effective as in normal slices. Intracellular recordings with KCl-filled electrodes in 23 slices perifused with Yamamoto medium containing 2 mM $CaCl_2$ confirmed that LVP and AVP excited a majority of responsive hippocampal neurones (15 cells) but weak inhibitory effects were also occasionally observed (9 cells). The peptides produced at times a slight membrane depolarization and a small increase in membrane conductance.

The results, which showed a predominant excitatory effect of vasopressin in the hippocampus, suggest moreover that hippocampal neurones possess vasopressin receptors resembling those of the V_2 category shown to be present on smooth muscle cells and on liver cells.

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138.6 ADRENOCORTICOTROPIN ALTERS MOTOR UNIT SIZE AND EFFICIENCY FOLLOWING DENERVATION OF RAT EXTENSOR DIGITORUM LONGUS MUSCLE. C. Saint-Come* and F.L. Strand. Dept. of Biology, New York Univ., New York 10003

Daily intraperitoneal administration (0.2U) of Adrenocorticotropin (ACTH 1-39) to male rats (180-200g) during recovery from crush denervation of the extensor digitorum longus (EDL) muscle improves the recovery of neuromuscular efficiency as measured by a ratio of maximum tension recorded from peroneal nerve stimulation to that obtained from direct massive stimulation of the muscle. In addition, ACTH 1-39 appears to increase the size and activity of regenerating motor units. Motor unit size and activity were monitored by delivering finely graded stimuli (0.05ms. duration) to the peroneal nerve by means of a pair of platinum electrodes at one minute intervals. Muscle action potentials were recorded by means of a fluid electrode system that permits simultaneous recording of isometric tension. Alternate proximal and distal stimulation of the peroneal nerve allowed calculation of the motor conduction velocity. While the indirectly evoked action potential and isometric contraction amplitude are both increased by peptide treatment, no other parameters of the electrical response (conduction velocity and duration) or of the contractile response (contraction time and half relaxation time) of the denervated muscle are affected. ACTH 1-39 does not alter any of the parameters of the contractile response of the EDL when the muscle is directly stimulated, supporting other evidence from this laboratory that the enhancement of neuromuscular function by ACTH is a neurogenic, not a myogenic phenomenon.

138.7 SPECIFIC ANTAGONISM OF SUBSTANCE P INDUCED EXCITATION OF LOCUS COERULEUS NEURONS BY (D-Pro^2, D-Trp^{7,9})-SP. G. Engberg, T.H. Svensson*, S. Rosell* and K. Folkers* (SPON: S. Grillner). Depts of Pharmacology, University of Göteborg and Karolinska Institute, Stockholm, Sweden and Institute for Biochemical Research, University of Texas, Austin, USA.

Several lines of evidence suggest that Substance P (SP) participates in synaptic transmission in the brain. Recent immunocytochemical studies have shown several brain nuclei, including the noradrenergic locus coeruleus (LC) which innervates almost the entire neuroaxis, to be enriched in SP-containing fibers. In addition, a great majority of LC neurons have been found to be specifically excited by microiontophoretically applied SP. A major drawback in SP research has been the unavailability of a specific SP-antagonist. However, recently (D-Pro^2, D-Trp^{7,9})-SP has been synthetized and this agent was found to block peripheral effects of SP. Here single cell recording techniques and microiontophoresis were used to establish whether this compound would antagonize the excitatory effect of SP on rat brain noradrenaline (NA) neurons in the LC. When microiontophoretically applied at low ejection currents SP consistently caused a rapidly increased firing rate of the LC neurons. Total recovery of base-line activity was seen within 30 sec after termination of ejection. The simultaneous iontophoretic application of (D-Pro^2, D-Trp^{7,9})-SP completely antagonized the SP-induced excitation of the NA cells, but did not affect the excitation of the LC neurons by glutamate or acetylcholine . When applied alone the SP-antagonist did not affect the spontaneous firing of the cells. Thus, (D-Pro^2, D-Trp^{7,9})-SP seems to be an effective and specific SP-antagonist in a brain nucleus, the LC, which receives a prominent SP-input. This input is probably not critical for maintenance of the spontaneous activity of the NA cells. (Supported by the Swedish Medical Research Council, project nos. 4747 and 4495)

138.8 AUTORADIOGRAPHIC LOCALIZATION OF CHOLECYSTOKININ RECEPTORS IN GUINEA PIG BRAIN. R.B. Innis, M.A. Zarbin, J.K. Wamsley, S.H. Snyder and M.J. Kuhar. Dept. Neuroscience, Johns Hopkins School of Medicine, Baltimore, Maryland 21205.

Cholecystokinin triacontatriapeptide (CCK-33) or its COOH-terminal derivative peptides are putative neurotransmitters in the mammalian central nervous system. Data which are compatible with this hypothesis are as follows. CCK-33 and its COOH-terminal octapeptide (CCK-8), as measured by radioimmunoassay, are present in high concentrations in the brain. The predominant form of cholecystokinin (CCK) in the brain appears to be CCK-8. Moreover, immunohistofluorescence studies indicate that CCK-like immunoreactivity is localized to neuronal perikarya and processes within the CNS. Recently, specific high affinity CCK receptors were detected in mammalian brain and pancreas with in vitro biochemical techniques. In an attempt to further analyze this peptide's role in neurotransmission, we have determined the distribution of CCK receptors within the CNS at the level of resolution of light microscopy. This in vitro autoradiographic method of receptor localization is quantitative and allows the labeling of receptors with a high degree of specificity.

Cholecystokinin receptors were found widely distributed throughout the central nervous system with enrichment in limbic, visual, and cortical areas. In the limbic system, relatively high densities of receptors are found in the medial and lateral mammillary nuclei, in the stratum lacunosum-moleculare of the hippocampus, in the polymorphic layer of the dentate gyrus, and in other areas. Within the visual system, receptors are found in the ganglion cell layer of the retina, in the optic tract, in pretectal areas, and in the superficial layer of the superior colliculus. In the cerebral cortex, CCK receptors have a distinct laminar distribution with particularly high densities over laminae IV and VI. Laminae II, III, and V have a lower density of receptors while lamina I has a negligible density of receptors. In cingulate cortex, a dense band of grains overlies the anatomically indistinguishable laminae II, III, and IV. An even higher density of autoradiographic grains is localized to lamina VI of cingulate cortex while a negligible density of grains is present in laminae I and V.

138.9 BRADYKININ AS A PEPTIDE TRANSMITTER: CHARACTERIZATION OF ^{3}H-BRADYKININ RECEPTOR BINDING. Donald C. Manning, Robert B. Innis and Solomon H. Snyder. Johns Hopkins University, Sch. of Medicine, Dept. of Neuroscience, Pharmacology and Psychiatry, Baltimore, Maryland 21205.

Bradykinin (BK), a nonapeptide well known to be one of the most potent algesic substances, has been postulated to be one of the primary stimuli for pain resulting from tissue damage. A role for BK as a central neurotransmitter is suggested by the existence of immunoreactive BK in specific neuronal systems in the brain (Proc. Natl. Acad. Sci., USA., 76:1489-1493, 1979) and the hypotensive, analgesic and hypothermic effects elicited by BK injections in the brain. Bradykinin may also act peripherally where it is known to stimulate both vagal and sympathetic chemosensitive A delta and C fibres in the heart and is proposed to function in mediating the pain associated with myocardial ischemia. Classically, BK is known to have very potent effects on smooth muscle of the guinea pig ileum and estrous rat uterus. It is in these tissues with the greatest receptor density where we have chosen to characerize BK binding initially.

We have used ^{3}H-bradykinin (^{3}H-BK) as a physiologic ligand, the newest batch having a specific activity of 65 Ci/mmole. In the guinea pig ileum ^{3}H-BK exhibits saturable binding to membrane homogenates having a K_D value of approximately 0.4 nM and a B_{max} of 25 pmoles/g tissue wet weight. Incubations were done in 25 mM TES buffer pH 6.5 containing 0.2% BSA, 1 mM DTT, 0.1 µM Captopril (SQ 14225) and 100 µM Bacitracin for 30 mins at 4° C. Membrane bound ^{3}H-BK was separated from unbound ^{3}H-BK by centrifugation.

We have initiated in vitro receptor autoradiography studies and preliminary data show binding as expected in the muscle layers of the guinea pig ileum but surprisingly we have found binding in the mucosal layers also. This mucosal binding is an intriguing and unexpected finding indicating some unknown function of BK in the villi possibly involving ion flux modulation.

Another unusual finding of this study is the inhibition of receptor binding by divalent cations. This is contrary to every other agonist investigated in this lab where divalent cations usually increase agonist binding. Calcium was the most potent of the divalent cations studied so far causing 50% inhibition of specific binding at approximately 1 mM, Mg was less potent. Bradykinin's influence on the contraction of smooth muscle and calcium's unusual potency at affecting BK receptor binding suggests a possible link between the receptor and the calcium conductance channel.

138.10 SOMATOSTATIN RECEPTORS IN BRAIN AND PANCREAS: DIFFERENT PHARMACOLOGICAL PROPERTIES, Jean-Claude Reubi*, Jean Rivier*, Marilyn Perrin*, Marvin Brown and Wylie Vale. Peptide Biology Laboratory, The Salk Institute, La Jolla, CA 92037.

Saturable and high affinity binding has been obtained for a somatostatin-28 (SS-28) analog, [Leu^8,D-Trp^{22},Tyr^{25}]-SS-28 in 2 different organ preparations likely to be rich in SS receptors: the rat brain (cortex) and the hamster insulinoma, the latter primarily composed of pancreatic β-cells. In both preparations, the specific binding is more than 65% of the total binding and is maximal after 1 hr incubation at 22° C. The K_D for SS-28 is in both cases approximately 1 nM; the number of binding sites correspond to 260 fmols/mg protein in rat cortex and 80 fmols/mg protein in hamster insulinoma. SS binding sites in the brain show a strong regional distribution: Hippocampus > amygdala > cortex > spinal cord > hypothalamus > cerebellum. Pharmacological specificity is present in both preparations since bombesin, LRF or des-Trp^8-SS-14, an SS analog inactive in all SS bioassays, are all inactive at 100 nM, whereas [D-Trp^8]-SS-14 or [D-Trp^{22}]-SS-28, two very potent analogs in SS bioassays, completely displace the radioligand. The following pharmacological differences exist however between both binding sites: whereas SS-14 is approximately equipotent with SS-28 in the brain, it is less potent in the insulinoma despite a similar degradation rate. SS-analogs which selectively inhibit pancreatic insulin release (i.e. Des-Asn^5[D-Trp^8,D-Ser^{13}]-SS-14 are more potent in insulinoma than brain. In contrast, [5-D-F-Trp^8]-SS, which is potent in inhibiting growth hormone release, is more potent in brain than in insulinoma. These results suggest that SS receptors in brain, where SS possibly subserves a neurotransmitter or neuromodulator role, are pharmacologically different from SS receptors located on pancreatic β-cells, which control insulin release.

The successful use of a SS-28 analog as radioligand together with the high binding affinity, particularly in insulinoma, of SS-28 and SS-28 analogs, is in agreement with in vivo bioassay data showing high potency of SS-28 in pancreas and brain, and raises the question whether SS-28 is a possible endogenous ligand for SS receptors.

138.11 CHOLECYSTOKININ RECEPTORS ARE REDUCED IN CEREBRAL CORTEX AND BASAL GANGLIA OF HUNTINGTON'S DISEASE. S. E. Hays*, and S. M. Paul*. (SPON: J. Daly). Clinical Psychobiology Branch, NIMH, Bethesda, MD 20205.

Accumulating evidence indicates that cholecystokinin (CCK) functions as a neurotransmitter or neuromodulator. This evidence includes the presence of specific CCK receptors which we and others have identified in rodent brain. We now have demonstrated the presence of CCK receptors in human brain, and have found that patients with Huntington's chorea have a marked reduction in receptor number, not only in basal ganglia (the region of major neuropathology in Huntington's disease), but in cerebral cortex as well.

Post-mortem tissue was obtained from brains of 8 patients with Huntington's disease and 8 controls matched for age, sex and autolysis time. Specific (displaceable) binding of $^{125}CCK_{33}$ was 62% lower in basal ganglia of Huntington's patients as compared with controls, and 33% lower in cerebral cortex of Huntington's vs. controls. Scatchard analysis demonstrated that these differences reflected reduced receptor number (B_{max}) while the apparent affinity (K_D) was not significantly different from controls.

Reduced CCK receptors in Huntington's basal ganglia indicate that CCK receptors are localized predominately to neuronal elements, and suggest a role for CCK in the regulation of motor function. The reduced cortical CCK receptors suggest that CCK also may be important in cognitive function, which frequently is impaired in Huntington's patients.

138.12 SUBSTANCE P RECEPTORS ON ADRENAL CHROMAFFIN CELLS. P. Boksa*, S. St. Pierre* and B.G. Livett (SPON: Barry S. Layton). Division of Neurology, The Montreal General Hospital, Montreal, Quebec, Canada.

We have previously shown that the undecapeptide substance P (SP) inhibits nicotine- or ACh-induced release of catecholamines from cultured bovine adrenal chromaffin cells. In order to further characterize the site at which SP exerts this action, we have now compared, in chromaffin cell cultures, the inhibitory actions of SP, SP-free acid and a series of SP analogues in which each amino acid of SP is replaced by L-alanine. Chromaffin cells were isolated by retrograde perfusion of adrenal medullae with collagenase, purified on Percoll, and maintained as monolayer cultures as described previously (Livett, B.G. et al., Nature 278: 256, 1977). On the day of the experiment, cultures were pre-loaded with (^{3}H)l-norepinephrine ((^{3}H)NE, 10^{-7}M), washed free of unbound radioactivity, and the nicotine ($5x10^{-6}$)-induced release of (^{3}H)NE was measured in the presence and absence of SP or its analogues. All of the analogues tested inhibited nicotine-induced NE release and their ID_{50}'s are shown in the following Table, where the analogues are listed according to their rank order of potency.

	ID_{50}		ID_{50}
1) (Ala^5)-SP	7.1×10^{-7}	7) (Ala^1)-SP	5.1×10^{-6}
2) SP	1.4×10^{-6}	8) (Ala^{11})-SP	7.3×10^{-6}
3) (Ala^2)-SP	1.6×10^{-6}	9) (Ala^8)-SP	8.0×10^{-6}
4) (Ala^6)-SP	2.2×10^{-6}	10) (Ala^{10})-SP	1.7×10^{-5}
5) (Ala^4)-SP	2.3×10^{-6}	11) (Ala^7)-SP	2.0×10^{-5}
6) (Ala^3)-SP	4.1×10^{-6}	12) SP-COOH	2.2×10^{-5}

These data indicate that the most important groups for conferring inhibition of NE release from chromaffin cells are the C-terminal amide and amino acids in positions 7,10,8 and 11; substitution of amino acids 1 to 6 led to less loss of activity. Similar results have been obtained with these analogues in other SP-responsive tissues including the guinea pig ileum, rabbit mesenteric vein and rat vas deferens (Couture, R. et al., Can. J. Physiol. Pharmacol. 57: 1427, 1979). We conclude that a specific SP receptor exists on adrenal chromaffin cells and that the binding site of this receptor shares similar structural requirements with the SP receptor found in other tissues.

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138.13 **CHARACTERIZATION OF CENTRAL INSULIN RECEPTORS.** Nancy R. Zahniser. Dept. Pharmacology, UCHSC, Denver, CO 80262.

Both insulin immunoreactivity and specific binding sites for ^{125}I-insulin have been identified in the mammalian brain. Although the function of insulin in the brain remains to be determined, it has been suggested that insulin is a neuromodulator or a neurotransmitter. This study was carried out to determine the localization of ^{125}I-insulin binding in rat cerebral cortex. Results from binding assays using crude nuclear and nonnuclear (synaptic/mitochondrial) membrane fractions of rat cerebral cortex were compared to those obtained using purified plasma membranes from rat liver. Studies of the inhibition of radiolabelled insulin binding by unlabelled insulin or glucagon were carried out over a concentration range of 170 fM - 17 μM unlabelled polypeptide. ^{125}I-Insulin binding to membranes from both brain and liver was inhibited by low concentrations (<0.15 nM) of insulin but was not inhibited by glucagon. Based on these experiments, specific binding of ^{125}I-insulin binding was defined as the difference in the amount of radioligand bound in the absence and presence of 1.7 μM insulin; it constituted approximately 60% of total binding to cortical membranes and 95% of total binding to hepatic membranes. In both brain and liver membrane preparations, specific ^{125}I-insulin (350 nM) binding reached equilibrium within 75 min at 15° and remained stable for at least 150 min. Routinely, membranes were incubated with ^{125}I-insulin for 90 min at 15° and binding reactions were terminated by rapid centrifugation of the membranes through 0.32 M sucrose. The IC_{50} values for insulin inhibition of ^{125}I-insulin binding were similar in both cortical synaptic membranes (5.7 ± 2.8 nM) and hepatic membranes (2.0 ± 1.1 nM). The Hill coefficients were less than one in both cases (synaptic membranes: 0.42 ± 0.04; hepatic membranes: 0.50 ± 0.03). These low Hill coefficients may indicate negative cooperativity, multiple binding sites or a two-step binding reaction. In the same series of experiments similar results were obtained with membranes from the nuclear fraction of the rat cortex. In this tissue the IC_{50} value for insulin was 8.7 ± 4.4 nM and the Hill coefficient was 0.32 ± 0.02. Scatchard plots of ^{125}I-insulin binding appeared curvilinear in all three membrane preparations. The density of insulin receptors in liver membranes was at least three-fold greater than that measured in either the cortical synaptic or nuclear membrane fractions. These results indicate that by using an iodinated ligand of high specific activity and a large amount of rat brain membrane protein (0.4 - 0.8 mg), specific ^{125}I-insulin binding sites can be measured and characterized in the brain. As previously reported (Havrankova et al., Nature 272: 827, 1978), the affinity of the receptors for insulin in the brain appears to be similar to that measured in the liver. The results also indicate that ^{125}I-insulin binding sites in rat cerebral cortex are rather uniformly distributed and are not restricted to synaptic membranes. Studies are currently being conducted to determine the function of these central insulin receptors.

139.1 THE EFFECTS OF HYPOPHYSECTOMY ON MULTIPLE OPIATE RECEPTORS. E. Young,* J. Lewis, J. Leibeskind and H. Akil (SPON: M. Gnegy). Mental Health Research Institute, Univ. of Michigan, Ann Arbor, MI 48109.

The existence of at least two subtypes of opiate receptors, the μ and δ receptors which preferentially bind alkaloid and opiate peptides, is supported by much data. However, no one has been able to selectively alter these receptor subtypes differentially. There is an old and complex literature on the effects of hypophysectomy and adrenalectomy on opiate analgesia. We have looked at the effects of hypophysectomy on ^{3}H morphine and ^{3}H D-Ala-D-leu enkephalin (DADL) binding, μ and δ receptor ligands respectively, across multiple brain regions.

Male albino hypophysectomized and sham operated control rats were obtained from Charles River laboratories. After ten days of adaptation, the animals were decapitated, their brains rapidly removed and dissected into the following brain regions: hippocampus, striatum, frontal cortex, midbrain medulla-pons, and thalamus. Opiate receptor binding assays were done on each brain region of hypophysectomized and control animals simultaneously with both ^{3}H morphine and ^{3}H DADL enkephalin. Scatchard plot analysis were done for each ligand for each brain region.

The results showed: 1) a decrease in μ receptor number in hypophysectomized rats in the following regions: striatum, hippocampus, medulla-pons and thalamus; 2) an increase in the affinity of the μ receptor for morphine in the following brain regions: hippocampus, medulla-pons and thalamus; 3) no change was found in μ receptor number or affinity in the cortex or midbrain; 4) no significant change in δ receptor affinity or number in any of the brain regions. Thus, hypophysectomy produces selective change in one subtype of opiate receptors (μ) varying across brain regions. We are currently investigating the effects of adrenalectomy to see if similar findings emerge.

139.2 REGULATION OF OPIATE RECEPTORS IN NEUROBLASTOMA X GLIMOA HYBRID CELLS IN TISSUE CULTURE, S. F. Atweh. Dept. of Neurology, Univ. of Chicago, Chicago, IL 60637.

Neuroblastoma X Glioma Hybrid (NG) cells grown in tissue culture bind opiate ligands. This binding satisfies pharmacological criteria characteristic of opiate receptors. Opiates inhibit the synthesis of cyclic-AMP by NG cells in response to stimulation by prostaglandin E_1. This inhibition exhibits time-related tolerance to the opiates. The phenomenon of tolerance, however, is not associated with changes in opiate receptor binding. The present study demonstrates some factors that regulate receptor binding in NG cells in culture.

NG cells were grown in a culture medium consisting of Dulbecco modified Eagles' medium supplemented with hypoxanthine, aminopterin, thymidine and 10% fetal bovine serum. Cells were washed and harvested from the flasks at various intervals after plating and sonicated in Tris-HCl buffer. Opiate receptor binding was measured by incubating the homogenate or the membrane fraction with ^{3}H-Etorphine for 30 minutes at 22°C. Memberanes and bound ligand were isolated by rapid filteration on GF/B filters using a suction machine and washed three times with cold buffer to minimize non-specific binding. Specific binding of ^{3}H-Etorphine was calculated by subtracting non-specific binding in the presence of saturating concentrations of Naloxone. Under these conditions NG cells from confluent flasks (12-15 days after plating) exhibited maximal binding of 12.6×10^4 ligand molecules per cell with a Kd of 0.7 nM. Receptor binding exhibited marked variations with the age of the cultured cells. Three days after plating NG cells exhibited very little specific binding; thereafter binding per cell increased with the age of the culture and plateaued after day 12. Increased binding related to the increasing density of the cells. Scatchard analysis revealed that this change was due to an increase in the number of receptor sites per cell with no change in affinty to Etorphine. Culturing the cells in the presence of 0.5 mM Dibutyrl Cyclic-AMP slowed their growth and increased the activity of choline acetyltransferase. The density of opiate receptors did not show a comparable increase, but remained a function of cell density. NG cells, 4 days after plating, were incubated in "conditioned" medium obtained from 10-day old flasks containing 15-20 million NG cells. Cells incubated in the "conditioned" medium for 24 hours exhibited twice as many opiate receptors as those culture in "fresh" medium.

These results suggest that NG cells in tissue culture can regulate their opiate receptors by secreting a factor into the medium which stimulates receptor formation and/or expression.

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139.3 OPIATE RECEPTOR HETEROGENEITY IN HUMAN BRAIN REGIONS. K.A. Bonnet*, J. Groth*, H. Mermelstein*, M. Cortes*and E.J.Simon Depts. Psychiatr. and Pharmacol. NYU Med. Ctr. New York, N.Y. 10016.

Evidence for the existence of several sub-classes of opiate receptors has been reported for the CNS and other tissues of several animal species. Cross-competition for receptor binding between enkephalins and opiate alkaloids has led to the postulation of μ (opiate-preferring) and δ (enkephalin-preferring) receptors. Differential distribution of these receptor sub-types between brain regions has been reported by us and others. We now report that a similar heterogeneity and differential distribution of opiate receptors exists in human brain regions. Four brain regions (thalamus, amygdala, striatum and frontal cortex) were dissected from brains obtained at autopsy from the Office of the Chief Medical Examiner of New York. Competition for receptor binding of unlabeled naloxone and D-ala^{2}D-leu^5 enkephalin (DADL) against tritiated naloxone and DADL was studied in each brain region. As in rat and guinea pig brain,naloxone was found to be considerably more effective (4-10 times) against [^{3}H]naloxone than against [^{3}H]DADL,and the reverse was true for DADL (10-20-fold better against [^{3}H]DADL). An interesting exception is the thalamus where naloxone is more effective against [^{3}H]DADL than against [^{3}H]naloxone, in fact, 10 times more potent against [^{3}H]DADL than in the other regions. These findings are in very good agreement with our results in rat brain. They suggest the existence of μ and δ receptors in human brain and their differential distribution, the thalamus showing a considerable preponderance of μ receptors. This is the first evidence for receptor heterogeneity in human brain. An understanding of the nature and distribution of opiate receptor sub-types may ultimately have considerable importance in clinical pharmacology, including drug addiction and pain management. (Supported by grant DA-00017 from the National Institute on Drug Abuse).

139.4 OPIOID RECEPTORS IN THE MONKEY BRAIN, PITUITARY AND RETINA: LOCALIZATION BY LIGHT MICROSCOPIC AUTORADIOGRAPHY. J.K. Wamsley, M.A. Zarbin*, W.S. Young, III and M.J. Kuhar. Dept. of Neurosci., Johns Hopkins Univ. Sch. of Med., Balto., MD 21205.

Opioid receptors were localized in the monkey brain, pituitary and retina by using both in vivo (Atweh and Kuhar, Brain Res., 124:53, 1977) and in vitro (Young and Kuhar, Brain Res., 179:255, 1979) labeling techniques of autoradiography. The tissues of three male cynomolgus monkeys (Macaca fasicicularis) were prepared and the opioid receptors were labeled with [^{3}H]-diprenorphine.

High concentrations of autoradiographic grains, associated with opioid receptors, were found in the substantia gelatinosa of the spinal cord. This high density extended out into the dorsal root. Only moderate grain densities were found in lamina III and IV (of Rexed), but the entire gray matter of the spinal cord (including the ventral horn) showed concentrations of grains greater than background. High densities of grains were also found in the spinal trigeminal nucleus, nucleus tractus solitarius and the lateral parabrachial nucleus (with fewer grains in the ventral parabrachial nucleus). The substantia grisea centralis of the periaqueductal gray matter had high grain densities near its rostral extent, just ventral to the posterior commissure. The medial nuclei of the thalamus, many nuclei of the hypothalamus, the substantia innominata and the amygdaloid complex also showed high grain concentrations. In the cortical areas examined there were marked differences in the grain concentrations between regions with the highest density occurring in the deep laminae of the cingulate gyrus.

In the monkey pituitary gland, high densities of autoradiographic grains were associated with the neurohypophysis with only background levels of grains occurring over the adenohypophysis and intermediate lobe. The nuclei of the hypothalamus associated with the neurohypophysis, the supraoptic and paraventricular nuclei, also had moderate to high grain densities. In the monkey retina, autoradiographic grains were associated with the ganglion cell layer and significant grain densities were identified in the optic nerve. Presumably, some of the latter are undergoing axonal flow.

These opioid binding sites correlate well with those observed in autoradiographic studies of the rat brain, although there are a few notable differences. Many of the opioid receptor distributions can also be correlated with anatomical loci of brain functions known to be influenced by administration of opiate compounds.

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139.5 EFFECTS OF GUANINE NUCLEOTIDES ON MULTIPLE OPIATE RECEPTOR BINDING SITES. S. R. Childers, J. Vagovic* and J. Jessup*. Dept. of Pharmacology, Univ. of Florida College of Med., Gainesville, Fla. 32610.

Guanine nucleotides, especially GTP, decrease binding of agonists to opiate receptors in brain and membranes. Multiple (μ and δ) opiate receptors have been identified in brain, but it is not clear that these different agonist sites are differentially regulated by GTP. C. B. Pert et al identified multiple Type 1 and Type 2 receptors distinguished by the presence and absence of GTP regulation. To further explore these concepts we compared GTP effects on the binding of a μ ligand, ^{3}H-morphine and a δ ligand, ^{3}H-D-Ala$_2$ D-Met$_5$ enkephalin (DADLE), to rat brain membranes. GTP effectively reduced the binding of both agonists, with slightly greater inhibition seen with ^{3}H-morphine (48% max inhibition) than with ^{3}H-DADLE (39% max inhibition) binding. The IC_{50} values for both GTP and Gpp(NH)p were similar in inhibiting both ^{3}H-ligands. Analysis of 10 regions of porcine brain with different concentrations of Gpp(NH)p inhibiting both ^{3}H-morphine and ^{3}H-DADLE binding revealed similar IC_{50} values and max inhibition values of Gpp(NH)p in all regions studied. Scatchard analyses showed that the effect of 50 μM GTP on ^{3}H-morphine was primarily to decrease the affinity, but not the number, of high affinity sites with little effect on low affinity sites. However, the effect of GTP on ^{3}H-DADLE was to decrease the number of high affinity sites by 40% without affecting affinity, while decreasing affinity of low affinity DADLE sites by 50%.

In other neurotransmitter systems, divalent cations antagonize the actions of GTP. In brain membranes, addition of either Ca^{++}, Mg^{++} or Mn^{++} had very little effect on the inhibition of ^{3}H-morphine binding by Gpp(NH)p. However, if membranes were first stripped of divalent cations by pre-incubation with 1 mM EDTA, the potency of Gpp(NH)p was increased compared with non-treated membranes, with a 2-3 fold decrease in Gpp(NH)p IC_{50} values and no change in max inhibition by Gpp(NH)p. The effect of EDTA was not duplicated by EGTA. In EDTA-treated membranes, the Hill slope of Gpp(NH)p inhibition of ^{3}H-morphine binding was dramatically reduced to 0.5 (compared with 0.9 in control membranes). These results suggest that GTP may exhibit different potency profiles in the absence of divalent cations, but may bind to a single lower affinity state in the presence of cations.

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139.6 The Binding and Analgesic Properties of a Sigma Opiate: SKF 10,047 K. Spiegel*, M. Buatti*, and G.W. Pasternak (SPON: R. Price). Cotzias Lab. of Neuro-Oncology, Sloan Kettering Institute and Cornell University Medical College.

Martin et al (JPET 197:517, 1976) classified opiates into three categories on the basis of their pharmacological properties and postulated a different receptor for each: mu (morphine), kappa (ketocyclazocine) and sigma (SKF 10,047 or N-allylnormetazocine). We have investigated the receptor binding and analgesia of the prototypic sigma opiate, SKF 10,047. ^{3}H-SKF 10,047 binds specifically and saturably to rat brain membranes. Unlabeled SKF 10,047 at low concentrations (IC_{50} 5 nM) inhibits ^{3}H-SKF 10,047 binding but at 1 μM does not inhibit binding to a greater extent than levallorphan, morphine or naloxone, also at 1 μM. Computer analysis of saturation experiments demonstrates two components: a high affinity site (K_D 0.3 nM) present at low levels in rat brain and a low affinity site (K_D 6 nM) present at far greater levels. ^{3}H-SKF 10,047 binding is quite sensitive to low concentrations of the proteolytic enzymes trypsin and chymotrypsin and the reagents N-ethylmaleimide and iodoacetamide. Like other opiates, sodium ions lower binding in a dose-dependent manner and this inhibition is substantially reversed by the addition of manganese ions. Naloxazone in vivo and in vitro irreversibly blocks high affinity ^{3}H-SKF 10,047 binding, suggesting that SKF 10,047, morphine, ethylketocyclazocine and the enkephalins bind with highest affinity to the same site. Competitive displacement of specific ^{3}H-SKF-10,047 binding by morphine, levallorphan, naloxone and ketocyclazocine is biphasic, suggesting multiple binding sites. Dextrallorphan is inactive. Blockade of the high affinity site by naloxazone results in the loss of morphine's ability to displace binding at low concentrations. The remaining binding, corresponding to the low affinity site, is far less sensitive to morphine (IC_{50} 40-70 nM compared to <1 nM for the high affinity site). In contrast, the low affinity binding remains quite sensitive to displacement with unlabeled SKF 10,047, suggesting that this low affinity site binds sigma opiates better than mu drugs and may represent the sigma receptor postulated by Martin et al. SKF 10,047 is a potent analgesic in the writhing assay (ED_{50} 0.55 $\pm$ 0.12 mg/kg) with a slope parallel to morphine (ED_{50} 0.23 $\pm$ 0.1 mg/kg). Naloxone (1mg/kg) blocks both morphine and SKF 10,047 analgesia equally well. Blockade of high affinity sites by naloxazone in vivo lowers the analgesic potency of SKF 10,047 5-fold in a manner similar to the decrease in morphine, enkephalin, ketocyclazocine and beta-endorphin analgesia, implying a single analgesic receptor for them all.

139.7 SULFHYDRYL-GROUP INVOLVEMENT IN OPIATE RECEPTOR LIGAND SELECTIVITY SHIFTS: IS THE "μ" CONFORMATION THE DESENSITIZED STATE OF THE "δ" CONFORMATION? W. D. Bowen* and C. B. Pert (SPON: J. Rosenblatt). Section on Biochemistry and Pharmacology, Biological Psychiatry Branch, NIMH, Bethesda, MD 20205.

We have recently reported (Bowen et al., Proc. Nat. Acad. Sci. USA, in press) that the ligand selectivity of opiate receptors in rat striatal patches can be shifted by the presence of allosteric effectors in the incubation medium: sodium ion, divalent cations and GTP shift the μ-like, alkaloid-preferring selectivity to a δ-like, enkephalin-preferring selectivity while promoting the coupling of opiate receptors to adenylate cyclase. Comparison of the striatal slice binding of the prototype μ-agonist, [^{3}H]dihydromorphine (DHM), with that of the prototype δ-agonist, [^{3}H]D-Ala^2-D-Leu^5-enkephalin (DENK), in the presence of various ions and sulfhydryl effectors was performed. When tissue slices are pre-incubated for 15 min at 25°C (50 mM Tris, pH 7.4), sodium chloride (100 mM) increases DENK (+32 ±5%) while reducing DHM (-52 ±7%); manganese ion (3 mM) enhances DHM (+14 ±3%) and does not significantly alter DENK; Na and Mn ions in combination appear synergistic, markedly enhancing (+72 ±9%) DENK while restoring DHM to control levels. Pretreatment of tissue slices with N-ethylmaleimide (NEM) (.05 mM) strikingly alters this pattern, causing sodium ion to inhibit DENK binding as well as DHM binding. Dithiothreitol (DTT) (3 mM) also perturbs this pattern [e.g., enhancing DENK (+46 ±5%) and inhibiting DHM (-10 ±3%) in the absence of added ions]. Hydrogen peroxide (10 mM) preincubation, which promotes disulfide formation, abolishes the effects of sodium ion on DENK; subsequent pre-incubation with DTT, which reduces disulfide bonds, reverses the effects of H_2O_2, restoring the normal ion effects. Thus, NEM, DTT and H_2O_2, all of which perturb the sulfhydryl-disulfide status of proteins, appear able in vitro to shift the ion-regulated equilibrium between μ- and δ-opiate receptor conformations. Striatal slices taken from rats subjected to a 5-min ice-water swim, a highly stressful manipulation that causes massive endorphin release into cerebrospinal fluid and concomitant endorphin depletion at several brain loci, reduces DENK binding (-18%) and increases DHM binding (+15%) compared to control rats ($p < 0.05$). The physiological significance of the μ/δ opiate receptor equilibrium may thus be related to the sensitivity state of opiate receptors in vivo, a state which may be regulated by a mechanism involving sulfhydryl groups.

139.8 CHARACTERIZATION AND VISUALIZATION OF THE "κ" OPIATE RECEPTOR IN RAT BRAIN. R. Quirion, M. Herkenham and C.B. Pert (SPON: M. Murphy). Section on Biochemistry and Pharmacology, Biological Psychiatry Branch and Laboratory of Neurophysiology, NIMH, Bethesda, MD 20205

Recent analyses of the binding of radiolabeled ethylketocyclazocine, a prototype "κ" agonist (in Martin's classification scheme) have been interpreted as supportive biochemical evidence for the existence of yet another unique opiate receptor (Kosterlitz and Patterson, Proc. R. Soc. Lond. 210:113-122, 1980; Woods et al., Eur. J. Pharmacol., in press). Indeed, specific [^{3}H]ethylketocyclazocine binding to 25 μ striatal slices of unfixed rat brain thaw-mounted onto slides cannot be readily displaced by morphine, endorphins, or enkephalins, while naloxone, etorphine and two benzomorphans with "κ" pharmacological properties, MR2034, MR2266, and ethylketocyclazocine itself, are potent displacers. Optimal (75-80%) specific binding of [^{3}H]ethylketocyclazocine (K_D = 2.6 nM; B_{max} = 0.13 pmoles/slice) is obtained at 4°C in the presence of sodium chloride (100 mM), incubation conditions which promote opiate antagonist binding (Creese et al., Life Sci. 16:1837-1842, 1975) and reduce the binding of [^{3}H]dihydromorphine and [^{3}H]D-Ala^2-D-Leu-enkephalinamide to undetectable levels. Visualization of these binding sites with tritum-sensitive film (LKB) reveals a striking, highly discrete brain distribution pattern (e.g., striatal patches, habenular stripe) which is indistinguishable from that of [^{3}H]naloxone (Herkenham and Pert, Proc. Nat. Acad. Sci. USA, 77:5532-5536, 1980). Our interpretation of these data is that "κ" receptors like "μ" and "δ" opiate receptors (Bowen et al., Proc. Nat. Acad. Sci. USA, in press) are different conformations of the GTP-sensitive Type 1 allosteric opiate receptor complex which can assume the "κ" ligand selectivity pattern.

139.9 SUBCELLULAR DISTRIBUTION OF CALMODULIN AND OPIATE RECEPTORS IN CULTURED-NEURONAL CELLS - EFFECT OF OPIATES: D.Baram* and R. Simantov. Dept. of Genetics, Weizmann Institute of Science, Rehovot 76100, Israel.

The antagonism between Ca^{++} ions and the in vivo analgesic action of morphine was extensively studied by Harris, Loh and Way, J.Pharm.Exp.Ther. 195: 488, 1976, and conceivably reflects the profound effect of morphine on the regional and subcellular localization of Ca^{++} in the brain. However, this antagonism seems to take place in sites which are distal to the opiate receptors themselves since Ca^{++} ions increase, rather than antagonize, the binding of opiate agonists-alkaloids or enkephalins- to the brain opiate receptors. The recent findings that many, if not most, of the Ca^{++} activities in mammalian cells take place via interaction of Ca^{++} with the polypeptide calmodulin, prompted us to study the localization and the possibility of receptor-mediated regulation of calmodulin by opiates. Cultured neuroblastoma-glioma cell hybrids were chosen since they possess high density of functional opiate receptors. Calmodulin activity was monitored by a two-steps enzymatic assay, using a purified rat brain phosphodiesterase and crotalus adamanteus 5'-nucleotidase. Binding studies with ^{3}H-D-ala^2-met-enkephalinamide at 0.5-10.0 nM were performed to determine the affinity and the number of opiate receptors. After differential centrifugation of the lyzed cells, the nuclear (P_1), mitochondrial-membrane (P_2), microsomal (P_3) and the cytosol (S) fractions were isolated and tested for calmodulin and opiate receptors. Calmodulin activity was detectable in the concentration order of $S>P_2>P_3>P_1$, whereas opiate receptors were enriched in the P_2 and P_3 fractions. Treatment of the cells with 10^{-5} M morphine sulfate for 48 hours had no effect on calmodulin levels of P_1, P_2, P_3, or S. No changes in the fractions were also observed after treatment with the opiate antagonist naloxone. The mitochondrial-membrane (P_2) fraction was further purified on a gradient of 0.4, 0.8 and 1.2 M sucrose. Five layers and the mitochondrial pellets that were obtained showed an uneven distribution of calmodulin and opiate receptors. Two layers (3 and 4) were the richest in opiate receptors, with one of them (4) being twice as rich as the other. However, calmodulin was highly enriched in layer 4 but was undetectable or in a very low concentration in layer 3. This shows that neuroblastoma-glioma cells contain opiate receptors which are located on membranes that are separable into two types according to the membrane size and/or density. That these two layers contain different amounts of calmodulin deserved further experiments aimed at elucidating whether the biochemical and pharmacological effects of morphine are associated with selective effects on calmodulin or opiate receptors localized in specific cell structures.

139.10 THE USE OF THIOL-SEPHAROSE AND DEAE-SEPHADEX BEADS FOR PURIFICATION OF SOLUBLE OPIATE RECEPTORS. M. Dornay* and R. Simantov (Spon: M. Hershkowitz). Department of Genetics, Weizmann Institute of Science, Rehovot, Israel.

Sulphydryl blocking compounds inhibit the interaction between opiate alkaloids or peptides and the membrane-bound rat brain opiate receptors. Selective protection of the opiate receptors by opiate ligands from SH agents was indicated by Kosterlitz's, Simon's and Snyder's groups. It was of interest, therefore, to study whether thiol-Sepharose B4 beads, that bind covalently compounds that contain SH groups, can be used for purification of opiate receptors. Membranes prepared from the whole rat brain, the hypothalamus-thalamus of the rat (mostly μ receptors) or the neuroblastoma-glioma cell hybrids (apparent δ receptors) were labled with ^{3}H-D-Ala^2-met-enkephalinamide and solubilized by Brij 36T. The solubilization was conducted in the presence of 4 different protease inhibitors to minimize proteolysis. The free ligand was removed by chromatography through Sephadex G-75 column and the void volume that contained the receptor-^{3}H-enkephalin macromolecule was partially purified on a Sepharose B6 column. Three peaks of radioactivity were observed in the effluent, two of them contained proteins and one was free ligand. One of these peaks had a stock radius of about 59Å and was indicated by additional experiments as the specific opiate receptor. This peak was loaded on thiol-Sepharose column in phosphate buffer, washed with the same buffer to remove the non-bound compounds and then eluted by a gradient of 0-50 mM dithiothreitol (DTT). That the radioactive material eluted from the columns was bound to macromolecular proteins was tested by ammonium sulfate precipitation and rapid filtration, a technique developed to separate between free and receptor-bound ligand. The percentage of receptor-^{3}H-enkephalin bound to thiol-Sepharose column and eluted by DTT was 4%, 35% and 34% for opiate receptors solubilized from neuroblastoma-glioma, whole rat brain minus cerebellum and the rat hypothalamus-thalamus, respectively. Thiol-Sepharose beads therefore bind a very small portion of the opiate receptors of neuroblastoma-glioma cells as compared to those prepared from rat brain. Similar experiments with DEAE-Sephadex A50 columns, eluted with 1 M KCl, also showed higher binding of opiate receptors solubilized from the whole rat brain or the hypothalamus-thalamus as compared to neuroblastoma-glioma receptors. Experiments are being carried out to observe whether soluble and active opiate receptors preserve their selective interaction with thiol-Sepharose or DEAE-Sephadex beads and whether this selectivity correlates with differential binding properties to opiate alkaloids and peptides.

139.11 MULTIPLE BENZOMORPHAN BINDING SITES ON NCB20 HYBRID CELL LINE. R.E. West, Jr.*, R.W. McLawhon*, G. Dawson*, and R.J. Miller*. (SPON: D.R. Brown) Depts. Pharmacology, Biochemistry, and Pediatrics, University of Chicago, Chicago, IL 60637.

D-ala^2-D-leu^5-enkephalin (DADL) and ethylketocyclazocine (EKC) bind to a single site on the NG108-15 and N4TG1 clonal cell lines. Specific binding of either compound is totally displaceable by the other. On the NCB20 cell line DADL binds to a single site (K_D=1nM, B_{max}=350 fm/mg prot). EKC binds to two sites on these cells (K_D=3nM, B_{max}=500 fm/mg; K_D=15nM, B_{max}=1400 fm/mg). In competition binding assays, DADL competes for only part of the EKC binding. Scatchard analysis of EKC binding in the presence of 10^{-5} M DADL yields a single low affinity EKC site (K_D=19nM, B_{max}=1250 fm/mg). Thus, it is the high affinity EKC site for which DADL competes. In its pharmacologic profile and in the effects of ions and nucleotides on it this site resembles the δ-opiate receptor described in the nervous system and on the other cell lines.

The lower affinity EKC site displays an unusual pharmacologic profile. Of the drugs and neurotransmitters so far tested, benzomorphans bind with highest affinity. Morphine, etorphine, and naloxone, as well as DADL do not compete for this site. PCP and ketamine compete with μM affinities. Stereoselectivity is the reverse of what might be expected. Mn and Mg inhibit binding to this site, while EDTA, but not EGTA, increases binding. Nucleotides have no effect.

Preliminary studies of basal and PGE_1-stimulated adenylate cyclase activity indicate that benzomorphans may be more efficacious than DADL, morphine or etorphine as inhibitors of adenylate cyclase in these cells.

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139.12 EXPOSURE OF OPIATE BINDING SITES ON GLIOMA CELL LINE. J. Shorr,*G.Korner,* U.Bachrach* and O.Abramsky, Lab. of Neuroimmunology, Dept.Neurology and the Dept. Biochemistry, Hebrew University-Hadassah Med. Center, Jerusalem, Israel.

Cultured neuroblastoma-glioma hybrid cells (strain NG108-15) and neuroblastoma cells (N4TG1) contain large numbers of opiate receptors: 100000 and 20000 Enk-binding sites per cell respectively (W.A. Klee and M.Nirenberg, P.N.A.S. USA 71: 3474, 1974). The neuroblastoma-glioma hybrid cell line NG-108-15 has been a result of hybridization between mouse neuroblastoma N18TG-2 and rat glioma C6BU-1. Both parental cell lines were poor in opiate receptor sites on their plasma membrane (W. A. Klee and M. Nirenberg, P.N.A.S. USA 71: 3474, 1974).

In general, hybrid cells display parental properties despite the increase in the number of chromosomes. On the other hand, there are cases in which cells display properties which are not detectable in either parents (J. A. Paterson and M. C. Weiss, P.N.A.S. USA 69: 571, 1972).

The question rises whether the binding of opiates to the hybrid cells is a novel property or whether it originates (in a latent stage) in one of the parental cell lines. To answer this question, Sendai Virus (which is used for the formation of hybrid lines) was added to cultured glioma cells, neuroblastoma and the hybrid of neuroblastoma and glioma cells. Hybridization was performed according to the method of S. K. Sharma, M. Nirenberg and W.A. Klee (P.N.A.S. USA 72: 590, 1975). Opiate binding assays were carried out by binding (^{3}H)naloxone (NEN) to the cells in 50 mM Tris buffer pH 7.4. For fusion experiments one-day-old cells (young) or five-day-old cells (old) were employed. These cells were obtained by splitting confluent culture in a ratio of 1:13. We found that in the old fused cells the binding capacity increased by more than 50% compared to the now fused controls. The maximal increase in the binding of the tritiated ligand to the cells was apparent 30 min after fusion. In young glioma cells the basal specific binding activity was higher than in old glioma cells; moreover fusion did not increase the binding activity compared with non-fused young cells. Neither fused neuroblastoma cells nor the hybrids of neuroblastoma and glioma increased their specific binding after incubation with the virus. These findings suggest that young glioma cells exhibit opiate binding. This property which is lost with age can be recovered by treating old cells with Sendai Virus.

139.13 OPIATE RECEPTOR DISTRIBUTION IN MAMMALIAN CORTEX: A COMPARATIVE AUTORADIOGRAPHIC STUDY. M. Herkenham, S. Moon Edley* and C. B. Pert. Laboratory of Neurophysiology and Biological Psychiatry Branch, NIMH Bethesda, MD 20205.

The method of opiate receptor localization by in vitro autoradiography (Herkenham and Pert, PNAS, 1980, 77, 5532) can be applied to human tissue, since receptor labeling by incubation of fresh, unfixed cryostat-cut brain sections is performed postmortem on glass slides. In this fashion, sections from the brains of human, squirrel monkey, cat and rat were incubated at 4°C in a pH 7.4 solution containing ^{3}H-naloxone, 100 mM NaCl and .05 M Tris buffer. They were subsequently rinsed in sequential dishes of 4°C phosphate-buffered saline, dried under a stream of air, fixed in hot paraformaldehyde vapors and defatted in xylene and alcohol rinses. Autoradiography by dipping into Kodak NTB-2 emulsion or apposition to LKB Ultrofilm in x-ray cassettes gave similar results, but the film, when analyzed by computerized densitometry, facilitates quantitative analysis of large brains and widespread cortical distributions.

In the mammals studied, ^{3}H-naloxone-labeled opiate receptors are found throughout all cortical areas, though differences in density between areas, laminae and species are noticeable. In both rat and cat, medial and orbital frontal, temporal and insular areas show dense ^{3}H-naloxone binding, while primary sensory areas are sparsely labeled. In the squirrel monkey, areas displaying a prominent granular layer IV are the more densely labeled, while limbic areas are relatively more sparsely labeled. Species differences in laminar distributions are very striking. Opiate receptors throughout most of the rat cortex have peak densities in layers I, III or upper V, and deep VI (Lewis et al., this mtg). In the cat, layer I binding is prominent only in frontal, cingulate and retrosplenial areas. There and elswhere, layer VI is densely labeled, though this deep band is only very striking in insular, temporal and parahippocampal areas. In the squirrel monkey, a receptor-dense band in upper layer V predominates in all granular cortical areas and is continuous with a broader and sparser layer V band in limbic temporal and midline areas. In the portions of human cortex examined (frontal and anterior temporal), peak labeling is found in layers II and IV. For all animals, sections incubated in ^{3}H-dihydromorphine show the same pattern as ^{3}H-naloxone labeling but those incubated in ^{3}H-D-ala-D-leu-enkephalin display diffuse, specific binding throughout all cortical areas and laminae. The widespread but laminated appearance of alkaloid binding may mark receptors postsynaptic to intrinsic cortical or extrinsic thalamic "opiatergic" neurons, while the ubiquitous peptide binding represents a functionally mysterious opiate receptor subtype.

139.14 ASSAY OF SOLUBILIZED OPIATE RECEPTORS BY ADSORPTION TO DEAE PAPER. I.F. James*. (SPON: A. Goldstein). Addiction Res. Fdn., 701 Welch Rd., Palo Alto, CA 94304.

Recently, Ruegg et al. reported the solubilization of opiate receptors from the toad, Bufo marinus (Eur. J. Pharmacol. 64:367, 1980). We now describe a rapid and convenient assay for solubilized receptors from toad brain, which is adapted from a method used previously in the study of acetylcholine receptors (Schmidt and Raftery, Anal. Biochem., 52:349, 1973).

Washed brain membranes were extracted with 0.5% (w/v) digitonin and insoluble material was removed by centrifugation ($_{2}$10 000g, 1 hour). The supernatant was then incubated with [^{3}H] etorphine (1 hour, 22°C) and binding to solubilized receptors measured by adsorption of ligand/receptor complex to DEAE paper.

Between 5% and 20% of stereospecific etorphine binding sites on washed brain membranes were solubilized by digitonin. Binding of etorphine to these sites was proportional to the concentration of solubilized protein up to 0.4 mg/ml and had a pH optimum of 7.2. Etorphine (1nM) could be displaced from solubilized binding sites by 1μM levallorphan, but not 1μM dextrallorphan, indicating that stereoselectivity of receptors was maintained.

Experiments are in progress to characterize toad brain opiate receptors before and after solubilization.

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139.15 REGULATION OF OPIATE RECEPTORS BY GONADAL HORMONES: POSSIBLE SPECIES DIFFERENCES? David L. Roberts and James A. Diez. School of Biology, Georgia Institute of Technology, Atlanta, GA 30332

It has been previously reported by Hahn and Fischman (Biochem. Biophys. Res. Comm. 90:819, 1979) that brain opiate receptor concentrations in adult male rats increase almost 100% after castration. This finding seems particularly intriguing in that it may provide a link in the mechanism of feedback control of gonadal steroids since with testosterone replacement therapy, receptor number in castrates was found to equal intact controls. We hypothesized that this finding would be generalizable to females as well as males in a related species, but we found no evidence for a major effect of gonadal hormones in controlling opiate receptors in male or female mouse brain.

Opiate receptors were assayed by incubating washed or unwashed membrane fragments from whole mouse brain(minus cerebellum)with 0.27-9.0 nM tritiated met-enkephalin or naloxone, for 1 hr at 22° or 3 hr at 4°. Non-radioactive morphine or met-enkephalin was used for determination of non-specific binding. The assay was conducted in a total volume of 1.2 ml with about 1 mg of protein per tube, well within the linear range for total binding at equillibrium. Data were analyzed by Scatchard plots and the kinetic constants (Kd, Bmax) for high and low affinity binding obtained were comparable to values in the literature.

In some experiments, gonadectomized mice were compared with sham-operated and intact controls; in other experiments, male and female mice were injected with estradiol benzoate and testosterone propionate(200 ug/kg, s.c.)and compared with vehicle and uninjected controls. Treatment times varied from 3 wks to 3 months for gonadectomy, and from 1-2 wks for hormone administration. Unlike the published work with rats cited above, none of the groups of mice used in our experiments showed differences in binding (Kd or Bmax) which appeared interesting or meaningful; differences in group means were typically less than 15%, a value similar to within-group individual variation. We are currently evaluating the effect of castration in male and female rats, with the aim of determining whether our findings represent a true species difference. Supported by a Biomedical Research Support Grant.

140.1 POSSIBLE SIGNIFICANCE OF BINOMIAL PARAMETERS n, p AND q AT A CENTRAL INHIBITORY SYNAPSE. H. Korn, D.S. Faber, A. Mallet* and A. Triller*, INSERM U3 and U194, CHU Pitié-Salpêtrière, 75013 Paris, France and SUNYAB, Buffalo, N.Y. 14214, USA.

The characteristics of transmitter release were studied at the level of the goldfish Mauthner cell's (M-cell) inhibitory synapses. This system allows simultaneous recordings from single pre and postsynaptic neurons and a statistical treatment of fluctuating postsynaptic potentials (Korn and Faber, Science, 194: 1166-1169, 1976). Electrodes filled respectively with KCl and with HRP were inserted in the M-cell soma and in interneurons belonging to either this neuron's collateral network or to a class of commissural second order vestibular cells, all being identified as mediating chemical inhibition of the M-cell given the presence of a Passive Hyperpolarizing Potential (Faber and Korn, Science, 179: 577-578, 1973; Korn and Faber, J. Neurophysiol. 38: 452-471, 1975). As recently reported (Korn, Triller, Mallet and Faber, Science, 1981, in press) we found that computer calculated binomial predictions provide a better description of the observed fluctuating inhibitory postsynaptic potentials (IPSPs) than did the Poisson model. Furthermore there is a one to one correspondence between the binomial parameter n and the total number of presynaptic terminal boutons established by the reconstructed HRP stained interneurons. This series of experiments included 19 cells and the number of boutons varied from 3 to 30. Thus, the binomial quantal unit represents the amount of transmitter emitted at each terminal by a single and evidently all or none releasing bouton. Two sets of evidence favour the hypothesis that such a quantal unit equals the content of only one presynaptic vesicle rather than a larger relatively fixed number of vesicles 1) with basic assumptions that one vesicle opens 2.000 channels, that a single channel conductance is of 20 pS and that the input resistance of the M-cell is of about 200 KΩ, calculations indicate that the effect of one released vesicle is a unitary IPSP amounting to .08% of the equilibrium potential. For a binomial product np of 5, the average unitary IPSP would then be 4% of the IPSP equilibrium potential, which is exactly in the range of those observed experimentally. 2) Presynaptic stimulations of increased frequencies (from 1 to 50 per sec) produce smaller IPSPs indicating a reduced mean quantal content. However and as expected, since the number of synaptic boutons is invariant, binomial n remains constant; in contrast, during the same synaptic depression the parameter p of the binomial equations, i.e. the probability of release at each terminal fell drastically in several cells while q, which defines the value of one quantum was left relatively unchanged.

140.2 MECHANISM FOR PRESYNAPTIC INHIBITION IN CRAYFISH CLAW OPENER MUSCLE. Douglas A. Baxter and George D. Bittner. Dept. of Zoology, The Univ. of Tex., Austin, TX. 78712.

Crayfish (P. simulans) opener muscle fibers are innervated by a single excitor axon and a single inhibitor axon. The inhibitor axon makes synapses not only on opener muscle fibers, but also the terminals of the excitor axon, thus providing both post- and presynaptic inhibition. We have made intracellular recordings from the excitor motor axon as it branches over the ventral surface of the opener muscle within a single space constant of the inhibitory axo-axonal synapses. Both hyper-and depolarizing presynaptic inhibitory potentials (PIPs) were recorded from different excitor axons during inhibitory stimulation. The sign and amplitude of the PIPs in different excitor axons varied with the resting membrane potential (Em) so that the apparent reversal potential of the PIPs was -70.5 mv.

The amplitude of the action potential in 25 excitor axons was 93±4 mv (mean ± s.d.), over-shooting the -74±7 mv resting potential. The spike was followed by a depolarizing after potential (DAP) of 10±2 mv at 2 msec after the rising phase of the spike, and a DAP duration of 30-50 msec. Excitatory action potentials were reduced in amplitude by 6.0±1.2 mv during presynaptic inhibition. Small alterations (1-3 msec) of the optimum delay between the inhibitor spike and the excitor spike produced less presynaptic inhibition and much less reduction of excitor spike amplitude. The reduction in excitor spike amplitude was independent of the amplitude and sign of the PIPs. These and other data suggest that the brief conductance change associated with presynaptic inhibition shunts the excitatory spike amplitude, and is the primary cellular mechanism responsible for presynaptic inhibition.

Finally, bath application of 0.5 mM gamma-aminobutyric acid (GABA) was found to shift the resting Em (ΔE_{GABA}) and reduce the amplitudes of the spike and DAP at all points along the excitor axon. The sign and amplitude of ΔE_{GABA} in different excitor axons varied with the resting Em so that the apparent GABA reversal potential was -68.8 mv. Bath application of GABA reduced the amplitude of the excitor spike by 10-20 mv (mean = 15 mv). In addition, GABA shifted the Em and reduced spike amplitude in the inhibitor axon by a similar amount. The extrajunctional GABA receptors were blocked by picrotoxin and appeared to increase the membrane permeability to chloride ion. Thus, the excitor and inhibitor axons appear to have similar GABA receptors distributed over their entire length.

140.3 SOMATOSTATIN AND GLUTAMIC ACID INCREASE EXCITABILITY OF SINGLE CUTANEOUS PRIMARY AFFERENT C- AND Aδ-FIBERS IN THE CAT SPINAL CORD. S. Jeftinija* and M. Randić. Dept. of Vet. Physiol. Pharmacol., Iowa State University, Ames, IA 50011.

Somatostatin and glutamic acid are present in the superficial laminae of the dorsal horn, the area where primary afferent fibers are known to terminate. While some dorsal horn neurons can be inhibited by a postsynaptic action of somatostatin, glutamate has the potent excitant effect on dorsal horn cells. In addition, amphibian dorsal roots and mammalian muscle afferent terminals are depolarized by glutamate. To determine whether somatostatin and glutamate exert a presynaptic action on the primary afferent fibers we measured the electrical excitability changes of intraspinal portions of single cutaneous afferent C- and Aδ-fibers during local application of these compounds.

In spinalized cats, small filaments of the sural nerve were prepared for recording and thresholds for antidromic activation of single afferent fibers were measured during intraspinal stimulation with a fine platinum microelectrode positioned within laminae I-III of the dorsal horn. Somatostatin (3.1 mM, pH 5.5) and L-glutamate (1.0 M, pH 7.0) were iontophoretically ejected through glass micropipettes glued to a stimulating electrode (intertip distance < 15 μm).

Out of 21 C-fibers tested, somatostatin (25-100 nA for 3 min) decreased threshold (to 67-95% of control) in a reversible and dose-dependent manner in 9 fibers, and increased threshold (to 109-124% of control) in 2 fibers. Decrease in threshold was also observed in 5 of 9 Aδ-fibers tested with somatostatin. L-glutamate (25-100 nA for 3 min) decreased threshold (to 67-93% of control) in 14 of 27 C-fibers and 5 of 8 Aδ-fibers.

Our data indicate that, in addition to previously demonstrated postsynaptic actions in the cat dorsal horn, somatostatin and L-glutamate have presynaptic actions to increase excitability in some cutaneous primary afferent C- and Aδ-fibers. Supported by NSF grant BNS 23871 and U.S. Dept. of Agriculture.

140.4 ELECTROGENIC MECHANISM OF PRIMARY AFFERENT DEPOLARIZATION T. Hashiguchi and A.L. Padjen. Department of Pharmacology and Therapeutics, McGill University, Montreal, Quebec.

The objective of this study was to analyse the ionic mechanism of primary afferent depolarization (PAD) by intra-fibre recording (microelectrodes filled with 3M KCl, 30-50 MΩ resistance) in the isolated hemisected frog spinal cord. Preparation was superfused with Ringer solution at 10°C. Membrane characteristics were determined by means of standard current clamp technique with pulses of 1.5 sec. Stable recordings (1-2 hrs) were obtained from more than 50 fibres with resting membrane potential -71.0 ± 6 mV (Mean ± S.D., n=49) and action potentials, evoked by root stimulation or direct current injection, of 97 ± 12 mV (n=51). All fibres were impaled at the dorsal root (DR) entry of 9th or 10th segment and had conduction velocity of >15 m/sec. I-V curves at steady state showed linear relationship in hyperpolarizing range of membrane potential (effective resistance, R_e=38 MΩ ± 16, n=11). Depolarizing pulses however revealed strong delayed rectification in all fibers studied. Adjacent DR stimulation (supramaximal for A fibres) evoked PAD (5.6 ± 3.2 mV, n=21) but ventral root stimulation evoked much smaller PAD only in some fibres. Most of the fibres showed spontaneous slow depolarizations often as large as evoked PADs. Linear part of I-V curves during PAD indicated a net decrease in R_e (to 68% ± 9 of control, n=11). Amplitude of PAD was dependent on membrane potential (increasing with hyperpolarization) but no reversal of PAD was obtained presumably because of delayed rectification. Extrapolation of the linear part of I-V relationship indicated an apparent reversal potential (E_r) of -45 ± 11 mV (n=11). PAD amplitude did not change when K sulfate (instead of KCl) filled microelectrodes were used but extrapolated E_r became more negative (-52 ± 8 mV, n=9).

These results are consistent with the idea that PAD results from an increase in primary afferents permeability to chloride ions.

Supported by MRC of Canada.

140.5 THE EFFECT OF BURST PATTERNED STIMULATION ON POST-TETANIC HYPERPOLARIZATION OF MAMMALIAN C-FIBERS. R. Siegel* and R.I. Birks, Physiology Department, McGill University, Montreal, Canada.

The effect of physiological modes of burst patterned stimulation on post-tetanic hyperpolarization (PTHP) of rabbit vagus nerve bathed in isethionate-Locke solution at 35°C was studied using the sucrose-gap technique and method of analysis of Rang and Ritchie (J. Physiol. 196, 183-222, 1968). Stimulation with 0.5 sec bursts at 20 and 40 Hz every 8 sec was compared to stimulation with equally spaced pulses at 1.25 and 2.5 Hz respectively. Single control bursts at 30 Hz for 5 sec were used to estimate maximum Na pump activity. In 20 trials in 10 experiments the amplitude of PTHP following each successive burst at 40 Hz increased during the first 90±15 sec (Mean ± S.D.) of burst patterned stimulation to a steady-state hyperpolarization 2.6± 0.4 times that in response to the first burst. The steady-state hyperpolarization was 46±5% that in response to a control 5 sec 30 Hz tetanus. A significantly smaller ($P < 0.01$) hyperpolarization occurred during stimulation at 2.5 Hz: the increase over 2 min was to a steady-state value 71±9% of that during the 40 Hz burst patterned stimulation. When the intraburst frequency was reduced from 40 to 20 Hz the hyperpolarization was reduced; but it was over twice that found in comparison trials at 1.25 Hz. Thus burst-patterning of this form produces a greater increase in electrogenic Na pump activity than occurs when the pulses are equally spaced at the same average frequency.

Burst patterned stimulation of preganglionic fibers to the cat superior cervical ganglion has been shown to increase acetylcholine release and synthesis at the unmyelinated terminals by as much as three times that found when the stimulation is with equally spaced pulses at the same average frequency (Birks, J. Physiol. 295, 51-52P, 1979). Indirect evidence indicated the increase in synthesis to be related to accelerated pump activity and the increase in release to increased $[Na^+]_i$. The present results indicate that the increase in sodium pump activity of small unmyelinated fibers is indeed greater when the stimulation is burst patterned. Furthermore, the amplitude of PTHP can be taken as an index of sodium load provided pump activity is proportional to $[Na^+]_i$ and both membrane resistance and pump ratio remain unchanged. On this basis the results also support the hypothesis of increased $[Na^+]_i$ during burst patterned activity.

This work was supported by the Medical Research Council of Canada.

140.6 FREQUENCY DEPENDENCE OF IA-MOTONEURON EPSPs, W.F. Collins III* and L.M. Mendell, Dept. Neurobiology and Behavior, SUNY, Stony Brook, N.Y. 11794.

EPSPs recorded from medial gastrocnemius (MG) motoneurons during stretch evoked and electrical stimulation of single MG group Ia fibers have been analyzed in an effort to characterize the central properties of Ia fibers and their terminals. Anesthetized cats whose spinal cords had been acutely transected at T13 were used since the resulting enlarged Ia EPSPs (Nelson, et al., J. Neurophysiol. 42, 1979) can be resolved without averaging. Electrical stimulation of single fibers was achieved by stimulating dissected dorsal root filaments shown by impulse discharge to contain only a single MG Ia fiber. The synaptic action of a single Ia fiber was confirmed either by the similarity of the spike triggered and the electrically evoked average EPSPs (1 afferent, 3 motoneurons) or by observing that the EPSP was all or none and unaffected by increasing stimulus intensity (1 afferent, 1 motoneuron). The rate of stimulation varied between $1Hz$ and $500Hz$. Both successive individual EPSPs and the same data averaged over several blocks of n stimuli (e.g. n=5;33) were analyzed. Several clear trends emerged in the course of these initial experiments. At higher repetition rates (e.g. $>40Hz$ at some connections, but varying from connection to connection) the EPSP declined over time with much of the decrease occurring within the first few (5-20) stimuli. After a variable interval a new stable mean amplitude level was achieved. During this period EPSP amplitude fluctuated from trial to trial. These fluctuations occurred in a random manner as analyzed by turning points and phase lengths (Mieri & Rahaminoff, J. Physiol. 278, 1976). The final EPSP average amplitude was highly dependent on the frequency of stimulation with high repetition rates being associated with smaller EPSP amplitudes. There was also a noticeable increase in the proportion of "failures" at high repetition rates as well as a decrease in the number of large EPSPs. At repetition rates up to $200Hz$ there was no measurable change in the latency or rise time of the EPSP averaged during the final portion of the train despite a considerable decrease in amplitude. At higher frequencies a small increase in latency ($< 100\mu sec$) and rise time ($< 200\mu sec$) was noted. However, these may be less reliable because the continued decline in EPSP amplitude results in a smaller signal to noise ratio. The mechanism of this decrease in Ia-motoneuron EPSP amplitude remains unclear. Continuing studies should help to determine whether alterations in impulse conduction at branch points, Ia terminal invasion and/or transmitter release at individual synaptic sites contribute to the observed frequency dependent amplitude decrease. (Supported by NIH grants NS 16996-01 and NS 06407-01.)

140.7 POST-TETANIC POTENTIATION OF Ia EPSPs: DIFFERENTIAL DISTRIBUTION AND POSSIBLE MECHANISMS. A. Lev-Tov*, M. J. Pinter* and R. E. Burke. Lab. Neural Control, NINCDS, NIH, Bethesda, MD 20205.

Post-tetanic potentiation (PTP) of Ia EPSPs has been reported to vary inversely with motoneuron input resistance (R_N; Lüscher et al., Nature 282:859, 1979). We studied 55 medial gastrocnemius (MG) motoneurons in pentobarbital-anesthetized cats with ventral roots cut. A conditioning train of pulses to the MG nerve at 500 Hz for 20 sec was followed by test pulses at 0.5-1.0 Hz. The largest sequential EPSPs during a 10 sec post- tetanus epoch were averaged (V_p) and normalized by control EPSPs (V_c). Despite considerable scatter, we found a negative linear correlation between PTP magnitude (V_p/V_c) and V_c ($r= -0.50$, $p<0.001$, 2-tailed test) and a weaker correlation between V_p/V_c and R_N ($r= -0.33$, $0.02>p>0.01$), implying a tendency for differential distribution of PTP. A complicating factor is the presence of significant depression after prolonged tetani. We found a strong positive correlation ($r= 0.74$; $p<0.001$) between normalized amplitude of the first post-tetanus EPSP (often smaller than control) and V_p/V_c. The observed PTP curve reflects depression as well as potentiation phenomena; both can affect the "peak" and either (or both) could cause differential effects. We found no clear correlations between "pure" synaptic depression (i.e., dependent on quantal release from individual boutons, tested by EPSP pairs at 250 ms interval) and either V_c or R_N. However, this synaptic depression was markedly enhanced during PTP, indicating increased transmitter release per bouton. There was no evidence that failure of action potential invasion contributes to post-tetanic depression since, in most cases, amplitudes of Ia prepotentials in the ventral horn, and of cord dorsum Ia volleys, were maximal immediately post-tetanus. Other possible sources of differential depression remain to be excluded. During PTP, the rise time and half-width of most EPSPs increased but there was no systematic relation between shape changes and V_p/V_c. The correlations between V_p/V_c and V_c or R_N may simply reflect differential scaling of non-linear voltage summation at Ia synapses, which terminate mainly on motoneuron dendrites. With PTP-enhanced transmitter release, non-linear summation would vary directly with local EPSP amplitudes and local input resistances, which are large in dendritic sites and are likely to scale inversely with motoneuron size. Supporting this notion, we found that the summation of MG and lateral gastrocnemius EPSPs was consideraly more non-linear when both were potentiated, as compared to control conditions. The relatively weak differential distribution of PTP in MG motoneurons seems compatible with conventional notions of augmented transmitter release during PTP.

140.8 FIBERS IN THE RIGHT VISCEROPLEURAL CONNECTIVE CAN ACCELERATE THE DECAY OF PTP OF SYNAPSE RC1-R15 OF APLYSIA CALIFORNICA. Jacques P. Tremblay and Gilles Grenon*, (Dept of Anatomy, Laval Univ, Quebec, P.Q. Canada)

A synapse called RC1-R15 can be activated by threshold stimulation of the right visceropleural connective (RC) of Aplysia californica. The activity of this synapse is recorded in cell R15 of the abdominal ganglion. Repeated stimulation of this synapse at 1 to 2 Hz produces successively a reduction of the RC1-R15 EPSPs (synaptic depression), an increase to a sustained plateau (frequency facilitation) and when the rate of stimulation is reduced a rapid increase followed by an exponential decay (posttetanic potentiation). In this poster, we show that stimulation of the RC 50% or 100% above the threshold for activating the fiber making the RC1-R15 synapse can accelerate the decay of PTP of synapse RC1-R15. This suprathreshold stimulation does not modify the synaptic depression and the frequency facilitation phenomena, nor does it affect the membrane resistance and potential of cell R15. Stimulation of RC below the threshold of synapse RC1-R15 can also reduce the PTP of synapse RC1-R15. High frequency (5 Hz) subthreshold stimulation of RC can also reduce the synaptic depression. However the subthreshold stimulation has no effect on frequency facilitation of RC1-R15. The effect of subthreshold stimulation on PTP is often correlated with the recording of a very small EPSP (sEPSP) in cell R15. This sEPSP is barely detected at the beginning of the subthreshold train of stimuli and has a long latency. The effects obtained with subthreshold and suprathreshold stimulation of the RC are abolished in sea water with a high concentration of Ca^{++} and of Mg^{++}. Both types of effects are attributed to the activation of fibers in the RC (called modulating fibers) which by the intermediary of one or several interneurons modulate presynaptically the transmission of synapse RC1-R15. The presence of these modulating fibers in the RC does introduce some noise in the study of PTP of synapse RC1-R15. The acceleration of PTP decay by these RC fibers does not however invalidate the main conclusions of our previous parametric studies of PTP at this synapse. These conclusions were: 1) PTP increases when the frequency of stimulation of RC1-R15 increases, 2) PTP increases when the number of stimuli increases, 3) PTP decays exponentially, 4) PTP decays more slowly following a train with a larger number of stimuli and 5) PTP decays more slowly following a train at an higher frequency of stimulation (Schlapfer et al., Brain Res. 109, 1-20, 1976). All these conclusions have been reached despite the acceleration of PTP decay by the RC fibers. The possible interactions of the modulating fibers of RC with pharmacological studies made at RC1-R15 will be discussed. Researchers are also caution about the possibility of undetected presynaptic modulating effects even in model systems where the synaptic activity is studied between two identified neurons.

140.9 POTASSIUM CURRENT (I_K) IN APLYSIA NEURON L_{10}: RELATIONSHIP TO SPIKE-AFTER-HYPERPOLARIZATION AND TO TRANSMITTER RELEASE. Dick Mooney* and Rafiq Waziri. Dept. of Psychiatry, Univ. of Iowa, Iowa City, IA 52242.

Interneuron L_{10} was voltage clamped in situ with recording and current-passing electrodes in the perikaryon. Monosynaptic IPSPs produced by L_{10} spikes were recorded in one of several follower cells with conventional double barreled electrodes. There are two principal K-conductances in L_{10}. The main one produces a delayed rectifying current; it is suppressed by intracellular or extracellular exposure to TEA, and by extracellular application of 4-aminopyridine or Ba^{++} ions. It is activated by depolarizing pulses and exhibits an expotential decay to brief pulses. Secondly, there is a smaller Ca^{++}-activated I_K which is apparent after somewhat longer depolarizing pulses, and which is suppressed by Co^{++} or Mn^{++} ions. There is a small residual outward I_K after both intracellular TEA and extracellular Co^{++} application. There is no appreciable "A current" activated by depolarizing pulses from hyperpolarized holding potentials, as there is in neuron R_{15}. The after hyperpolarization of the spike has the combined properties of the two principal I_Ks. The delayed rectifying current, measured after brief depolarizing pulses and prior to transmitter release, is correlated with transmitter release, notably under conditions where membrane potential is varied or when L_{10} repeatedly fires. The significance of this correlation is yet unknown.

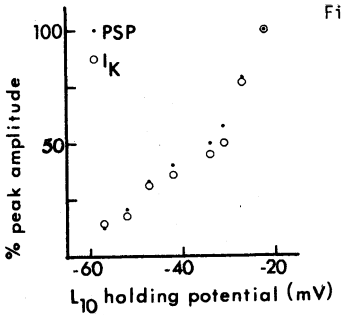

Fig. 1 Normalized PSP and I_K (tail current) amplitudes show a similar dependence on the steady holding potential which preceded and followed a 5 msec. depolarizing pulse.

140.10 TETANUS TOXIN PRODUCES BLOCKADE OF SYNAPTIC TRANSMISSION IN MOUSE SPINAL CORD NEURONS IN CULTURE. G.K.Bergey, P.G.Nelson R.L.Macdonald and W.H.Habig*. Lab. of Devel. Neurobiol., NICHD and Bact. Toxins Br., Bureau of Biologics, Bethesda, MD 20205.

The effects of tetanus toxin on synaptic transmission were investigated using fetal mouse spinal cord neurons grown in dissociated cell cultures. Spinal cord cultures were prepared and maintained in 10% horse serum and Eagle's minimal essential medium (MEM) for four to ten weeks. Prior to addition of tetanus toxin for experiments the cultures were washed X3 and placed in 1% fetal calf serum in MEM. Tetanus toxin (final conc. 10^{-5} to 10^{-9} g/ml) in 1% FCS-MEM was added to the cultures and intracellular recordings were made at various times using 4M KAc filled microelectrodes and a conventional bridge circuit. Spinal cord neurons in this culture system demonstrate prominent synaptic activity; over 80% reveal evidence of spontaneous PSPs.

Intracellular recordings from spinal cord neurons in the above concentrations of tetanus toxin revealed increased excitation manifest by paroxysmal depolarizing events (PDE) in the early hours of toxin exposure (Macdonald, Bergey and Habig, Neurology 29:588, 1979). Continued recording revealed diminished frequency of PDEs after several hours. Recordings from spinal cord neurons after 6 hr to 4 days of toxin treatment revealed an absence of spontaneous synaptic activity. No EPSPs or IPSPs were demonstrable in these chronically treated neurons (n=85). No morphological changes were apparent; membrane potentials and excitability were not affected. Reponses to iontophoretically applied glutamate, GABA and glycine were readily elicited at iontophoretic currents comparable to control situations.

An assay of evoked synaptic activity demonstrated 11 evoked monosynaptic connections in 20 control cell pairs. In contrast, after 24 hrs of tetanus toxin treatment (10^{-7} g/ml) no synaptic connections could be evoked by intracellular stimulation of 21 cell pairs (sig. to <.001 by corrected Chi square).

Neurons remained synaptically quiescent after tetanus toxin treatment despite removal of exogenous toxin and subsequent periods of up to 24 days in medium with antitoxin.

To examine whether the toxin-induced synaptic blockade was a result of effects upon calcium mechanisms, the effects of tetanus toxin on the calcium component of the spinal cord neuron action potential (Heyer, et.al. Br. Res. in press) were examined in the presence of 10 μM TTX, 25 mM TEA and 10 mM calcium. Neither the dV/dt nor the maximum duration of the calcium spike was significantly affected by 24 hr treatment with tetanus toxin.

The apparent presynaptic locus for tetanus toxin blockade of synaptic transmission in spinal cord neurons may involve mechanisms distinct from those mediating calcium flux.

140.11 EFFECTS OF VARIATIONS OF EXTRACELLULAR POTASSIUM ACTIVITY ($[K^+]_o$) ON SYNAPTIC TRANSMISSION AND $[Ca^{2+}]$ RESPONSES IN HIPPOCAMPAL TISSUE IN VITRO. G.L. King and G.G. Somjen. Dept. of Physiology Duke University Medical Center, Durham, NC 27710.

Extracellular recordings were made from the stratum radiatum (SR) and the stratum pyramidale (SP) of the CA1 region of rat hippocampal slices maintained in vitro. Responses were evoked by focal microstimulation of afferent fibers in SR. "Output", expressed as the amplitude of either the compound EPSP or the compound postsynaptic spike, were plotted as a function of "input" (presynaptic compound spike) or of stimulating pulse intensity. $[K^+]_o$ was monitored with ion-selective microelectrodes in the tissue. Either lowering or raising $[K^+]_o$ from the control level (3.0 mM) enhanced the field EPSP (detected as an increased slope of the input-output curve), which suggested increased transmitter release per presynaptic impulse. Raised or lowered $[K^+]_o$ did not affect the input-output curves in a linear manner; no prediction could be made from measured $[K^+]_o$ as to the magnitude of increased transmitter release. The excitability of presynaptic fibers was also increased when $[K^+]_o$ was raised. At 9.0 mM $[K^+]_o$ and above, spontaneous seizure-like discharges were observed. We also measured $[Ca^{2+}]_o$ with ion-selective microelectrodes. Repetitive stimulation of presynaptic fibers causes a decrease of $[Ca^{2+}]_o$ in hippocampal slices (Benninger et al., Brain Res.187:165, 1980). These stimulus-evoked $[Ca^{2+}]_o$ responses were larger in SP than in SR. $\Delta[Ca^{2+}]_o$ became detectable only when the stimulation was of sufficient intensity to evoke a postsynaptic action potential. Raising $[K^+]_o$ greatly enhanced the $\Delta[Ca^{2+}]_o$ evoked by afferent stimulation. In contrast, lowering $[K^+]_o$ reduced the $\Delta[Ca^{2+}]_o$ evoked by afferent stimulation, in spite of the enhanced EPSP, but concordant with the diminished spike discharge. We conclude: (1) That, besides its well-known action on electrical excitability, $[K^+]_o$ also has an important role in regulating transmitter release (see also: Cooke and Quastel, J. Physiol. 228:435, 1973). (2) That, in CA1 region, the quantitatively most important process contributing to the lowering of $[Ca^{2+}]_o$ during stimulation is the action potential discharge of cell bodies and/or proximal dendrites. (3) That the 5 mM $[K^+]_o$ and 2 mM $[Ca^{2+}]_o$ customarily used in bathing media for in vitro CNS tissue significantly alter synaptic transmission compared to the physiological 3 mM $[K^+]_o$ and 1.2 mM $[Ca^{2+}]_o$ (see also: Dingledine & Somjen, Brain Res. 207:218,1981). Supported by Grant#NS 11933 of the NINCDS.

140.12 LEPTINOTARSIN ACTIVATES TWO MECHANISMS OF QUANTAL RELEASE AT THE RAT NEUROMUSCULAR JUNCTION. J. R. Stimers. Dept. Biological Sciences, University of Southern California, Los Angeles, CA 90007.

Leptinotarsin (LPT) causes a biphasic increase in MEPP frequency, followed by a total cessation of release. In the present study, LPT was found to act during the first phase of release by opening Ca channels in a voltage-independent manner, activating the normal Ca-dependent release mechanism. During phase two, release occurs by a mechanism which does not have the normal Ca^{2+} or voltage dependence.

When the external Ca^{2+} concentration is lowered, the peak MEPP frequency of phase one diminishes and is not distinguishable from control at Ca^{2+} concentrations less than 10^{-5}M. The peak MEPP frequency of phase two is first reduced at less than 10^{-6}M Ca^{2+} and is still above control frequency at 10^{-9}M. Cd^{2+} added to normal Ringer gives results similar to 10^{-9}M Ca^{2+}. Furthermore, increasing Ca^{2+} to 20mM enhances phase one, while depressing phase two to low levels.

Using electrotonic polarization of the endplate, in the presence of TTX, the MEPP frequency during phase one of the LPT response is enhanced by depolarization and unaffected by hyperpolarization, even though the release rate is very high. Phase two, however, shows no change in MEPP frequency with either hyperpolarization or depolarization, even when higher currents are used. Application of 50mM K^+ before phase one increases MEPP frequency which is quickly followed by a vigorous LPT response. The same solution applied between phases one and two does not increase MEPP frequency and the expected phase two increase is inhibited. In the same experiment, if K^+ is returned to the normal value (5mM), MEPP frequency increases to the usual phase two level.

These data indicate that depolarization of the endplate is not solely responsible for the biphasic increase in MEPP frequency elicited by LPT. Furthermore, phase one has properties similar to those seen with normal Ca-dependent release. Therefore, it is concluded that LPT acts during phase one by inducing a Ca conductance in the presynaptic membrane. Since this conductance is blocked by Cd^{2+}, Ca^{2+} may be passing through the normal Ca channels. Phase two, however, is inhibited by depolarization with K^+ and has only micromolar dependence upon external Ca^{2+}.

140.13 NON-QUANTAL ACH RELEASE IN THE MOUSE DIAPHRAGM: EFFECTS OF DENERVATION AND BOTULINUM TOXIN. E.F. Stanley, & D.B. Drachman, Dept. of Neurology, Johns Hopkins Sch. of Med., Baltimore, MD 21205.

The spontaneous release of acetylcholine (ACh) from motor nerve terminals is now thought to occur by two mechanisms: a) quantal release, giving rise to mepps; and b) non-quantal release (Katz & Miledi, Proc. Roy. Soc. Lond. B 196:156-172, 1977).

We have studied the effects of denervation and botulinum toxin on these 2 forms of spontaneous ACh release in the mouse diaphragm by a sensitive visual method. This method is based on the observation that ACh can produce structural changes at the neuromuscular junction (see also O'Brien et al., J. Physiol. 295:92p, 1979). The mouse diaphragm is removed whole, and is incubated in oxygenated Ringer's solution with DFP, 1.5 x 10^{-4} - a potent cholinesterase inhibitor - (and tetrodotoxin 0.5 µg/ml, to prevent nerve firing) for 12 to 15 min, and then fixed in 5% glutaraldehyde. The endplate "swellings" are readily seen with the stereomicroscope, or even with the naked eye. We have confirmed the specific role of ACh in producing the swellings, since they do not form: a) in the presence of curare, or b) after prolonged denervation. Moreover, the addition of ACh to denervated diaphragms produces identical swellings. Thus, we have been able to use the occurrence of endplate swellings as an indicator of spontaneous ACh release.

Our findings show that:

1) Botulinum toxin blocks quantal ACh release nearly completely. However, swellings still occur, which can be attributed to persisting non-quantal ACh release.

2) Following denervation ACh release continues for up to 9 hrs, which correlates with mepp activity and hence quantal ACh release. However, non-quantal ACh release ceases far sooner, at 3 1/2 hrs.

These findings fit well with our previous observations suggesting an important role for non-quantal ACh release in the neural regulation of the resting membrane potential and extrajunctional ACh receptors of skeletal muscle (Stanley et al., Soc. Neurosci. Abstr. 6:384, 1980; Pestronk et al., Exp. Neurol. 70, 690-696, 1980.)

140.14 EVIDENCE THAT DECREASES IN INTRACELLULAR pH RAPIDLY INHIBIT TRANSMISSION IN THE GUINEA-PIG HIPPOCAMPAL SLICE. Peter Lipton and Donna Korol* Dept. of Physiology, Univ. of Wisconsin, Madison,Wisc. 53706

Decreases in intracellular pH (pHi) accompany states of compromised energy metabolism,increased neural activity and hypercapnia in brain tissue. We have been studying possible effects of dec reased pHi on monosynaptic transmission in the guinea-pig hippocampal slice. Slices are maintained at 37° by superfusion with a bicarbonate buffer at pH 7.4; the perforant path is stimulated and the evoked response is recorded in the dentate granule cell layer. The size of the population spike is monitored.

Decreasing extracellular pH by .4 units was accomplished either by increasing CO_2 concentration x3.5 (from 5% to 18%) or by decreasing buffer HCO_3^- concentration x 3.5 (from 26 to 7.5mM). Both of these changes produced identical effects on the evoked response; the population spike was reduced to about 55% of its control level with a half-time between 30 and 60 seconds. The decreases in pH did not affect the anti-dromic response in the dentate granule cells within 5 minutes. After this time there was a small (10-20 percent) decrease. Thus the action of the decreased pH seems to be on small neuronal elements. Other workers have studied effects of decreasing extracellular pH on synaptic transmission and have concluded that inhibition probably results from an extracellular, rather than intracellular action of the acidity. We tried to distinguish between these two sites of action.

Elevating both buffer CO_2 and HCO_3^- by x 3.5 decreases pH_i by .35 units (Whittingham and Lipton, unpublished) without changing extracellular pH. This buffer reduced the population spike by 60% with a half-time of 60 secs.

Increasing HCO_3^- may signifigantly lower buffer free Ca^{2+} (Schaer,Pflug. Archiv.,347:249) and we find that a 50% reduction in buffer Ca^{2+} from its normal 2.4 mM rapidly inhibits transmission. However, we find that the elevated CO_2/HCO_3^- buffer has an identical effect on transmission even if buffer Ca is raised to 5mM. (Reducing buffer Ca from 5 to 2.4mM does not affect transmission). In further control studies we found that simply substituting another anion (isethionate) for Cl^- has only a very small effect on transmission.

Thus, decreasing pHi by .35 units appears to significantly attenuate neural transmission. The fact that increasing CO_2 alone similarly inhibited transmission is consistent with this conclusion and suggests that decreasing extracellular pH does not affect transmission. We conclude that decreasing pH by lowering HCO_3^- rapidly lowers pHi. This will be tested.

140.15 STIMULATION OF CHOLINERGIC SYNAPTIC VESICLE Ca^{2+}/Mg^{2+} ATPase ACTIVITY BY BICARBONATE. Joan E. Rothlein and Stanley M. Parsons. Dept. of Chemistry, Univ. of California, Santa Barbara CA 93106.

The Ca^{2+}/Mg^{2+} ATPase associated with cholinergic synaptic vesicles from Torpedo californica is stimulated by several anions, of which bicarbonate is the most effective. Since Torpedo vesicles have been shown to specifically take up exogenous acetylcholine by a process stimulated by bicarbonate and either MgATP or CaATP (Parsons, S.M. and Koenigsberger, R. (1980), PNAS 77,6234-6238), additional studies exploring the mechanism of bicarbonate stimulation of the vesicular ATPase were performed. Basal Mg^{2+} and Ca^{2+} ATPase activities were stimulated by bicarbonate in a saturable manner with an apparent dissociation constant of 5mM for a two-fold maximal stimulation. In the absence of bicarbonate the double reciprocal plot of ATPase activity versus ATP concentration was biphasic with Michaelis constants of 0.048 mM and 0.58mM and maximum rates of 40 and 135 nmoles ATP hydrolyzed/min/mg protein. The double reciprocal plot became nearly linear in the presence of bicarbonate with a Michaelis constant of 0.06mM and a maximum rate of 250 nmoles ATP hydrolyzed/min/mg protein. Basal and bicarbonate stimulated activities were maximal at pH 7.0 and unaffected by sulfhydryl reagents. Classic ATPase inhibitors, NBD-Cl and DCCD, and anion-channel blockers, SITS and DIDS, were more effective in inhibiting bicarbonate stimulated ATPase activities than basal activities.

The vesicle ATPase was stimulated 3-4 fold by several non-ionic detergents and solubilized by 1.6% Lubrol WX or Igepal DM 710. The solubilized vesicle protein contained up to 90% of the total ATPase activity. The solubilized ATPase activity was stimulated by bicarbonate, stabilized by ATP, and appeared as a single broad band in equilibrium buoyant density centrifugation. Thus, bicarbonate binds directly to the ATPase to stimulate its activity.

140.16 KINETIC ANALYSIS OF Ca^{++} EFFLUX FROM SYNAPTOSOMES. S.M. Shreeve* and D.H. Ross (SPON: A. Modak). Div. Molec. Pharmacol., Univ. Tx. Hlth. Sci. Ctr., San Antonio, TX 78284.

Ca^{++} efflux from synaptosomes has been believed to occur primarily via Na^+-Ca^{++} exchange. Recent studies have demonstrated the existence of a high affinity ATPase along the inner portion of synaptic membranes. This enzyme is stimulated by low concentration of Ca^{++} (0.1 - 5.0 µM) and is believed activated by both cytosolic ATP and Ca^{++}. Our interest in studying Ca^{++} efflux was to determine whether or not a portion of the Ca^{++} efflux was ATP-dependent, as has previously been shown in squid axon (Nature 278: 271, 1979). Synaptosomes were isolated from brain homogenates by sucrose gradient centrifugation. Synaptosomes were loaded for two minutes with $^{45}Ca^{++}$ under a depolarizing concentration of K^+ (55 mM) at 37°C, pH 7.4. Efflux was started by the addition of buffer containing 132 mM Na^+, 5 mM K^+, 3 mM EGTA and physiologic concentration of standard components. $^{45}Ca^{++}$ efflux was measured at regular intervals, from 5 sec to 300 sec. Nonspecifically bound Ca^{++} was corrected for by loading the synaptosomes at 0°C and performing the efflux experiments at the appropriate temperature. Efflux of Ca^{++} occurred in three distinct phases, to which rate constants were assigned: K_1 (fast), K_2 (intermediate) and K_3 (slow). The time course for these phases were: K_1, 0-10 sec; K_2, 10-50 sec and K_3,> 50 sec. 65% of Ca^{++} efflux occurred during K_1, while 10% was seen at K_2 and 25% at K_3.

Efflux was studied after choline substitution, glucose deprivation and sodium azide (Na^+N_3) treatment. K_1 efflux was seen to be Na^+- and glucose-dependent, but was unaffected by Na^+N_3. K_2 efflux was Na^+-dependent, glucose independent and inhibited by Na^+N_3. K_3 efflux was unaffected by any treatment. Efflux at 0°C eliminated K_1 and K_2 but not K_3. The results suggest K_1 efflux may be ATP-dependent while K_2 is Na^+-dependent. K_3 was unaffected by any treatment and may reflect osmotic leak of Ca^{++}. These studies support the contention that Ca^{++} efflux from synaptosomes may involve a high affinity Ca^{++}-dependent ATPase.

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140.17 PHOSPHORYLATION OF SYNAPSIN I (PROTEIN I) IN HIGHLY PURIFIED SYNAPTIC VESICLES AND OTHER SUBCELLULAR FRACTIONS FROM RAT BRAIN. W. B. Huttner, P. De Camilli*, W. Schiebler* and P. Greengard. Department of Pharmacology and Section of Cell Biology, Yale University School of Medicine, New Haven, CT 06510.

Synapsin I, previously referred to as Protein I, is a neuron-specific protein, present in most, and possibly in all, axon terminals, where it appears to be associated primarily with neurotransmitter vesicles (De Camilli et al.; Huttner et al., manuscripts in preparation). Synapsin I has been shown to be phosphorylated by calcium/calmodulin-dependent protein kinases in three distinct sites (sites 1, 2, 3) and by cAMP-dependent protein kinase in one site (site 1) (Proc. Natl. Acad. Sci. U.S.A., 76, 5402-5406, 1979; J. Biol. Chem., 256, 1482-1488, 1981). In the present study, the phosphorylation of Synapsin I in these sites was investigated in various subcellular fractions from rat brain including a highly purified synaptic vesicle fraction obtained after chromatography on controlled pore glass. During subcellular fractionation the enrichment of Synapsin I paralleled the enrichment of synaptic vesicles. Synapsin I in the highly pure synaptic vesicles could not be phosphorylated at site 1 by either calcium/calmodulin-dependent or cAMP-dependent protein kinases, in contrast to Synapsin I recovered in more crude synaptic membrane fractions. We are currently investigating the silent nature of site 1 of Synapsin I recovered in the synaptic vesicle fraction, to see if it reflects some physiological process occurring in vivo. The silent nature of the phosphorylation of this site 1 does not seem to result from limited proteolysis or presence of a protein kinase inhibitor and is unaffected by prior treatment of vesicle-associated Synapsin I with purified phosphatase.

Synapsin I could be released from isolated synaptic vesicles under appropriate experimental conditions by moderate increases of ionic strength. This effect of ionic strength was potentiated when sites 2 and 3 were in the phosphorylated form.

140.18 THE EFFECT OF REPEATED AMPHETAMINE ADMINISTRATION ON STEREOTYPED BEHAVIOR AND RESPONSE TO APOMORPHINE. P. G. Conway* and N. J. Uretsky. Division of Pharmacology, Ohio State University College of Pharmacy, Columbus, Ohio 43210.

The repeated administration of amphetamine has been shown to induce psychotic behavior in humans and to enhance, rather than diminish, stereotyped behavior in rats. The mechanism of this paradoxical "reverse tolerant" effect is at present unclear, but could be explained by amphetamine-induced subsensitivity of autoreceptors on dopaminergic nerve terminals. To test this hypothesis, rats were pretreated with amphetamine (5.0 mg/kg, i.p.) or saline (0.1 ml/100 g) twice daily at various rate schedules (5 days, 2 day withdrawal; 5 days, 9 day withdrawal; 10 days, 4 day withdrawal). After the withdrawal period, all animals were challenged with various doses of amphetamine (1.0, 3.0, 5.0 or 10.0, mg/kg, i.p.) and stereotypy was monitored for 90 minutes. At all doses and rate schedules studied, involving daily amphetamine administration, the amphetamine pretreated rats exhibited enhanced stereotypy compared to saline pretreated rats. However several injections of amphetamine in one day did not produce this effect after a withdrawal period of 6 days. If this enhancement of stereotypy were due to dopaminergic autoreceptor subsensitivity, then apomorphine's ability to stimulate these receptors and produce a decrease in striatal content of 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) as well as a decrease in locomotor activity should be reduced. Indeed, blocking dopaminergic presynaptic receptors with the neuroleptic molindone (1.0 mg/kg, i.p.) inhibited the apomorphine-induced decrease in striatal content of DOPAC and HVA. In contrast, a low dose of apomorphine (50 µg/kg s.c.) decreased locomotor activity and reduced DOPAC and HVA striatal content comparably in both saline and amphetamine pretreated animals. Also it would be expected that apomorphine's ability to inhibit γ-butyrolactone (GBL)-induced dihydroxyphenylalanine (DOPA) accumulation be reduced in amphetamine pretreated animals. However apomorphine, in a dose related manner, reduced striatal GBL stimulated DOPA accumulation by the same extent in saline and amphetamine pretreated animals. Based on these studies, amphetamine-induced behavioral facilitation does not appear to be mediated by an alteration in striatal dopaminergic presynaptic receptors. Supported by NS grant 13888.

140.19 EFFECT OF THE COMMON FOOD DYE, ERYTHROSIN B (F, D & C RED NO.3), ON TRANSMITTER RELEASE FROM MAMMALIAN CEREBRAL CORTICAL TISSUE. Patricia D. Wade and Philip Siekevitz. Rockefeller University, 1230 York Avenue, New York, NY 10021.

Erythrosin B is a tetraiodinated derivative of fluorescein which is used in food as a coloring agent. We find it to cause release of the transmitters examined, GABA and norepinephrine, from an in vitro cerebral cortical preparation. A mixture of coloring agents including Erythrosin B exacerbates learning defects in children having the hyperactivity syndrome (Science 1980, 207:1485), and, significantly, induces at moderate doses behavioral hyperactivity in certain children with no previous pathology (Science 1980, 207:1487). Erythrosin B at 10µM or greater causes an increase in miniature endplate potential frequency at the frog neuromuscular junction (Science 1980, 207:1489), and is reportedly taken up into brain from the circulation nearly (.82X) as well as water (PNAS 1977, 74:2914).

After preincubation with [^{3}H]norepinephrine or [^{3}H]GABA, and subsequent rinsing of the tissue slices, 9 minutes exposure to 100µM Erythrosin B caused peak release which is estimated to be 3X (norepinephrine) or 10X (GABA) above baseline (Procedure as described for other compounds in Wade, Fritz and Siekevitz, Brain Res. in press.). The parent compound fluorescein did not appear to cause release at 1mM at the sensitivity of the assay. Other derivatives of fluorescein, Eosin YS (tetrabrominated fluorescein) and phloxine B (tetrabrominated, tetrachlorinated fluorescein), were found to cause GABA release at 100µM. Thus the halogen substitutions appear to be important for the property of release. The release was found to occur equally well in the absence of added Ca^{++} (studied for GABA only) and could not be blocked by 10^{-6}M tetrodotoxin, both of which results suggest that the induction of release is not via voltage-dependent Na^+ channels. Erythrosin B is apparently similar to black widow spider venom and the sulfhydryl-oxidizing agent diamide in not requiring Ca^{++} for release and presumably in not requiring Na^+ channel-related depolarization. To summarize, the release effect of Erythrosin B in central nervous system is not transmitter-specific, and, it could be argued, occurs in the estimated dose range in which peripheral physiological and hyperactivity effects are observed.

Supported by N.Y. State Health Program fellowship no. 1803 and Muscular Dystrophy Association fellowship to P.D.W. and NIH grant no. NS-12716-04 to P.S.

140.20 BIPHASIC DOPAMINE RELEASE FROM STRIATAL SYNAPTOSOMES PARALLELS Ca ENTRY. P. Drapeau* and M.P. Blaustein. Dept. of Physiology, Univ. of Maryland Sch. of Med., Baltimore, MD 21201.

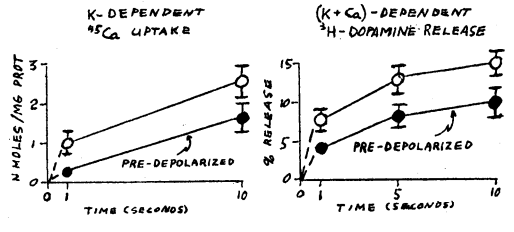
Ca uptake and dopamine release from rat striatal synaptosomes were examined over a 10 sec interval. Synaptosomes were loaded with ^{3}H-dopamine by incubation in 0.1 µM ^{3}H-dopamine solution. Release was subsequently evoked by incubation in K-rich media. Both high K-stimulated ^{45}Ca uptake and ^{3}H-dopamine release occurred in two phases (see figure below): i) a "fast" phase, which showed voltage-sensitive inactivation within one sec, and ii) a "slow" phase, which lasted for 10 sec. The rate constants for release were about 0.08 s^{-1} for the fast phase, 0.01 s^{-1} for the slow phase (averaged between 1 and 10 sec), and 0.001 s^{-1} for the basal efflux of ^{3}H-dopamine.

The evoked release of dopamine was blocked by the Ca channel antagonists Ni, Mg, Mn, Co, and Cd; Sr and Ba substituted for Ca in supporting K-stimulated release. These divalent cations have been shown to have similar effects on Ca entry into rat forebrain synaptosomes (Nachshen and Blaustein, J. Gen. Physiol. 76: 709-728, 1980).

The two phases of evoked dopamine release increased in magnitude as K was increased from 5 to 75mM, and had a $K_{0.5}$ for Ca of 0.2-0.7mM. Similar effects of varying K and Ca on both phases of Ca entry have been observed in rat forebrain synaptosomes.

The data indicate that Ca entry is rate-limiting for dopamine release; however, the kinetics of Ca entry and dopamine release are not linearly related.

Supported by NIH grant NS-16106. PD is a recipient of a MRC of Canada Fellowship.



140.21 PRESYNAPTIC CALCIUM CHANNEL INACTIVATION AT THE SQUID GIANT SYNAPSE. G. Augustine, R. Eckert and R. Zucker, Catalina Winter Squid Program; Department of Biology, UCLA; and Department of Physiology-Anatomy, UC, Berkeley.

Voltage-gated calcium channels inactivate in neuronal soma membranes. Because of the importance of Ca currents in regulating transmitter release, we attempted to determine if Ca channels in a presynaptic terminal also inactivate. Experiments were performed on the giant synapse of the Pacific squid Loligo opalescens. Standard procedures were used to record from pre- and postsynaptic elements, with the preterminal voltage clamped using either 2 or 3 microelectrode techniques. External TTX (2 x 10^{-7} g/ml), 3-,4-diaminopyridine (2 mM) and injected TEA were used to block sodium and potassium currents.

Depolarizing pulses to approximately 0 mV from a holding potential (V_h) of -65 or -70 mV elicited inward currents. These currents were presumably carried by Ca ions, because they were eliminated by 2mM $CdCl_2$. During prolonged depolarizations the Ca current relaxed exponentially, with a time constant of approximately 0.1s. Two lines of evidence indicate that the relaxation is due primarily to Ca channel inactivation, rather than concurrent activation of an outward (potassium) current. First, no outward current was seen in these preparations after treatment with cadmium, which blocks Ca currents while sparing voltage-gated potassium current. Second, Ca 'tail' currents recorded at the end of depolarizing pulses with $V_h = E_K$ decreased in amplitude as pulse duration increased. These tail currents were blocked by cadmium, and had time constants of 1 ms or less.

Inactivation was also observed with other procedures. A decrease in amplitude of the inward current occurred if the test pulse was preceded by a depolarizing prepulse. This inactivation increased monotonically as the amplitude or duration of the prepulse increased (and decreased as the interval between the two pulses was increased). Inactivation was also seen when V_h was lowered. The Ca current was maximal with V_h = -70 mV, was reduced at -50 mV, and was nearly eliminated at V_h = -30 mV.

Thus, Ca channels of the presynaptic terminal of the squid giant synapse appear to inactivate, as do most other voltage-gated Ca channels that have been investigated. Inactivation is not likely to affect transmitter released from this synapse during a single impulse, due to the brief duration of the action potential compared to the time course of inactivation, but may play a role in decreasing transmitter release during prolonged trains of presynaptic impulses.

Supported by Muscular Dystrophy Foundation Postdoctoral Fellowship, USPHS grants S-S07-RR07009 and NS 15114.

140.22 THE EFFECT OF ANTIPSYCHOTIC AGENTS ON TRANSMISSION AT THE NEUROMUSCULAR JUNCTION OF THE FROG. N. Stockbridge* and J.W. Moore. Dept. of Physiology, Duke University Medical Center, Durham, NC 27710.

Many studies of transmission at the frog neuromuscular junction have shown calcium to play a key role in the release of the chemical messenger. In low calcium solutions, the amount of transmitter released is proportional to about the fourth power of the external calcium concentration (Dodge & Rahamimoff, 1967; Andreu & Barrett, 1981). This fourth power relationship suggested to us that calmodulin, the ubiquitous intracellular calcium-binding protein with its four calcium binding sites, might be involved in the process of transmitter release.

Experiments have been conducted to test this hypothesis of calmodulin involvement in transmitter release. Trifluoperazine is an antipsychotic which binds to calmodulin and prevents its participation in *in vitro* reactions (Levin & Weiss, 1977). At the frog neuromuscular junction, trifluoperazine (20-30 uM) reversibly decreases the observed endplate potential response to nerve stimuli. This action of trifluoperazine does not appear to be due to a decrease in the sensitivity of the postsynaptic membrane to the transmitter substance, since miniature endplate potentials are undiminished in amplitude by trifluoperazine concentrations several-fold higher than that necessary to block evoked release. Nor does trifluoperazine appear to block of the propagation of action potentials in the presynaptic motor fiber, since extracellular recordings of the nerve terminal potential are unaffected by these concentrations of the drug. At a concentration of 200 uM, trifluoperazine sulfoxide, a derivative of trifluoperazine with much decreased affinity for calmodulin, has no affect on transmission. Other calmodulin-binding drugs also have effects on transmission generally consistent with expectations based upon their *in vitro* behavior.

141.1

WITHDRAWN

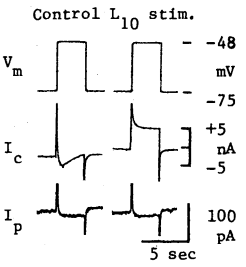
141.2 PATCH CLAMP ANALYSIS OF SOMATIC CURRENTS IN MOLLUSCAN BURSTING PACEMAKER NEURONS: RESPONSES TO NEUROTRANSMITTER AND SLOW SYNAPTIC INPUT. William W. Anderson and Wilkie A. Wilson*. Epilepsy Center, V.A. Hospital, Durham, NC 27705.

Voltage clamp analysis of molluscan bursting pacemaker neurons indicates that certain neurotransmitters and slow synaptic inputs inactivate slow inward currents at depolarized voltages (Wilson and Wachtel, Science, 202:772, 1978). Because the transmitters were iontophoresed into the neuropil, because molluscan somata usually do not have synaptic contacts, and because molluscan soma membrane has slow inward currents, W & W suggested that synaptic and neurotransmitter inactivation of inward currents measured from the whole cell could be due to either inactivation of inward current channels at subsynaptic neuropil sites, or to the inactivation of these channels throughout the cell, including in the soma membrane, by the spread of an intracellular messenger.

We are investigating these possibilities by voltage clamping Aplysia neurons with two intrasomatic microelectrodes and measuring soma membrane currents with a 50-100 uM patch electrode connected to a virtual ground operational amplifier. Dopamine was iontophoresed onto neuropil processes of R_{15}, and slow synaptic input was produced in L_3 by stimulating the presynaptic cell L_{10}.

Short duration stimulation (ca. 20 sec) of L_{10}, which is sufficient to change the whole cell current (I_c) of L_3 from inward to outward during a depolarizing clamp pulse, causes at most only a slight change in the L_3 somatic patch current (I_p), which remains inward (see figure). In these cases, synaptic inactivation of the inward current measured from the whole cell is thus primarily due to the inactivation of nonsomatic, neuropil currents. Possible changes in somatic patch currents with longer periods of synaptic stimulation is under investigation.

Iontophoresis of dopamine onto R_{15} processes in the neuropil does cause clear changes in somatic patch current. Patch current goes from inward to outward for a -50 to -10 mV clamp pulse. Dopamine receptors are not thought to be located on the soma membrane of R_{15}. Whether dopamine was stimulating neuropil receptors and changing soma conductances via an intracellular messenger, or was leaking under the patch and stimulating previously undetected somatic dopamine receptors is not known, but is under study.



Supported by a NIH Postdoctoral Fellowship to WWA, and NIH Grant 15212 and a grant from the VA to WAW.

141.3 CA DEPENDENCY OF ACETYLCHOLINE (ACH) INDUCED K RESPONSES. A.M. Williamson and D.O. Carpenter. Div. of Laboratories and Research, NYS Dept. of Health, Albany, New York 12201.

In Aplysia neurons, there are three kinds of responses to Ach due to conductance increases to Na, Cl, and K. Although the Na and Cl responses are similar in time course, the K response shows a delay in its onset which cannot be explained by an unusual placement of receptors within membrane invaginations or by restrictions in the diffusion path to the receptors. The time to peak of the K response is longer (8-10 sec.) than for the Na or Cl responses (1-2 sec.). The K responses are also sensitive to cooling, being abolished at 8°C. The slow time course and temperature sensitivity are suggestive of a second messenger and/or metabolic dependency, although there is no evidence that cyclic nucleotides are involved in the response. Another possibility is that the K response is mediated by Ca accumulation within the cell or by its release from internal stores. Ca-activated K currents were first discovered by Meech and have since been seen in other systems.

We conducted experiments on the K response to iontophoretically applied Ach on medial cells of the pleural ganglia of Aplysia in order to evaluate the involvement of Ca in the response. The cells were bathed in artificial sea water (ASW) at or near room temperature, and recordings were made under current or voltage clamp conditions. We have previously shown that $MgCl_2$- mannitol sea water blocked the K response. Since Mg is known to block Ca dependent processes we examined the effect of other Ca blockers. The addition of 1-3 mM Cd ions to ASW produced virtually complete but reversible inhibition of the response. The addition of 25 mM Co ions which are known to block voltage dependent Ca currents in Aplysia reversibly reduced the K current by 50%. Manipulations in the Ca concentration from .1 to 10X normal did not produce any significant change in the response. Ca-free solutions with .1 mM EGTA, a potenet Ca chelator, produced little change on the magnitide of the response, but did lengthen the time course. Intracellular injection of EGTA did not alter the response.

The results obtained with Mg, Cd, and Co ions suggest that there is Ca dependency to the K response. Those divalent ions are known to block Ca entry through the membrane. However, the mechanism does not appear to involve a simple Ca-activated K conductance since varying the Ca concentration, and/or adding EGTA had no effect, and no inward current was observed. It is possible that those divalent ions displace Ca from a binding site on the receptor or in the membrane and prevent receptor activation. A less likely possibility is that those ions block the K channel.

141.4 BIOCHEMICAL, PHARMACOLOGICAL AND ELECTROPHYSIOLOGICAL STUDIES ON ISOLATED HORIZONTAL CELLS FROM THE TELEOST RETINA. J. E. Dowling, E. M. Lasater, R. Van Buskirk*, K. J. Watling* and M. Zeldin*. The Biological Laboratories, Harvard University, Cambridge, MA

One of the major challenges of current neuroscience research is to correlate biochemical and physiological processes at the level of single neurons. We are currently approaching this problem using preparations of horizontal cells isolated from the teleost retina.

Carp retinal neurons are dissociated by a combination of enzymic and mechanical means, and horizontal cells are isolated by velocity sedimentation in a Ficoll gradient at unit gravity. Electron microscopy shows the cells are intact with no impinging presynaptic terminals.

When incubated with 2 mM IBMX, the isolated horizontal cells demonstrate dopamine-dependent cyclic AMP accumulation. Cyclic AMP levels are routinely raised 20-40 times over basal levels by 100 μM dopamine. The response (EC50~ 10 μM) is potently blocked by haloperidol, fluphenazine or (+) butaclamol.

Dopamine also induces changes in protein phosphorylation in isolated horizontal cells. The incorporation of ^{32}Pi into various phosphoproteins was examined by SDS polyacrylamide gel electrophoresis. Cells incubated in the absence of dopamine demonstrate two major phosphoproteins, one (A) with a molecular size of approximately 300 KD, and a second (B) with a size of approximately 68 KD. Exposure of the cells to 10-100 μM dopamine results in the appearance of component C with a molecular size greater than 400 KD and the disappearance of phosphoproteins A and B. Haloperidol blocks the dopamine dependent disappearance of A and appearance of C.

Other neurotransmitters were examined for their ability to increase cyclic AMP levels in isolated horizontal cells. Of these only vasoactive intestinal peptide (VIP) is effective. The VIP and dopamine responses are not additive, but haloperidol does not block the VIP response. This suggests the presence of separate VIP and dopamine receptors on isolated horizontal cells associated with a common adenylate cyclase pool.

Finally, intracellular recordings from isolated horizontal cells maintained in tissue culture for 2-4 days show that about one-quarter of the cells are responsive to dopamine. Dopamine increases membrane resistance, and most often hyperpolarizes the cell. L-Glutamate, on the other hand, depolarizes virtually all cultured horizontal cells while D-glutamate, GABA and glycine are without effects.

141.5 INTRACELLULAR MEASUREMENTS OF CHLORIDE ACTIVITY ON THE HORIZONTAL CELLS OF THE VERTEBRATE (FISH) RETINA.
M.B.A. Djamgoz*and P.J. Laming* (SPON: E.M. Lasater). Dept. of Pure and Applied Biology, Imperial College, London SW72BB England.

Ionic substitution experiments have shown that both sodium and chloride ions might be required for the generation of light-evoked horizontal cell responses (S-potentials) in vertebrate retinae (Kaneko, A. & Shimazaki, H., J. Physiol. Lond., 252:509-522, 1975; Waloga, G. & Pak, W.L., J. Gen. Physiol., 71:69-92, 1978; Miller, R.F. & Dacheux, R.F., J. Gen. Physiol., 67:639-659, 1976). In the present study, the role of chloride ions was investigated by measuring intracellular and extracellular ionic activities in L- and C-type horizontal cells.

Isolated cyprinid (roach) retinae were placed receptor side upwards in a transparent recording chamber and maintained in moist air. Retinae were specifically not treated with physiological salines so 'normal' ionic activity values could be measured. Horizontal cells were impaled with double-barrelled chloride sensitive microelectrodes based on the Corning liquid ion-exchanger 477315. The light stimulus comprised a 1.5 mm spot of continuously variable wavelength and intensity. Ion-sensitive microelectrodes were calibrated in a series of solutions of constant ionic strength both before and after an experiment, and data were considered only when the two calibrations did not differ by more than 5 mV. Results were obtained from 78 red-sensitive L_1-units and 9 biphasic C-units in 11 retinae.

The average extracellular chloride activity was 112 mM and this was constant throughout the retina. Intracellular chloride activities in both types of unit were consistently less : 60 mM for L_1-units and 54 mM for C-units. The calculated chloride equilibrium potentials (E_{Cl}'s) were -19 mV and -20 mV; the corresponding dark membrane potentials (E_m's) of these units were -34 mV and -35 mV averaged, respectively. In both types of unit, therefore, the chloride ions are not in equilibrium with the membrane potentials (E_{Cl} is more positive than E_m by 15 mV), and can potentially contribute to the synaptic generation of S-potentials.

141.6 EXTERNAL CALCIUM INFLUENCES THE CHARACTERISTICS OF FAST SYNAPTIC CURRENTS AT THE SYMPATHETIC GANGLION. Elizabeth A. Connor* and Rodney L. Parsons (SPON: G. Webb), Depts. of Physiol. and Biophysics and Anatomy and Neurobiol., Univ. of Vermont, Burlington, Vermont 05405.

Calcium has been reported to influence the timecourse of the nicotinic fast excitatory postsynaptic current (EPSC) decay in sympathetic ganglion cells in addition to its critical presynaptic role in transmitter release (Kuba and Nishi, 1979). In preliminary experiments we noted that calcium not only prolonged fast EPSC timecourse, but in many cells exposed to elevated calcium, the EPSC no longer decayed as a single exponential. In these cells, the decay had two distinct exponential components; one faster and one slower than the control rate of decay. In the present study, we have investigated the alteration of fast EPSCs by calcium (0.9mM to 9.0mM) in more detail. Fast EPSCs were recorded from voltage-clamped B cells (-100 to +30mV) in the IX and X sympathetic ganglia of the bullfrog, Rana catesbeiana, maintained in a HEPES-buffered solution at 21-23°C. Peak EPSC size and decay τ increased with an elevation of external calcium in the concentration range 0.9 to 5.4mM. No further increase in EPSC size occurred with increase in calcium from 5.4 to 9.0mM as reported previously (Kuba and Nishi, 1979). The EPSC decay was a single exponential function in all cells maintained in 0.9 to 1.8mM calcium, but at concentrations $\geq$ 3.6mM calcium, an increasing percentage of cells had EPSCs exhibiting a double exponential decay. Prolongation of the decay timecourse in high calcium can not be attributed simply to the increase in peak EPSC size as EPSCs potentiated 2-5 fold by preganglionic stimulation at 30Hz for 4 seconds decayed as a single exponential with the time constant value similar prior to and after the period of stimulation. A similar alteration in fast EPSC decay occurred in cells exposed to 1.8mM calcium and 8.0mM strontium. In these cells, peak EPSC size was not significantly different from controls but the decay was either markedly prolonged when the EPSC decayed as a single exponential or was comprised of two exponential components, one faster and one slower than control rate of decay. In the strontium-treated cells, the slow component time constant, but not the fast, appeared to increase with hyperpolarization. The complex decay in high calcium or strontium was not due to an increased voltage deviation in these cells or an increased transmitter release, but may represent a direct action of these divalent cations on kinetics of the fast EPSC decay.
(Supported by NIH Grant NS-14552)

141.7 NONCHOLINERGIC "LATE-SLOW"-EPSP IN MAMMALIAN SYMPATHETIC GANGLION. John H. Ashe and B. Libet. Department of Physiology, School of Medicine, University of California, San Francisco, CA. 94143.

Orthodromic input, during cholinergic blockade by d-tubocurarine (50 µM) plus quinuclinidyl benzilate (QNB, 0.05 µM) can elicit a relatively large, slow depolarization, recorded extracellularly as a "late-late-negative" (LLN) response of the superior cervical ganglion of rabbit. LLN is also unaffected by adrenergic antagonists (phenoxybenzamine 10 µM, or sotalol, 10 µM). LLN is a graded response, with latencies of 1 to 3 sec and durations up to 20 min or more. That LLN represents a neuronal PSP is indicated by a) the ineffectiveness of antidromic stimulation of postganglionic axons; b) the absence of LLN in presynaptic recordings (electrodes on ganglion and preganglionic nerve); c) the decrement of LLN along postganglionic nerve, with a length constant the same as for the known PSP's. LLN clearly represents an equivalent of the "late-s-EPSP" described for frog paravertebral (Nishi and Koketsu, 1968) and guinea pig inferior mesenteric (Neild, 1978) ganglion cells; we propose the name "slow-slow" or "ss-EPSP".

The major determinant of both amplitude and duration of LLN is the total number of stimulus pulses (applied to cervical sympathetic nerve). Indeed, pulse frequencies as low as 1 pps can elicit almost as large a response as higher frequencies (20-40 pps), if a sufficiently long train containing an equal number of pulses is applied. However, latency and rise-time are shorter with higher stimulus pulse frequency.

The ss-EPSP features, including effectiveness of low frequency orthodromic volleys and amplitudes estimated to reach 25-50% of the action potential, indicate that this very slow noncholinergic PSP could play a significant role in normal ganglionic functions. (Supported by U.S.P.H.S. research grant NS-00884.)

141.8 The ionic mechanism of the non-cholinergic excitatory potential in mammalian sympathetic ganglion cells. Z. G. Jiang*, N. J. Dun and M. A. Simmons. Dept. of Pharmacol. Loyola Univ., Maywood, Il 60153.

Repetitive preganglionic nerve stimulation (10-30 Hz 2-8 sec) of the inferior mesenteric ganglia of the guinea pig elicited a slow excitatory potential which was not blocked by nicotinic (d-tubocurarine, 0.1 mM) and muscarinic (atropine, 1 µM) antagonists. The amplitude and duration of the non-cholinergic excitatory potential ranged from 2 to 15 mV, and 20 sec to 3 min, respectively. In many instances, spontaneous spike discharges occurred during the generation of non-cholinergic potential; the spontaneous discharges were not blocked by cholinergic antagonists, but readily abolished by membrane hyperpolarization. In a portion of the neurons tested, the input resistance showed a biphasic change: an initial decrease was followed by a more prolonged increase. This biphasic membrane resistance change could also be demonstrated when the membrane potential was clamped at rest manually. In the remaining neurons, the non-cholinergic excitatory potential was associated with either a monophasic increase of input resistance or no detectable change in both unclamped and clamped conditions. The amplitude of the non-cholinergic potential was augmented upon membrane hyperpolarization to the level of K equilibrium potential; the extrapolated equilibrium potential was about -40 mV. In a few neurons, the non-cholinergic response was either not significantly altered or diminished upon conditioning hyperpolarization. In high K (10-15 mM)Krebs solution, the amplitude of non-cholinergic potential was reduced, however, the respone was increased upon membrane hyperpolarization. When the concentration of Na in the perfusing Krebs solution was reduced to 1/3, the non-cholinergic potential was attenuated, whereas, it was not appreciably affected in a low Cl solution. Our results indicate that the ionic mechanism underlying the non-cholinergic potential may be similar to that of the membrane depolarization induced by substance P on these neurons, i.e. a combined mechanism of G_{Na} activation and G_K inactivation. (Supported by NS15848).

141.9 POSTSYNAPTIC HYPERPOLARIZATION DOES NOT AFFECT LONG-TERM ENHANCEMENT OF CA1 SYNAPSES. B.L. McNaughton, C.A. Barnes, H. Wigström*. Dept. Anatomy and Embryology Univ. College London, and Dept. Physiology, Univ. of Göteborg, Sweden.

The long-term enhancement of synaptic efficacy which follows high frequency activation of certain hippocampal synapses has been suggested to constitute a workable neural substrate for associative memory. Aside from the long lasting (although not permanent) nature of this process, one of the major grounds for the assertion that it models associative memory is that it appears to require the association of afferent fibre activity for its generation (McNaughton, Douglas & Goddard, B. Res. 157, 277-293, 1978; Levy & Steward, B. Res. 175, 233-245, 1979).

Virtually all workable neural models for associative memory postulate that some form of postsynaptic integration process must regulate the synaptic change by which information is to be stored. Although the co-operative nature of the enhancement process suggests that postsynaptic integration is required, this has not been demonstrated and viable presynaptic explanations remain.

We have examined, in CA1 pyramidal cells recorded from intracellularly in vitro, the effect of 1.0 nA hyperpolarizing current applied during high frequency activation of fibres in Stratum Radiatum. A non-overlapping set of fibres in the same layer received the identical stimulation without membrane hyperpolarization. Thus, each cell served as its own control.

The hyperpolarizing current, which was sufficient to cause a significant shift in membrane potential at the site of synaptic input, and to reduce or abolish cell discharge during the stimulus train, had no effect whatsoever on the magnitude of the synaptic enhancement observed 15 minutes later. Thus, if postsynaptic integration is involved in the control of enhancement, the signals integrated are likely to be chemical rather than electrical.

141.10 AMMONIA AND POSTSYNAPTIC INHIBITION. W. Raabe and S. Lin*. Depts. Neurology, VA Med. Ctr., Univ. of Minnesota 55417.

Ammonia intoxication has been shown to inactivate the extrusion of Cl^- from central neurons, thus shifting the equilibrium potential of the IPSP, E_{IPSP}, to the level of the resting membrane potential. This effect of ammonia abolishes the hyperpolarizing action of postsynaptic inhibition as well as its potency to suppress neuronal excitation. We measured the concentrations of ammonia, glutamate and glutamine associated with ammonia induced shifts of the E_{IPSP} to the resting membrane potential.

Cats were anesthetized with pentobarbital and respirated artificially. Mono- or polysynaptic IPSPs were recorded from spinal mononeurons. Ammonium acetate was given slowly i.v. until the E_{IPSP} shifted to the level of the resting membrane potential. The administration of ammonium acetate was then terminated. The recovery of the E_{IPSP} occurred as an average after 35 min. The average dose of ammonium acetate required to shift the E_{IPSP} was 2.75 mmol/kg. The resting membrane potential showed no significant changes during ammonia administration. The shift of the E_{IPSP} to the resting membrane potential was not associated with changes of the slope of IPSP size vs. membrane potential during applied current steps. The lumbar spinal cord was frozen in situ with liquid nitrogen before, during and after recovery of the changes in the E_{IPSP}.

With the shift of the E_{IPSP} to the resting membrane potential ammonia concentrations increased from 0.64 $\pm$ 0.02 µM/g (N=5) to 2.32 $\pm$ 0.42 µM/g (N=4), $p < 0.02$; glutamine increased from 5.26 $\pm$ 0.55 µM/g to 8.27 $\pm$ 0.87 µM/g, $p < 0.02$. With recovery of the E_{IPSP} ammonia decreased to 1.60 $\pm$ 0.07 µM/g (N=4); glutamine remained high 7.52 $\pm$ 0.93 µM/g. Glutamate did not change with the shifts of E_{IPSP}; control: 6.62 $\pm$ 0.56, E_{IPSP} shifted: 5.07 $\pm$ 0.48, E_{IPSP} recovery: 5.45 $\pm$ 0.42 µM/g.

An increase of tissue ammonia concentrations to 360% of normal inactivates the extrusion of Cl^- from neurons and thus abolishes hyperpolarizing postsynaptic inhibition. The changes of ammonia concentrations affecting inhibition in the spinal cord correspond qualitatively and quantitatively to those observed to affect postsynaptic inhibition in the cerebral cortex (Brain Res. 210: 311, 1981). It is concluded that in encephalopathies which increase ammonia in the CNS beyond 350% of normal, e.g. severe hypoxia, severe hypoglycemia, portacaval shunting, the effect of ammonia on postsynaptic inhibition contributes to the neurological abnormalities observed.

141.11 METHYLTRANSFERASE INHIBITION AND ACETYLCHOLINE-INDUCED MUSCLE CONTRACTION. G. Magilen, L. Ziskind-Conhaim, I. Diamond, and A.S. Gordon. Depts. of Neurology, Physiology, and Pharmacology. Univ. of Calif., San Francisco, CA 94143. 141.11

It has been suggested that methyltransferase (MT) enzymes play a role in chemotaxis, mitogenesis, hormone responsiveness and neurotransmission. We have investigated the possibility that MT activity is involved in acetylcholine (ACh)-induced myotube depolarization and contraction. In this study we have used primary myotubes grown from chick embryo thigh muscles cultured for 5-7 days. Muscle contraction was evoked when 1M ACh was applied focally by pressure injection from a micropipet. The role of MT reactions in ACh-evoked depolarization and contraction was determined by using a mixture of 0.1mM erythro-9-(2-hydroxy-3-nonyl) adenine, 0.1mM homocysteine thiolactone, and 1.0mM adenosine which together inhibit the activity of methyltransferases. ACh-induced muscle contraction was inhibited within 10 minutes after application of the inhibitor mixture. However, muscle contraction produced by mechanical stimulation was not affected by the inhibition mixture. The inhibition of contraction was not due to desensitization or a change of the resting membrane potential. Intracellular recordings showed that neither ACh-induced subthreshold depolarizations produced by injection of 1mM ACh nor action potentials were affected by the inhibitor mixture. The ACh-induced contraction was restored after washing out the inhibitors.

If the inhibition of contraction was caused by inhibition of MT activity, then the washing procedure which restores ACh-induced contraction should also restore the MT activity. We found that carboxy-MT and lipid-MT activities were decreased 93% and 92% respectively by the mixture of compounds. In contrast to the reversible inhibition of contraction, the inhibition of MT activity was not reversible. Therefore, the inhibition of contraction was not due to the inhibition of either carboxy-MT or lipid-MT activities.

From our results we conclude that the primary function of the acetylcholine receptor is not affected by inhibition of MT activity. Moreover, carboxy-MT and lipid-MT activities are not involved in the inhibition of ACh-induced muscle contraction caused by this mixture of compounds. Therefore, these compounds must be inhibiting contraction by acting on some other process.

141.12 PHYSIOLOGICAL REGULATION OF GLUTAMATE RECEPTOR BINDING. Baudry, M., Halpain, S.*, Arst, D.*, Smith, E.*, and Lynch, G. Department of Psychobiology, University of Calif., Irvine, Ca. 92717

The hippocampus exhibits various forms of physiological plasticity among which is long-term potentiation (LTP) of synaptic transmission following brief bursts of high-frequency stimulation of various pathways. An increasing body of evidence suggests that glutamate (or a closely related compound) is the neurotransmitter used by the pathways exhibiting the LTP phenomenon. Hippocampal synaptic membranes possess a high-affinity Na-independent ^{3}H-glutamate binding site which has properties expected of a postsynaptic glutamate receptor. We previously showed that micromolar concentration of calcium ions are able to increase the number of glutamate binding sites possibly by stimulating a membrane-bound Ca++-dependent proteinase (Baudry et al., Science, 1981, in press) and proposed that this could be the mechanism underlying LTP. We now report changes in glutamate receptor binding under various physiological or quasi-physiological situations.

First, using a new version of the hippocampal slice preparation in which only the CA_1 field is dissected and incubated in the recording chamber, we were able to replicate the finding that high-frequency stimulation results in an increased (+30%) ^{3}H-glutamate binding to hippocampal membranes. This increased binding reflects an increase in the maximal number of sites without changes in their affinity for the ligand. This effect is not found after low-frequency stimulation or when the high-frequency stimulation is performed in low calcium medium, two conditions which are not accompanied by LTP.

Second, incubation of hippocampal slices in the presence of low concentrations of glutamate (0.5 mM) or of various glutamate agonists also induced an increase in the maximal number of binding sites to synaptic membranes without changes in their affinity for the ligand. Finally, ^{3}H-glutamate binding increases progressively as a function of age; in this case, also, the increased binding represents an increased number of sites without changes in their affinity for glutamate. Moreover, in these latter two conditions, the binding measured in the presence of a saturating concentration of calcium (250 µM) does not exhibit the increased binding and this does not result from a qualitative difference in the ability of calcium to stimulate ^{3}H-glutamate binding. These data support the idea that there may exist in hippocampal membranes a fixed amount of glutamate receptors, only a fraction of them being available at any time, and that certainforms of synaptic activity are able to modify this fraction.

Supported by Grant NIMH-MH19793-10 and NIA-AG000538-05.

141.13 CHARACTERISTICS OF A CALCIUM-STIMULATED PROTEASE FROM SYNAPTIC MEMBRANES. M. Bundman, M. Baudry, E. Smith*, D. Heck* and G. Lynch, Dept. of Psychobiology, Univ. of Ca., Irvine, Ca. 92717.

Previous studies have established that calcium (~10μM) increases the number of sodium-independent binding sites for glutamate in several regions of the rat forebrain and that this effect is blocked by inhibitors of certain classes of proteases. Subsequent studies revealed that micromolar levels of calcium induced the degradation of a high molecular weight doublet protein and again, this effect was blocked by protease inhibitors. These results suggested that some neuronal membranes contain a protease which is involved in the regulation of receptors and which is activted by calcium in concentration ranges not far removed from those which might occur under physiological conditions. The experiments to be described intended to test the correlation between calcium induced proteolysis and calcium induced stimulation of glutamate binding by comparing the effects of several manipulations on these two processes.

Time course studies indicated that Ca^{2+} (100 μM) induces a a maximal effect after 15 minutes of incubation at 30°C. The proteolytic process is extremely temperature-sensitive with essentially no activity at 20°C and maximal activity at approximately 30°C; similarly Ca^{2+}-stimulation of glutamate binding is virtually absent at 20°C. The effects of several divalent cations were also tested on this proteolytic activity. Mn^{2+} (1mM) induces a 40-45% decrease in the amount of the substrate doublet protein, an effect similar to that of Ca^{2+} (0.1mM); Sr^{2+} (1mM) also substitutes for Ca^{2+} whereas Mg^{2+}, Ba^{2+}, Co^{2+}, Cu^{2+}, Ni^{2+}, and Zn^{2+} are ineffective at concentrations up to 1 mM. Comparable results have been found with the stimulation of glutamate binding. The Ca^{2+}-stimulation of both the proteolytic process and the glutamate binding are inhibited by the thiol protease inhibitors leupeptin and N-ethylmaleimide (0.1mM).

These data indicate that these two calcium-dependent processes, the stimulation of glutamate binding and the proteolytic activity, share a number of properties, including similar dependencies on divalent cations, inhibitors and temperature. Therefore, they add further support to our hypothesis that the calcium-induced increase in the number of glutamate receptors results from the proteolysis of a specific membrane protein. The precise identification of the protease and its substrate should provide a better understanding of the mechanisms by which calcium ions participate in the regulation of cell surface receptors and the role that this effect might play in synaptic physiology.

Supported by NIMH grant (MH1973-10).

141.14 EFFECTS OF LINCOSAMIDE ANALOGS ON ENDPLATE CHANNELS: CORRELATION WITH LIPID SOLUBILITY. J. F. Fiekers, I. G. Marshall*, and R. L. Parsons. Department of Anatomy and Neurobiology, Univ. of Vermont, College of Medicine, Burlington, VT 05405, and Dept. Physiol. and Pharmacol., Univ. of Strathclyde, Glasgow Scotland.

The lincosamide antibiotics, lincomycin and clindamycin, differ in molecular structure by the respective chemical substituent in position 7: lincomycin-hydroxyl, clindamycin-chloride. This chemical modification markedly influences the characteristics of MEPC decay and the shape of the power spectra obtained from noise analysis. Lincomycin splits MEPC decay into an initial rapid phase followed by a prolonged phase. Clindamycin increases the rate of MEPC decay without measurably altering its exponential nature. For each antibiotic the cut-off frequency of each spectral component is predicted by the rate of the corresponding constituent of the MEPC decay. Previously, we have shown (Fiekers, et.al., Fed. Proc. 40: 1981) that the postsynaptic block produced by lincomycin can be described by a sequential kinetic blocking model in which lincomycin interacts with the open state of the endplate channel complex. The present experiments were undertaken to explore the importance of chemical substitution at position 7 on endplate currents. The effects of two lincosamide analogs, epilincomycin and deoxylincomycin on the amplitude and kinetics of MEPCs and ACh-induced EPC fluctuations were studied in voltage-clamped snake costocutaneous neuromuscular junctions. These analogs split the MEPC decay into two components, one faster and one slower than the control decay rate. Either increasing the concentration of each analog or membrane hyperpolarization increased the decay rate of the fast component and decreased the rate of the slow component. The ratio of the respective amplitudes of each component (I_f/I_s) increased with hyperpolarization. On the basis of the electrophysiological analysis of the component rates and amplitudes the compounds could be arranged in the following sequence

lincomycin < epilincomycin << deoxylincomycin < clindamycin

The decay of currents recorded in deoxylincomycin, therefore, consisted primarily of a fast decay component and a small, slow tail component. Currents recorded in lincomycin consisted of a slow current component comprising a greater portion of the total current decay. Analysis of the lipid solubility using thin layer chromatographic techniques resulted in an identical sequence (clindamycin being the most lipophilic). The correlation between lipid solubility and the results on endplate current kinetics suggests that hydrophobic interactions with the endplate membrane may be important in the action of the lincosamide group of antibiotics. Supported by the MDA NATO, and PHS NS 14552

141.15 A CONSTRAINT ON SYNAPTIC ACTION IN APLYSIA: IMPLICATIONS FOR NERVOUS SYSTEM ORGANIZATION. Michael M. Segal and John Koester. Center for Neurobiology & Behavior, Physiology Dept., College of Physicians & Surgeons, Columbia University, N.Y., N.Y.

What constraints limit the types of synapses that can form in a nervous system? It is known that a postsynaptic neuron can have two types of receptors to the same neurotransmitter and the neuron can receive inputs from different cells using that neurotransmitter. In such a case, can one presynaptic cell depolarize and the other hyperpolarize, each by selectively activating one type of postsynaptic receptor? In other words, when a postsynaptic neuron concentrates receptors at synapses, does it concentrate one type of receptor to a particular transmitter at one synapse and a different type of receptor to the same transmitter at another synapse? To approach this question we have investigated neurons in the abdominal ganglion of Aplysia californica that receive inputs from two or more of the cholinergic neurons L10, L16, L24, Int XIII and Int XX.

For any given postsynaptic cell, we found that different cholinergic inputs converging onto the cell all produce the same type of synaptic action. This is true even for cells with more than one ACh receptor type. Cell L7 has both excitatory (E) and inhibitory (I) ACh receptors, and the 4 cholinergic neurons synapsing onto L7 all activate both receptor types, producing conjoint E-I synaptic potentials. Cells RD_{G} and LD_{G2} have two types of inhibitory ACh receptors, and each cell receives two cholinergic synaptic inputs, with each input activating both types of inhibitory ACh receptors.

If all cholinergic inputs to a given cell produce the same type of synaptic action, one would predict that all synaptic actions of different types are produced by non-cholinergic cells. Consequently we examined 3 neurons (L28, L32, LE) that elicit pure E responses in L7. We found that these presynaptic cells are all non-cholinergic.

These data lead to the hypothesis that a postsynaptic cell does not segregate one type of ACh receptor to one synapse and a different ACh receptor type to other synapses. If this hypothesis holds generally, it could, taken together with Dale's principle, provide insights into two organizational principles of nervous systems: (1) If a cholinergic interneuron excites a cell that another interneuron inhibits, the other interneuron must use a second transmitter. If each of these two interneurons produces an action on any other cell that differs from that produced by a third interneuron, then that interneuron must use a third transmitter; (2) If two cells use the same transmitter and influence a particular postsynaptic neuron in different ways, one would expect that at least one of the connections will be polysynaptic, involving an interneuron with a different transmitter. Thus, the constraint on synaptic action we have observed could explain a need for many neurotransmitters and a need for certain interneurons found in neural circuits. Supported by NIH grants GM 07367 and NS 14385.

144 SYMPOSIUM

MEMORY AND BRAIN MECHANISMS. L.R. Squire (Chairman; VA Medical Center, UCSD), B.A. Milner (Montreal Neurological Institute), D.S. Olton (John Hopkins Univ.), M. Mishkin (NIMH), E. Tulving* (Univ. of Toronto).

Brain mechanisms involved in mammalian memory functions will be considered from the point of view of neuropsychological studies of rats, non-human primates, and humans. Dr. Milner will discuss the role of medial temporal lobe structures in human memory based on a review of the early findings and a presentation of the most recent work in this area. Dr. Olton will discuss the effects of hippocampal lesions in rats, reviewing efforts to find parallels between the experimental animal and human literature. Dr. Mishkin will discuss cortico-limbic interaction and memory in non-human primates, reviewing efforts to establish a monkey model of human global amnesia. Dr. Squire will discuss amnesia in human and non-human primates, reviewing how the facts of amnesia have elucidated the organization of memory and its neural substrate. Dr. Tulving will discuss memory and brain mechanisms from the perspective of cognitive psychology, emphasizing the useful interaction that can occur between the experimental study of normal memory and the neuropsychological study of amnesia.

145 SYMPOSIUM

APPROACHES TO STUDYING GENE EXPRESSION AND REGULATION DURING NEURONAL DIFFERENTIATION AND FUNCTIONING. P.H. O'Lague (Chairman; UCLA); L. Greene* (New York Univ. Med. Ctr.); T. Joh* (Cornell Med. School); J. Roberts* (College of Physicians and Surgeons, Columbia Univ.).

The overall objective of this symposium is to present an introduction to several model systems and several current molecular biological approaches that appear useful in studying various aspects of neuronal differentiation and neuronal functioning at the level of the genome.

Determining the role of nerve growth factor (NGF), a presumed trophic substance, in the regulation of the growth and differentiation of its target neurons is an intensive area of current neurobiological research. L. Greene will describe short-term transcription-independent events and long-term transcription-dependent events both mediated by NGF and both of biological significance to neuronal development. The regulation and properties of catecholamine synthesizing enzymes will be discussed by T. Joh who will present biochemical and molecular biological evidence including peptide analysis, antibody cross-reactivity, and cDNA cloning for a common ancestral gene for the enzymes tyrosine hydroxylase, dopamine- -hydroxylase, and phenyl-N-methyltransferase. Molecular techniques have recently become available for quantitating gene expression in the brain and J. Roberts will describe the current methods for this quantitation by using as an illustrative example his work with peptide hormones such as ACTH, vasopressin, GnRH, among others. The production of large cells by chemically-induced fusion of cells sensitive to NGF has recently proven useful in studying aspects of neuronal differentiation. P. O'Lague will discuss the use of large clonal (PC-12) cells in studying the effects of intracellular injection of macromolecules, including NGF among others, on the acquisition of neuron-like properties and the effect of multiple nuclei on NGF-induced growth.

146 WORKSHOP

THALAMIC MECHANISMS OF PAIN SENSATION K.J. Berkley, (Chairman, Fl. St. Univ.), H.J. Ralston, III (UCSF), W.D. Willis, Jr. (Univ. Tx. Med. Br., Galveston), L. Kruger (UCLA), D. Albe-Fessard* (Univ. P. & M. Curie, Paris).

This workshop is designed to provide an overall view of what is currently known (and unknown) about thalamic mechanisms of pain sensation and to relate this information to what is known about such mechanisms in other parts of the central nervous system. Each speaker will address a different aspect of the topic by summarizing current knowledge and by describing some of the relevant problem areas under investigation in the speaker's own laboratory. A general discussion period will follow each lecture, and, if time permits, there will be another period for general discussion at the conclusion of the workshop.

Following some brief introductory remarks on the general status of the field by K.J. Berkley, H.J. Ralston, III will review our current knowledge of connective arrangements within the thalamus as they relate to "pain pathways". In the context of this review, he will also describe some of his recent ultrastructural work (involving intracellular injections of HRP) on local neuronal circuits in some of these thalamic regions. Next, W.D. Willis, Jr. will review our current knowledge of the pain-related response properties of neurons in somatic sensory portions of the thalamus. Included in this review, he will describe some recent electrophysiological findings on the responses of neurons located in both the lateral and medial thalamus of primates to noxious mechanical and thermal cutaneous stimuli. Next, L. Kruger will review our current knowledge of the thalamic mechanisms of pain that are specific to the trigeminal system. He will bring into this discussion some recent anatomical and electrophysiological work that is related to these mechanisms in cats and rats. Next, D. Albe-Fessard will review current ideas of pain mechanisms in the thalamus as they have been derived from clinical findings in humans and from electrophysiological and behavioral studies in animals. She will also discuss some of her relevant new findings on animal models of desafferentation pain and on inhibitory and facilitatory controls acting at thalamic levels. K.J. Berkley will conclude the session by summarizing the proceedings and discussing new directions for further research.

147.1 INHIBITION IN THE HIPPOCAMPAL SLICE IS REDUCED BY PHENCYCLIDINE. G. W. Bourne, Y. Theoret*, B. Esplin and R. Capek. Dept. of Pharmacology & Therapeutics, McGill University, Montreal, Quebec.

The frequently abused general anesthetic and psychotomimetic drug phencyclidine (PCP) can induce numerous signs of excessive stimulation of the central nervous system including convulsions when administered in high doses. The hippocampus may be the major locus of PCP evoked seizure activity because this structure has a low seizure threshold, contains large concentrations of stereospecific PCP binding sites (Vincent et al., Proc. Nat. Acad. Sci. 76, 4678) and responds by large increases in energy metabolism to PCP administration (Meiback et al., Nature, 282, 625). Therefore the possibility that PCP, as numerous convulsants, induces seizures by depression of inhibition was examined in the hippocampal slice in vitro preparation.

The hippocampus of male Sprague Dawley rats was sliced in transverse sections to a thickness of 425 µM. The slices were placed on a nylon mesh, were perfused at a rate of 1-2 ml/min with a 95% O_2 and 5% CO_2 saturated Krebs solution and were maintained at 36°C. Field potentials were recorded extracellularly by a 3 M NaCl filled glass micropipette. The population spikes were elicited orthodromically by stimulation of the Schaffer collaterals or antidromically by stimulation of the alveus. Inhibition of the orthodromic response was produced by a conditioning orthodromic or antidromic stimulus 5 to 40 ms prior to the stimulus evoking the test response.

Bath applied PCP in concentrations of 5 to 50 µM did not significantly alter the orthodromic or antidromic evoked responses in CA1 cells. However, these concentrations of PCP markedly reduced inhibition produced by orthodromic or antidromic stimulation at all time intervals tested. The reduction of inhibition commenced 15 minutes after PCP application. This effect was fully reversible, after a 60 minute washout period with Krebs solution the inhibition was returned to control levels. The inhibition reduced by PCP was restored by diazepam (50 µM), despite the continued presence of PCP in the perfusion medium.

These results indicate that decrease in inhibition may be responsible for PCP induced seizure activity. Since PCP did not interfere with responses elicited by iontophoretic administration of γ-aminobutyric acid (GABA) (Raja, S.N. & Guyenet, P.G., 1980, Neurosci. Abst. 270, 1) it is likely that depression of GABA release either by a direct action on the GABA release mechanism or through a depressant action on the recurrent inhibitory pathway may be the underlying mechanism of this action of PCP.
(Supported by the Medical Research Council of Canada).

147.2 EFFECTS OF PHENCYCLIDINE AND KETAMINE ON THE HIPPOCAMPUS. S.N. Raja*, J.L. Stringer* and P.G. Guyenet. Univ. Virginia School of Med., Dept. of Pharmacol., Charlottesville, VA 22908.

The effects of phencyclidine (PCP) and ketamine (K) on synaptic transmission were investigated at the level of two excitatory connections of the hippocampal formation: the interhippocampal projection from contralateral CA3 (cCA3) to CA1 and the entorhino-dentate pathway.

In urethane-anesthetized rats, PCP i.v. decreased by 16-40% the population EPSP elicited in the stratum radiatum of CA1 by cCA3 stimulation and reduced the amplitude of the corresponding population spike recorded at the level of the CA1 pyramidal (P) cell bodies by up to 97% (ED_{50}: 1.83 mg/kg).

Single-unit analysis of CA1 P-cell discharges indicated that i.v. PCP (0.5-3 mg/kg) reduced their spontaneous firing, their orthodromic activation by cCA3 stimulation and their excitation by iontophoretically applied glutamate and ACh but did not alter the size of the extracellular action potentials.

Similar effects on both field potentials and single-unit activity in the CA1 area were also observed following the administration of larger i.v. doses of K (ED_{50}: 7.2 mg/kg) but the effects of the latter drug were of considerably shorter duration than those of PCP.

The maintained activity of CA1 P-cells and the discharges induced by iontophoretically applied ACh and Glu were also decreased following iontophoretic administration of PCP and K in doses which did not produce any alteration in spike size.

PCP i.v. also decreased neurotransmission in the entorhinodentate pathway as evidenced by a reduction of both the rate of rise of the population EPSP and of the size of the population spike recorded in the dorsal granule cell layer of the dentate gyrus. The linear relationship between these two parameters was not affected by the drug, suggesting that the decrease in the evoked discharges of granule cells was mainly due to a reduction in dendritic depolarization.

In conclusion, PCP was shown to powerfully depress excitatory transmission in the hippocampus when administered in a dose range identical to that previously used to demonstrate behavioral alterations in the same species. The effects of PCP were similar to those of K but the former drug was both more powerful and longer acting. The effects of both PCP and K are presumably due in large part to a direct postsynaptic action. (DA 02310).

147.3 Δ^9-TETRAHYDROCANNABINOL (THC) INDUCED CHANGES IN BASAL AND NOREPINEPHRINE-STIMULATED ADENYLATE CYCLASE ACTIVITIES IN MOUSE CEREBRAL CORTEX. Cecilia J. Hillard and Alan S. Bloom. Department of Pharmacology and Toxicology, Medical College of Wisconsin, Milwaukee, WI 53226.

We have previously reported that two psychoactive cannabinoids, THC and 11-OH-THC, increased the binding of ^{3}H-dihydroalprenolol (^{3}H-DHA) to beta-adrenergic receptors of mouse cerebral cortical homogenates (Kiernan, CJ and Bloom, AS, Fed. Proc., 39:849, 1980). This increase in in vitro ^{3}H-DHA binding was found to be due to an increase in affinity with no change in the total density (Bmax) of binding sites. Conversely, cannabidiol, a cannabinoid devoid of THC-like psychoactivity, had no effect on ^{3}H-DHA binding. We have now investigated the effects of THC in vitro on both basal and norepinephrine-stimulated adenylate cyclase activities in mouse cerebral cortical homogenates.

Male ICR mice were used in these studies. The animals were sacrificed and cerebral cortices were removed and homogenized. Homogenates were incubated with drug, 0.75 mM ATP and 0.1 mM GTP for 2.5 minutes at 30°C. The cAMP produced during the incubation was measured using the competitive protein binding method of Brown (Brown et al., In: Advances in Cyclic Nucleotide Research, Vol. 2, ed. by P. Greengard and G.A. Robinson, p. 25-40, Raven Press, N.Y., 1972). THC was administered using an emulphor-ethanol-buffer vehicle.

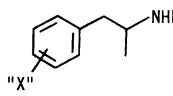
Mean basal adenylate cyclase activity in the absence of drug was 33.77 pmol/mg protein/min. THC at concentrations of 10 µM-30 µM increased basal adenylate cyclase activity to 140-170% of vehicle control, with the increase at 30 µM being statistically significant. The increase was biphasic, both lower and higher concentrations of THC had no effect on basal adenylate cyclase activity. The increase in basal adenylate cyclase activity could not be blocked by 10 µM dl-propranolol.

Mean adenylate cyclase activity in the presence of 10 µM l-norepinephrine was 48.51 pmol/mg protein/min, an increase of 43.6% over basal activity. At concentrations of 1 µM-30 µM, THC appeared to abolish the norepinephrine-stimulated component of adenylate cyclase activity since no additional increase over the change in basal adenylate cyclase activity was seen. At 100 µM THC, adenylate cyclase activity returned to control values. These results indicate that THC has effects on both basal and norepinephrine-stimulated adenylate cyclase activities that may be independent of the drug's effect on binding to the beta-adrenergic receptor. (Supported by USPHS grant DA-00124).

147.4 EFFECTS OF CERTAIN HALLUCINOGENIC AMPHETAMINE ANALOGUES ON THE RELEASE OF ^{3}H-5-HT FROM RAT BRAIN SYNAPTOSOMES. D.E. Nichols, D.H. Lloyd*, M.B. Nichols*, and G.K.W. Yim, Depts of Medicinal Chemistry and Pharmacognosy and Pharmacology and Toxicology, Purdue University, West Lafayette, Indiana 47907

It is generally believed that the mechanism of action for hallucinogenic amphetamines involves direct interaction with central serotonin receptors. However, some compounds have been shown to release endogenous transmitter. The extent to which this is a significant component of the action is unclear. PMA and MDA are two widely-abused hallucinogens which have been implicated in several overdose deaths. The former compound has been shown to be a powerful in vivo releaser of central 5-HT and peripheral NE. MDA has been shown both to release peripheral NE and to block reuptake of NE into synaptosomes.

The behavioral effects of hallucinogenic amphetamines are selectively elicited by the R-(-) enantiomer. It was therefore intriguing that N-methylation of MDA reverses this selectivity. That is, it is the S-(+) isomer of N-methyl MDA which is active. The object of this work was to study the effect of R and S MDA, R and S N-methyl MDA (MDMA), R and S PMA and some related compounds on the release of ^{3}H-5-HT or ^{3}H-DA from rat brain synaptosomes. Synaptosomes were prepared by the method of Whittaker and Barker. Following incubation with ^{3}H-5-HT, synaptosomes were placed into a superfusion apparatus as described by Raiteri et al. Amount of released transmitter in aliquots of superfusate was measured and rates of release were determined. The results of the studies with ^{3}H-5-HT are shown below, expressed as percent increase in release rate, as compared with controls.



R=H, X=3,4-OCH_2O- R and S MDA
R=CH_3, X=3,4-OCH_2O- R and S MDMA
R-H, X=4-OCH_3 R and S PMA

Percent Increase in ^{3}H-5-HT Efflux rate

Cmpd type	Substitution "X"		
	3,4-OCH_2O-	4-OCH_3	3,4-OCH_2O-, N-CH_3
R-amphetamine	99	86	75
S-amphetamine	86	76	75
α,α-dimethyl phenethylamine	9	6	6

This work was supported, in part, by Pharmacol. Toxicol. Training grant GM-709504 and USPHS grant DA-02189.

147.5 CHARACTERIZATION AND VISUALIZATION OF (^{3}H)Brom-LSD BINDING TO RAT BRAIN CORONAL SLICES. S. G. Beck, R. C. Meibach, S. Maayani, and J. P. Green. Dept. of Pharmacology, Mount Sinai School of Medicine, N. Y., N. Y. 10029.

Binding of (^{3}H)brom-LSD (i.e.,(^{3}H)BOL, 47 Ci/mmole) to rat brain coronal slices (32μ) taken from the level of striatum was characterized in respect to K_D, kinetics and dissociation. The (^{3}H)BOL was prepared by the Research Triangle Institute of NIH by bromination of (^{3}H)LSD and purified by reversed phase (C18) HPLC. Specific binding was determined in the presence of D-LSD (10^{-6}M). Incubations were carried out at room temperature (23 ± 2^{o}C) in 300 mOsmolar Tris Maleate, pH 7.4 and rinsed three times in the same ice cold (4 ± 1^{o}C, pH 7.4) buffer for 10 minutes each, after which the slices were wiped with GF/C filters and the radioactivity counted. For two determinations the K_D was found to be 6 and 10 nM with a B_{max} of 187 and 278 fmole/slice. Binding appeared to be maximal at 90 min. No specific binding was found to boiled slices.

In vitro radioautographic analysis was conducted by the technique described by Young and Kuhar (Brain Res., 1979, 179, 255) and by fixing the slices in paraformaldehyde fumes after incubation and directly dipping the fixed slices in emulsion (M. Herkenham, personal communication). Coronal rat brain slices were incubated for 90 min. in (^{3}H)BOL (6 nM, 11 Ci/mmole). Alternate sections were incubated with the same concentration of (^{3}H)BOL plus unlabeled BOL (10^{-7}M), unlabeled D-LSD (10^{-7}M) or unlabeled 5-HT (10^{-6}M). The concentrations of the masking ligands were determined from competition experiments on slices taken from the level of the striatum. Like LSD, (^{3}H)BOL binding sites were found in all layers of the cortex, with a dense band in layer 4, in the septal nucleus, caudate, superior colliculus, raphe, substantia nigra, and interpeduncular nucleus. Label was also found in the hippocampus. As with 5-HT, grains were also found in the subiculum. LSD appeared to mask most of the binding of (^{3}H)BOL, except in isolated areas. In contrast, 5-HT did not mask (^{3}H)BOL binding sites when used at a concentration which had previously been shown to mask (^{3}H)LSD binding sites (Meibach, et.al., Eur. J. of Pharm., 1980, 67, 371). Supported by NIDA DA-01875 and NIDA DA-07135.

147.6 DIFFERENTIAL TOLERANCE TO ANALGESIC AND PHYSIOLOGICAL EFFECTS OF HALLUCINOGENS IN RATS. D.A. Gorelick# and A.B. Hine+. Depts. of Pharmacol. and Psychiatry, Albert Einstein Col. of Med., Bronx, N.Y. 10461, and N.Y.U. Sch. of Med., N.Y., N.Y. 10026.

Roughly equipotent doses (μmoles/kg i.p.) of the hallucinogens mescaline (80), 2,5-dimethoxy-4-methylamphetamine (DOM) (80), N,N-dimethyltryptamine (DMT) (80), and LSD (0.080) were given to adult, male Sprague-Dawley rats. Analgesia was measured by the tail flick method of D'Amour and Smith, heart rate by subcutaneous wire electrodes in the chest, body temperature by electronic tele-thermometer probe inserted into the rectum, and locomotor activity by placing subjects for 10 minutes in an annular photocell activity chamber. Measurements were made immediately prior to injection (baseline) and 20 minutes later. A control group received saline injection. Tolerance was studied by testing some subjects after 5 days of daily drug injection. Data were analyzed in terms of % change from baseline.

All drugs produced analgesia, bradycardia, hypothermia, and decreased locomotor activity when given acutely. When mescaline and DOM were given chronically, a differential pattern of tolerance was found. There was no tolerance to the bradycardic effect of mescaline and DOM, nor to DOM's effect of decreasing locomotor activity. There was complete tolerance to mescaline's hypothermic effect, and partial tolerance to DOM's analgesic effect. These results are consistent with previous reports of differential tolerance to behavioral effects of hallucinogens in rats.

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147.7 COMPARISON OF CENTRAL AND PERIPHERAL BINDING SITES FOR COCAINE, M.E.A. Reith*, H. Sershen* and A. Lajtha. Center for Neurochemistry, Rockland Research Institute, Ward's Island, New York, New York 10035.

We have shown previously that mouse brain contains membrane-associated binding sites that are relatively specific for cocaine. Analogs of cocaine are available, some with potent central stimulatory activity and others with only local anesthetic activity. Only the analogs with central activity appreciably compete with cocaine for its bindings sites on brain membranes; both the central effect and the binding show stereospecificity.

The present work indicates that preparations of membranes from mouse liver also bind cocaine in a saturable manner. However, the affinity of the binding is approximately 8-fold lower than that observed in brain. In comparison with those in brain, the binding sites in liver have only moderate stereospecificity, and they discriminate less between the centrally active compounds and the centrally inert analogs. The binding sites in liver may be enzymes involved in the metabolism of cocaine, although after incubation of (^{3}H)cocaine with liver membranes more than 82% of the radioactivity of free and membrane-bound (^{3}H)cocaine cochromatographed with (^{3}H) cocaine itself in various solvents. It is unlikely that the binding sites for cocaine in liver represent uptake sites, since 1) we found no active transport system for cocaine in liver slices, 2) the binding appeared to be Na^+ - independent, and 3) frozen/thawed membranes did not show diminished binding capacities.

Since low binding affinites, as observed in binding of cocaine to liver membranes, may result from fast dissociation rates, we investigated the possibility that bound radioactivity had been released during the separation of bound and free ligand in the filtration method by comparing filtration with centrifugation techniques. Equilibrium binding experiments on liver membranes indicated, with filtration: K_d=5.0± 0.7μM (average ± SEM), B_{max}= 31.8 ± 7.2 pmol/mg of protein, and with centrifugation: K_d=5.2 ± 1.0 μM, B_{max}=57.6 ± 5.2 pmol/mg of protein. Therefore, although both methods give identical values for the affinity constants, there is 45% loss of bound radioactivity upon filtration.

In conclusion, liver membranes have saturable binding sites for cocaine and there is no correlation between the potencies of cocaine analogs in inhibiting the liver binding and their central activities. The function of peripheral receptors for centrally active drugs such as cocaine remains unclear, but is reminiscent of the finding of peripheral sites for benzodiazepines and phencyclidines.

148.1 HUMAN VISUAL EVOKED POTENTIAL AUGMENTING-REDUCING AT THE OCCIPUT AND VERTEX. J. H. Lukas. US Army Human Engineering Laboratory, Aberdeen Proving Ground, MD 21005.

Lukas and Siegel (Science, 198, 73, 1977) reported a significant relationship between cortical augmenting-reducing (A-R) recorded from paralyzed cats and their previously recorded behavioral reactions to novel or aversive stimuli. Reducers were not as emotional or aggressive as the augmenters and demonstrated cortical inhibition during intense sensory stimulation or reticular activation. The present study examined human A-R to changes in luminance utilizing rigorous stimulus control and adequate recording procedures to minimize artifactual sources of variance in the visual evoked potential (VEP) amplitude (Tepas, et al., EEG & Clin. Neurophysiol., 36, 533, 1974).

Ten subjects observed with the right eye, 50 msec light flashes presented 1/sec by a Maxwellian-view optical system. A fixation point was provided within the center of the 22° light beam which was carefully aligned to pass within the pupil. Intensity was varied across a 5 log range in 1.0 log unit steps from a maximum of 599,000 trolands by interposing appropriate neutral density filters. Following 5 minutes dark adaptation, the flashes were presented in counterbalanced ascending-descending intensity series. The VEPs recorded from O_z and C_z (right ear reference) were tape recorded (1-100 Hz) and signal averaged in groups of 100 artifact free sweeps. Linear regression analyses were computed for the latency and amplitude of the first major O_z component (N70-P100) and the C_z component typically measured in human A-R studies (P100-N200).

Latency for the O_z and C_z components decreased with increasing light intensity. Pooled latency data provided correlations of -.96 and -.94 and slopes of -9.1 and -8.4 msec/log. The main effects for cortical area and intensity were statistically significant; whereas, the interaction term was not significant, indicating that the slope functions for latency did not differ at O_z and C_z. The amplitude data from O_z and C_z were positively correlated with intensity. Pooled amplitude data provide correlations of .96 and .95 and slopes of 1.3 and .7 uV/log. The main effects for cortical area, intensity and their interaction were all significant. Although the O_z and C_z data were recorded simultaneously, the C_z slope was less than half that of the O_z slope in 7 subjects. VEP amplitude variability tended to increase at O_z and decrease at C_z with increasing luminance. However, with one exception at O_z, VEP variability was not significantly correlated with intensity. Therefore, different A-R slopes recorded at C_z cannot be accounted for solely by inadequate stimulus control or VEP variability, and may represent true alterations in cortical functioning.

148.2 HUMAN EVOKED SPINAL CORD DORSUM POTENTIALS RECORDED DURING NEUROSURGERY. J. Ovelmen Levitt*, R. Sharpe*, and B. S. Nashold. Div. of Neurosurgery, Duke Univ. Med. Ctr., Durham, N. C. 27710.

Subdural electrical potentials have been recorded, using monopolar platinum electrodes, from the cervical spinal cord in 8 patients undergoing a neurosurgical spinal cord operation to relieve intractable pain. The evoked responses were recorded to assess the functional capacity of the spinal cord before, during and after the surgery. The responses from the intact side are the subject of this report. Stimuli were applied percutaneously or transcutaneously to peripheral nerves or directly to the spinal cord itself. The radial, median and ulnar nerves were alternately, supramaximally, stimulated (10-12 mamp, .5 msec, 7-19 Hz) and recordings were made at cervical levels C5, C6, C7 C8 for each nerve. The potentials were amplified by means of Grass P15 preamplifiers, and averaged 128 to 1024 times using a Nicolet 1170 signal averager. The duration, amplitude and morphology of the potentials were noted. The place of maximal response depended on the peripheral nerve stimulated with radial representation being the most rostral, and the ulnar the most caudal. The amplitudes varied from 30 uV to 180 uV. The morphology and duration of the evoked potentials was similar to that obtained from primates (McCouch, Austin, Lee and Lee, 1958), with components which could be related to primary afferent conduction and to dorsal horn neuronal activity. Conducted responses after tibial nerve stimulation were also recorded from the spinal cord surface over the posterior columns. The conduction distance, latency and velocity of all potentials were determined. Average conduction velocity for arm nerves was 65.8 M/sec. The spinal cord conduction through the operative area was also monitored by stimulating the cord directly at C8 and recording with a bipolar lead at C5. The techniques for recording and evaluating evoked electrical potentials in order to monitor the functional capacity of the human spinal cord are available and interpretation is facilitated through reference to the results of animal experimentation.

148.3 INTERHEMISPHERIC EVOKED POTENTIALS IN THE RAT: RELATION TO BEHAVIOR AND EFFECTS OF ATROPINE AND RESERPINE. G.C. Harvey*, L.S. Leung and C.H. Vanderwolf. Dept. Psychol., Univ. Western Ontario, London, Ontario, Canada N6A 5C2

Cathodal stimulus pulses applied 1 mm below the surface of the rat parietal cortex produced an interhemispheric response (IHR) in the contralateral cortex. In surface records the IHR consisted of 3 components: a) a negative component peaking at a latency of 10-20 msec; b) a positive component; and c) a long-lasting second negative component peaking at 50-100 msec. Records from 1 mm below the surface revealed a negative component peaking at 10-20 msec, followed by a large positive component peaking at 50-100 msec. The IHR is known to vary in relation to behavior (Racine, R., Tuff, L., & Zaide, J. Canad. J. Neurol. Sci. 2: 395-405, 1975). A frame-by-frame video analysis showed that the late component (LC) at 50-100 msec, recorded bipolarly, was maximal during alert immobility, but was strongly suppressed during Type 1 behavior (spontaneous walking, head movement, struggling when held). Type 2 behavior (rotary forepaw movement during face-washing, licking the paws, chewing food, drinking water) was associated with very little suppression of the LC. Using averaged evoked responses we observed a behavior-related suppression of the peak amplitude of the LC which varied with stimulus current. The suppression ranged from 100% at 1.5 x threshold to 48% at 5.5 x threshold. The degree of suppression was measured by comparing the IHR during struggling versus immobility. Atropine SO_4 (50 mg/kg i.p.) reduced the behavior-related suppression of the LC and increased the amplitude of the early component at 10-20 msec. There was little change in the LC amplitude during immobility. Reserpine (10 mg/kg i.p.) alone had little effect on the behavior-related suppression of the LC. Combining reserpine and atropine eliminated the suppression completely; even during struggling the IHR resembled that of an undrugged immobile rat. Cholinergic and aminergic reticulocortical pathways may modulate the IHR in relation to behavior.

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148.4 EFFECTS OF STIMULATION OF NUCLEUS TRACTUS SOLITARIUS WITH NALOXONE UPON MORPHINE-INDUCED EEG SYNCHRONIZATION IN THE RAT+. M. L. Kelly*, J. D. Bronzino, C. Cordova*, N. Oley (SPON: D. Adams). Depts. of Engineering and Psychology, Trinity College, Hartford, CT 06106.

There is no doubt that the enkephalins and a number of other opioid peptides are highly concentrated in specific regions within the brain and spinal cord (Wall and Woolf, Nature, 1980, 287, 185-186). The region of the nucleus tractus solitarius (NTS) has been included among these opiate receptor areas (Atweh and Kuhar, Brain Res., 1977, 124, 63-67). This region has also been implicated in the establishment and maintenance of EEG synchronization (Bronzino, et al., Am. J. Physiol. , 1972, 223, 376-383). To study the role of these NTS opiate receptors we have investigated the effect of intracerebral administration of naloxone, an opiate receptor antagonist, upon the analgesia and EEG synchronization induced by systemic administration of morphine.

Fourteen male albino rats were chronically implanted with bipolar recording electrodes in the parietal cortex and hippocampus, as well as a chemical stimulating cannula in the region of the NTS. After recovery from surgery all animals were exposed to each of the following treatments: (1) 30 mg/kg of morphine (i.p.); (2) 10 µg naloxone (i.c.) plus 30 mg/kg morphine (i.p.) (3) 30 µg naloxone (i.c.) plus 30 mg/kg morphine (i.p.) and (4) Ringer's (i.c.) plus 30 mg/kg morphine (i.p.). Treatments were given one week apart. The order of naloxone doses was reversed in several animals to control for morphine tolerance, and the volume of the intracerebral injections was held constant at 0.25 µl. Following injections, 4 hrs of EEG activity were recorded. EEG records were evaluated using visual scoring, as well as amplitude (Bronzino et al., Proc. IEEE-GMEB, 1980, 186-189) and spectral analysis techniques.

Intracerebral injections of naloxone 5 mins prior to systemic administration of morphine significantly reduced the total amount of time spent in EEG synchrony (i.e., high voltage, low frequency (HVLF) activity). The percentage of animals reaching HVLF activity was also significantly reduced. These results indicate that the region of the NTS mediates the production of specific changes in the EEG (i.e., HVLF activity) following systemic administration of morphine. The ability of intracerebral naloxone to reduce rather than to abolish morphine-produced EEG synchronization suggests the the region of the NTS is only one part of the total pathway responsible for this synchronization.

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148.5 HIPPOCAMPAL THETA RHYTHM: PHASE-RELATIONS OF NEURON FIRING AND CONDUCTANCE IN URETHANIZED RATS. S. Wolfson, S.E. Fox and J.B. Ranck, Jr., Dept. of Physiol., Downstate Med. Ctr., SUNY, Brooklyn, N.Y. 11203.

There are two types of hippocampal theta rhythm (or rhythmic slow activity, RSA) in the rat. One type occurs during immobility and can be abolished by atropine or driven by urethane anesthesia (atropine-sensitive RSA).[1] A second type occurs during walking. The present study uses extra- and intracellular recordings from hippocampal neurons to determine the phase-relations between atropine-sensitive RSA and action potentials or conductance changes. Rats were anesthetized with urethane. Fixed hippocampal macroelectrodes were implanted in dentate and CA1 to record RSA from both regions versus a distant reference. Stimulating electrodes were implanted in afferent and efferent pathways. Hippocampal neurons were identified by electrophysiological characteristics[2] with histological localization.

The phase relations were as follows:

	firing of projection cells	firing of interneurons	max. somatic conductance
CA1	+ (pyramids)	-	- (pyramids)
DENTATE	- (granules)	+	+ (pyramids)

+ & - refer to positive and negative peaks of dentate RSA

The major finding shown above is the occurrence of the maximum firing rate for CA1 interneurons and dentate granule cells on the negative phase of dentate RSA. This is in substantial agreement with results from urethanized rabbits,[3] but is in sharp contrast to our data for RSA in rats during treadmill walking:[4] all hippocampal neurons (pyramidal cells, granule cells and interneurons) in both CA1 and dentate were most likely to fire on the positive phase of dentate RSA. There are clearly radical differences in the synaptic mechanisms generating the two types of RSA. The extracellular data are supported by intracellular recordings. Intracellular impedance analysis[5] in pyramidal cells showed changes in impedance at up to 100 Hz, phase-locked to RSA. Changes in impedance at such high frequencies must be produced by conductance changes on the soma.[5] This coincidence of the maximum somatic conductance of pyramids and the maximum firing rate of local interneurons supports the hypothesis that projection cells are being inhibited by our putative interneurons on the phase at which the interneurons fire most rapidly. (Supported by NIH grant NS 14497 and NSF grant BNS 77-09375 to J.B.Ranck, and NIH grant NS 10987 to V.E. Amassian.)

[1]Vanderwolf, C.H., J. Comp. Physiol. Psychol., 88: 300-323, 1975.
[2]Fox, S.E. and Ranck, J.B., Exp. Brain Res. 41: 399-410, 1981.
[3]Bland, B.H., et al., Exp. Brain Res., 38: 205-219, 1980.
[4]Wolfson, S., et al., Neurosci. Abstr., 5: 285, 1979.
[5]Fox, S.E. and Chan, C.Y., this volume.

148.6 WET DOG SHAKES CAUSED BY HIPPOCAMPAL ACTIVATION ARE INHIBITED IN RATS WITH KAINIC ACID LESIONS. B.P. Damiano* and J.D. Connor. Dept. Pharmacol., Penn State Univ. Col. of Med., Hershey, PA.

Although wet dog shakes (WDS) occur during morphine withdrawal in rats, they can be caused by a variety of pharmacological and environmental stimuli. WDS have also been noted in association with hippocampal epileptiform activity (MacLean, 1957). The purpose of the present study was to determine: 1) if stimulation of hippocampal neurons through the perforant path (PP), an excitatory pathway into the hippocampus, causes WDS and, 2) if lesions of CA3 pyramidal cells, the main output cells of the hippocampus, prevent these WDS. Awake, unrestrained rats with implanted electrodes were tested for their responsiveness to various paradigms of PP stimulation (n=16). The characteristic evoked granule cell (GC) field potential with its population spike was monitored. Stimulation at 0.1 Hz for 2 min, which evoked large GC spikes, rarely caused WDS. However, stimulation at 1 Hz for 2 min caused 3-16 WDS depending on the size of the evoked GC spike. When WDS occurred, they were not usually related temporally to the GC spike. Twin pulse stimulation (1 Hz, 50-80 msec apart) was particularly effective in potentiating the GC spike and in causing WDS (6-21 in 2 min). Twin pulse stimulation (2 Hz, 50-80 msec apart) was also effective in causing WDS but often induced epileptiform activity. Higher frequency stimulation (10 Hz for 5-20 sec) consistently produced epileptiform activity with afterdischarges. Within 3 min of the stimulation, 8-32 WDS were observed, mostly in a cluster immediately following the train or afterdischarge. Direct stimulation of the GC through the recording electrode (10 Hz for 10-20 sec) produced a similar WDS response (8-21 shakes within 3 min). In another set of experiments, rats received bilateral icv injections of CSF (n=4) or kainic acid (400 ng, n=8). After 12-18 days, WDS in response to the various paradigms of PP and GC stimulation was inhibited by 80-95% in kainic acid lesioned rats vs CSF controls. Conversely, evoked GC spiking, epileptiform activity and afterdischarges were usually greater in kainate treated rats. Light microscopy revealed the characteristic kainate-induced CA3 pyramidal cell loss in the hippocampus. These results demonstrate that low frequency activation of hippocampal GC causes WDS in the absence of epileptiform activity. Granule cell axons innervate ipsilateral CA3 pyramidal cells. Although icv kainic acid lesions other brain areas, severe CA3 pyramidal cell loss coupled with blockade of WDS indicates that hippocampal output from the CA3 pyramidal cells may be involved in the WDS produced by hippocampal activation. (Supported by USPHS DA 02007).

148.7 MODULATION OF BACKGROUND ACTIVITY IN A HIPPOCAMPAL NEURAL NETWORK. J. L. Giacchino and J. M. Horowitz. Dept. of Animal Physiology, University of California, Davis, CA 95616.

Previous studies have described damped oscillatory evoked potentials in hippocampal networks (Biol. Cybernetics 37:115-124, 1980). In addition to associating a model with experimental data, the effect of background activity on hippocampal networks has been considered from a mathematical point of view (Intern. J. Neurosci. 5:113-123, 1973). The intent of this study was to determine if experimental data (PST histograms from pyramidal cells) supported model predictions.

Tungsten microelectrodes were used to record PST histograms from hippocampal cells following fornix, commissural or raphe stimulation. In rats anesthetized with sodium pentobarbital, a single unit was isolated using a WPI window discriminator and PST histograms were constructed using a Tracor-Northern digital signal analyzer. Following fornix stimulation in the rat, PST histograms had multiple peaks, with successive peaks showing smaller amplitude and larger width. A pulse train delivered over the commissure provided a means of augmenting the excitation of hippocampal cells. PST histograms for fornix stimulation with this augmentation were modified in that the time between successive peaks was shortened in 6 out of 8 cases. Cells that could be driven by fornix stimulation could also be categorized according to responses of the neuron to concurrent fornix and raphe stimulation. Class one neurons responded with an increase in latency after the concurrent stimulus and class two neurons exhibited decreased latency and increased cell activity.

These data are consistent with the behavior of a hippocampal network composed of pyramidal cells which excite interneurons that in turn inhibit the pyramidal cells. That is, the second and following peaks in the pyramidal cell PST histograms evoked by fornix stimulation may reflect rebound excitation. The PST histograms can be modified by background activity over commissural and brainstem pathways as predicted by the network simulation.

148.8 SPECTRAL CHARACTERISTICS OF HIPPOCAMPAL EEG IN BEHAVING RATS. L. S. Leung. Dept. Psychology, University of Western Ontario, London, Canada N6A 5C2.

Using electrophysiological criteria, 100 μm diameter wire-electrodes were implanted dorsal and ventral to the pyramidal cell layer of the hippocampal CA1 region of the rat. After recovery, the EEG at the electrodes were recorded during the following behaviors: slow-wave sleep (SWS), awake-immobility (AI), grooming, walking, rapid-eye-movement sleep (REM) and awakening from SWS(AW). EEG was also recorded following i.p. injection of sodium pentobarbital (10-20 mg/kg) and atropine sulfate (25 mg/kg). The EEGs were analyzed by fast Fourier transform by a PDP-11 or -12 computer. The power spectra and the coherence and phase spectra between two electrodes were plotted for 0.5-100 c/sec at a resolution of 1-2.2 c/sec and > 60 degrees of freedom.

At least three major components of the hippocampal EEG were inferred: 1. Irregular slow activity (ISA) - mainly power of 0.5-25 c/sec, with low dorsoventral coherence (coherence between EEG at electrodes dorsal and ventral to the pyramidal cell layer) ISA decreased generally in the behavioral sequence: SWS, AI, grooming, REM or walk or AW. Awakening from SWS caused a great decrease of ISA. 2. Rhythmical slow activity (RSA) or theta - characterized by a sharp power peak at 5-8 c/sec and a second harmonic at 12-18 c/sec. Dorsoventral coherence was high at the theta harmonics with a phase difference of 135°-180°. A small RSA peak at 5-7 c/sec could sometimes be observed during grooming or AI. 3. Fast activity (FA) - of about 20-80 c/sec, low peak power ($\leq$ 1/10 of theta peak power) and low dorsoventral coherence FA was larger during walking or REM than during AI, SWS, or AW. FA was similar to the oscillatory CA1 average evoked potentials (AEPs) elicited by electrical stimulation of the stratum radiatum (Leung, L.S., Brain Res., 198:95) in the following ways: (a) similar frequency range, (b) similar correlation with behavior, (c) similar changes following pentobarbital or atropine injections, and (d) a recurrent-inhibition, differential-equation model of the CA1 region (Leung, L.S., Brain Res., 205: 194) predicts both oscillatory AEP and fast EEG and their behavioral correlation. FA increased in power but decreased in peak frequency after pentobarbital. It decreased in power and frequency following atropine (cf. Leung, L.S. and Vanderwolf, C.H., Brain Res., 198:119).

The quantitative spectral analysis allows finer details of the EEG, e.g. FA, frequency contents of ISA, second harmonic of RSA, to be distinguished and studied in different behaviors.

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148.9 SPECTRAL SIGNATURES OF COGNITIVE ACTIVITY: SPATIAL MAPPING OF EEG CHANGES CORRELATED WITH TASK DIFFICULTY AND PUPILLOMETRIC RESPONSE. D. Barth* and J. Beatty, Human Neurophysiology Laboratory, Dept. of Psychology, University of California, Los Angeles, CA. 90024.

The performance of complex cognitive functions such as mental multiplication has been shown to elicit widespread momentary increases in sympathetic and decreases in parasympathetic activity that reflect the processing demands imposed by the task. These changes have been interpreted as peripheral signs of activity in the ascending reticular activating system. The current study was designed to investigate the influence of mental arithmetic on the pattern of activity in the central nervous system using spatial mapping and spectral analysis of the electroencephalogram (EEG).

Twelve right handed people were instructed to multiply 28 "easy" (one-digit by one-digit) and "difficult" (one-digit by two-digit) multiplications in a randomly presented sequence. Concurrent measurements were made of EEG (full International 10-20 System) and pupil diameter. The spectral density in each of the traditional EEG frequency bands was computed from Fourier analysis of samples, separately for each quartile of the range in pupil diameter. Four-way analysis of variance demonstrated that there was a significant frontal suppression of spectral density in the combined frequency bands associated with both the presence and degree of mental workload, as indicated by pupil diameter. This effect primarily consisted of changes in the delta, alpha, and beta bands. Left hemispheric suppression and right hemispheric enhancement of beta frequency spectral density accompanied task performance and increased with mental effort.

Mental multiplication was best discriminated from baseline activity by reduced spectral density in the delta and beta bands over left frontal (F7) and in the beta band left anterior temporal (T3) areas. Posterior enhancements in the beta (T5) and theta (P3, T6) spectral density were also significant. Lowest and highest pupil quartile ranges were best differentiated by reduced right frontal (F8) alpha and increased left posterior temporal (T6) theta density.

This experiment demonstrates a clear pattern of central nervous system activity associated with the presence and difficulty level of a mental multiplication task. The ANOVA and discriminant analysis indicate the differential involvement of both frontal and left hemispheric areas in the task. Posterior enhancement of EEG density may indicate a task related deactivation of cortical tissue.

148.10 ELECTROPHYSIOLOGICAL CORRELATES OF SELECTIVE ATTENTION TO SPEECH SOUNDS. P. W. Dickstein, J. C. Hansen, C. Berka* and S. A. Hillyard. Dept. of Neurosciences, Univ. of California, San Diego, La Jolla, CALIF 92093.

Selectively attending to tone bursts has been reported to enhance the measured negativity of the N1 peak (latency 90-150 msec poststimulus) of the auditory event related potential (ERP) in man. This finding has been interpreted as an electrophysiological correlate of Broadbent's stimulus set attention, which is presumed to involve a rapid selection between attended and unattended channels of stimuli based on analyses of their simple physical attributes such as pitch or location. Recent studies suggest that the enhancement of the measured N1 amplitude to attended tone bursts results from the elicitation of a long-lasting, endogenous negative wave rather than a gating of the auditory evoked potential.

This study was undertaken to find out whether selectively attending to speech sounds distinguished by relatively complex physical features engenders the same ERP components as does attending to tone bursts that differ in pitch. Twelve subjects listened to three different randomized sequences of stimuli consisting of: (1) the syllables /ba/ and /pa/, (2) the syllables /da/ and /ga/ and (3) 375 Hz and 300 Hz tone bursts. All stimuli were presented binaurally at 50 dB SL, and the subject was instructed to listen selectively to one of the two stimulus classes at a time, pressing a button to infrequent, slightly longer duration stimuli of that type. ERPs elicited by each class of stimulus were recorded from midline, left and right scalp locations, and difference waves were formed comparing the ERP to each stimulus when it was attended versus when it was unattended. The attention effect was quantified as a root-mean square measure of the difference wave over the latency range 150-550 msec.

In all three conditions, the attended stimulus elicited a significantly enlarged negative ERP in relation to the unattended stimulus. This negativity had a bilaterally symmetrical scalp distribution for all stimuli. Thus, the electrophysiological correlates of selective attention were qualitatively similar for selections between syllables that differed either in voice onset time (/pa/, /ba/) or direction of formant transition (/ga/, /da/) and for selections between tones differing in frequency. This suggests that the attentional mechanisms involved in selections between complex, speech-related cues and simple pitch cues share similar characteristics. A further implication is that the concept of stimulus set attention may have to be broadened to include complex as well as simple stimulus attributes.

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148.11 ENDOGENOUS AUDITORY EVOKED POTENTIALS IN THE CAT. N.K. Squires* and J.S. Buchwald. Mental Retardation Research Center, Brain Research Institute, Dept. of Physiology, UCLA Medical Center, Los Angeles, CA 90024.

Evoked potentials in the cat were examined under conditions parallel to those which are necessary for obtaining endogenous brain activity in human subjects. Clicks of two intensities were presented as task-irrelevant stimuli, while a 4 KHz, 1 sec tone was the CS in a classical eye-blink conditioning procedure. The three stimuli were presented in random order with an ISI of 1.5 sec. In each block of 500 stimuli one of the click intensities occurred frequently (P = .80), the other rarely (P = .15), and the CS with a probability of .05. Evoked potentials were recorded from a stainless-steel screw implanted in the skull just lateral to the midline on the inter-aural line (vertex) referenced to the bulla. Eyeblinks were recorded with bipolar EMG electrodes inserted into the orbicularis oculi. The evoked response to either the loud or soft click was enhanced when the click was rare compared to when it was frequent. This enhancement to the rare stimulus appeared for four peaks in the waveform, a negative wave at approximately 40 msec, a positive wave at 70 msec, a negative wave at 110 msec, and a positive wave at 300 msec. The enhancement did not appear in a cat presented with the same series of rare and frequent stimuli in the absence of the conditioning procedure. Conditioned eye blinks were confined to the CS, and could not account for the waveform increase to the rare click. When the rare event was the omission of a click from an ongoing constant train of clicks, a long-latency (100-500 msec) positive wave was produced. These long-latency potentials resemble those seen in the scalp-recorded evoked response of human subjects under similar conditions of stimulus rareness and attention to the modality of the stimuli. In the human these have been called "endogenous", event related potentials since they vary more with the psychological context in which a stimulus occurs than with the physical characteristics of the evoking event. Finally, in the cat certain components of the these long-latency responses were differentially altered by the administration of pentobarbital and ketamine.

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148.12 AUDITORY BRAINSTEM AND ENDOGENOUS EVOKED POTENTIALS IN AGED CATS. J.B. Harrison and J.S. Buchwald, Brain Research Institute, Mental Retardation Research Center and Department of Physiology, UCLA Medical Center, Los Angeles, CA 90024.

Auditory brainstem responses (ABRs) and endogenous potentials were compared in aged cats (10 to 23 years) and young adult cats of known age. An implanted vertex electrode was referenced to the left bulla. The head was held by bars extending into metal sleeves implanted in an acrylic head mount. Click thresholds were higher in the old cats with a mean difference of 35 dB SPL and little overlap between the two groups. Stimulus intensities were set relative to individual thresholds.

With 0.1ms click stimuli, 15, 30 and 45 dB above threshold, ABRs were generally comparable in the young and aged cats. Endogenous potentials were compared between the young and aged cats with the procedure used by Squires and Buchwald (Neurosci. Abst., 1981). Blocks of 500 stimuli were presented in random order with loud and soft clicks on a schedule of frequent (P = .80) or rare (P = .15). A 4KHz CS (P = .05) was paired with a brief shock train to the orbicularis oculi to establish a classical conditioned eyeblink response. In alternate blocks the loud or soft click was the rare stimulus. In some blocks the rare stimulus was absence of a stimulus. Eye movements and the orbicularis oculi EMG were recorded. At latencies shorter than 40ms no evoked response components were dependent on stimulus probability. In the young cats, a negative wave at approximately 40ms, a positive wave at 100ms, a negativity at 150ms and a positivity at 300ms were enhanced by the rare stimulus. The omitted stimulus elicited a late positivity at 250 to 600ms. In the aged cats, the rare stimulus enhanced a negative wave at approximately 50ms, a positive wave at 200 to 400ms, and a positive wave at 500 to 1000ms. A positivity at 500 to 600ms was produced by the omitted stimulus. Thus, the endogenous potentials of the aged cats were marked by diminution or absence of some waves and increased latency of others. These abnormalities were not accompanied by aberrant ABRs, which suggests that aging changes of the endogenous potentials are independent of the auditory brainstem pathway. (This work was supported by USPHS Grants AG1754 and HD04612).

148.13 ANALYSIS OF SINGLE AUDITORY EVOKED RESPONSES TO LINGUISTIC AND NONLINGUISTIC STIMULI IN AUTISTIC AND CONTROL CHILDREN. R.M. Edwards*, P. Tanguay*, S. Hecht*, R. Olch*, J. Vidal and J. Buchwald (SPON: J. Marsh). Depts. of Neuroscience, Psychiatry, Engineering and Physiology, UCLA, Los Angeles, Calif. 90024.

Children with early infantile autism characteristically are impaired in the understanding and use of language. To study this impairment, differences in the auditory evoked responses (AERs) of these children to linguistic and nonlinguistic stimuli were assessed using a stepwise discriminant analysis (SDA).

The AERs of ten autistic children and ten age and sex matched control children were recorded from six electrode configurations, F7, F8, T3, T4, a point midway between T6 and C4, and a point midway between T5 and C3, all referenced to the ipsilateral mastoid. The stimuli, two words and two musical chords, were presented under two conditions: condition 1, a simple listening task, and condition 2, an auditory discrimination task involving a motor response to a fifth stimulus, another word. Each AER consisted of 50 msec prestimulus baseline EEG and 500 msec post-stimulus EEG.

SDAs were applied to each subject's data separately for both conditions. The single AERs from all six channels were first analyzed simultaneously, then the AERs from the left hemisphere, and lastly those from the right. The results were assessed for within group differences and between group differences.

The results show first that the SDA classified the stimuli at a level above chance for all conditions. Secondly, no consistent pattern of higher classification of one stimulus type compared to the other was found. Thirdly, no differences between the two hemisphere's analyses were found. A difference between the two recording conditions was found in that the electrode sites of the best discriminators were equally distributed between the two hemispheres for condition 1, but were found predominantly on the right hemisphere for condition 2.

The autistic group differed from the control group in having a higher per cent correct classification by the SDA, a larger number of high F level discriminant variables occurring in the early part of the AER, and slower motor response times and more response errors for condition 2. No differences between the groups were found in the discriminability of words and chords, in the electrode site or latencies of best discriminant variables, or the separate hemispheric analyses.

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148.14 PSYCHOPHYSIOLOGICAL INDICES OF LEVELS OF PROCESSING. Anne Barry* and Michael T. Harvey.* (SPON: Gail R. Marsh). Department of Psychology, Duke Univ., Durham, NC 27706.

Ten subjects participated in an evoked-potential experiment exploring brain indices of 'depth' of processing (Craik, F.I.M. & Lockhart, R.S., J. Verb. Learn. and Verb. Behav., 11:671, 1972). Common English word-pairs were presented in a match-mismatch decision during which processing occurred at one of three levels of porcessing: SIZE, RHYME, or VERB-NONVERB. Word size varied in every trial, even when the subject's matching task was at a deeper level of processing. Behavioral data supported previous findings about levels of processing: words encoded more deeply were better remembered. Task 'depth' produced systematic significant effects on several components elicited after presentation of the second word, as well as on the CNV recorded during the preparatory interval between task instruction and the first word in the pair. Effects of task-irrelevant word size during rhyme and verb-nonverb decisions appeared in an early positive component (P200) and for components as late as 400 msec. post-stimulus. Results both support and fail to support certain findings of previous studies (e.g. Sanquist, T.F. et al., Psychophysiology: 17, 568, 1980).

148.15 CHANGES IN THE COHERENCE SPECTRA OF THE EEG AND AUDITORY EVOKED POTENTIALS OF THE CEREBRAL CORTEX AND AMYGDALA RELATED TO REPEATED DOSES OF d-AMPHETAMINE. R. J. Morgan, C. C. Turbes and G. T. Schneider*. Dept. of Physiology and Biophysics, Colorado State University, Ft. Collins, Colorado 80523.

The coherence function measures and the coherence spectra estimates how well each frequency component of one signal is phase locked to the corresponding frequency components of another signal. Due to normalization, all information about the relative amplitude of the frequency components is lost. In these experiments we examine the action of repeated doses of d- and l-amphetamine on the coherence spectra of the EEG and auditory evoked potentials (AER) of the sigmoid gyrus and basal nucleus of the amygdala. Data from eight cats are used in these experiments. Recordings are made with a polygraph and an FM tape recorder using hardwire and radio telemetry methods. The EEG and AER potentials were selected and analog to digitally converted. The Fast Fourier Transform (FFT) algorithm processing is done in the frequency domain. The EEG and AER averaged coherence spectra of the sigmoid gyrus and amygdala showed similar constituent frequency components phase locked between the two brain regions. There were coherence peaks at 1 Hz to 5 Hz, 12 Hz to 16 Hz and 43 Hz to 45 Hz. The ranges in percent coherence varied with the behavioral state and the sampling procedure before and after d- and l-amphetamine show a decrease in coherence of all but the 1 Hz to 5 Hz at the first dose of amphetamine. There is an increase in percent coherence in the component frequencies at 43 Hz to 100 Hz. There is a coherence peak at 76 Hz to 80 Hz during the second and third doses of the amphetamines in both the EEG and AER. The amphetamines show changes in the coherence spectra between brain regions that play a role in the processing of sensory information related to behavior. These studies give further indication of the functional interaction of these brain regions during sensory information processing, behavior and the action of certain drugs.

148.16 THEORETICAL AND EXPERIMENTAL SPATIAL CORRELATION AND COHERENCE FUNCTIONS OF EEG DUE TO STOCHASTIC FIELDS OF CORTICAL GENERATORS. R. D. Katznelson. Dept. of Elec. Eng. & Comp. Sci., Univ. of California at San Diego, La Jolla, CA 92093.

The study of cortical activity through the use of EEG measurements often require volume conduction (VC) considerations, (Nunez, P.L. and contrib. by Katznelson, R.D., Electric Fields of the Brain Oxford Univ. Press, 1981). It is the underlying neural activity that one is usually interested in characterizing, given the observed potential data. Since usually only statistical measures of EEG are obtained, the approach taken by many researchers is to study the temporal stochastic properties of EEG. However, very little work was done on the EEG as a stochastic field with spatial parameters in addition to the time parameter. In this study, theoretical spatial correlation functions (SCF) of stochastic EEG fields are derived for potentials measured in cortical depths, pial surface and the scalp. It is shown that obtaining these SCF's consists of two distinct problems: (1) Obtaining the SCF of cortical neural generators and (2) VC effects on broadening the cortical SCF. The method of spatial power spectral decomposition is used for specific examples of VC effects on surface SCF's obtained for planar stratified cortical geometry and for a three concentric sphere model representing the brain, skull and scalp. In the isotropic planar geometry example it is shown that for uncorrelated (independent) neural current dipole layer situated at depth h, the surface potential SCF is proportional to

$$R(r) \sim [1+(r/2h)^2]^{-3/2}$$

where r is the distance between the two measuring points. Similar expressions are derived for other geometrical configurations. It is also shown that the coherence (COH) (as measured by Lopes da Silva and others) is essentially a spatial correlation coefficient and that it is real (phase angle of 0 or 180 deg.) for surface isotropic cortical geometries. Since R(r) above corresponds to a correlation length of about 2h, it is expected that realistic cortical SCF's (which are not infinitesimally sharp as in the uncorrelated source layer), would produce surface SCF's wider than 2h. Hence, lower bounds for correlation lengths and COH values are obtained and the implications to COH spectra in animal brains and cerebral lateralization measures in humans are discussed. Some experimental COH spectra are presented and finally problem (1) above is addressed and a neural model to account for the decrease in observed COH with temporal frequency is presented.

149.1 RATE-DEPENDENT AND SCHEDULE-DEPENDENT EFFECTS OF SEROTONIN ANTAGONISTS ON OPERANT BEHAVIOR. P.C. Mele*and M.A. Caplan. Dept. Psych., Adelphi Univ., Garden City, N.Y. 11530

Drugs which deplete or antagonize serotonin (5HT) may alter operant behavior when administered alone or in combination with amphetamine. However, the behavioral variables which mediate the effects of these drug treatments are not well understood. The present study examined baseline rate of responding and reinforcement contingency as factors which may influence the behavioral effects of the structurally distinct 5HT antagonists methysergide (M) and cinanserin (C).

A multiple fixed-interval 2 min differential-reinforcement-of-low-rate 18 sec schedule of sweetened milk delivery (mult FI DRL) was used to maintain responding in rats. M (1-17 mg/kg) increased the moderate FI rates of responding without altering the positively accelerated pattern of responding and produced only minor alterations in the low DRL rates. C altered FI and DRL responding primarily at the highest dose tested (64 mg/kg); 8-32 mg/kg did not consistently alter behavior. 64 mg/kg of C increased the low DRL rates and shortened interresponse times; FI responding was disrupted such that low rates occurring early in the interval were increased and higher rates occurring later in the interval were decreased. The effects of 64 mg/kg of C were similar to those found with amphetamine (A, 0.25-3 mg/kg) and chlordiazepoxide (2.5-20 mg/kg). Thus, the effects of C on mult FI DRL performance appear to be rate-dependent whereas those of M are schedule-dependent.

The combined administration of A plus M (10 mg/kg) or C (32 mg/kg) generally produced similar effects in both components of the multiple schedule. For FI, response rates were typically reduced relative to those obtained with A alone. For DRL, the drug combinations frequently produced potentiated rate increases at low doses of A; at higher A dose combinations the rate increasing effects seen with A alone were attenuated. It is suggested that the effects of the drug combinations, like the effects of amphetamine alone, are best described as rate-dependent.

The qualitative differences between the effects of M and C may be due to differences in their pharmacological actions (e.g. Clineschmidt and Lotti. Br. J. Pharmac., 50, 311-313,1974). In contrast, the similarity in the effects of the drug combinations suggests that 5HT antagonism was the common mechanism of action which altered the effects of amphetamine.

149.2 MEDIAL-LATERAL DIFFERENCES IN SUBSTANTIA NIGRA MECHANISMS MEDIATING CIRCLING. F. Vaccarino and K. B. J. Franklin* (SPON: T.L. Sourkes). Dept. of Psychol., McGill Univ., Montreal, Quebec

It is well known that when drugs or lesions cause an imbalance in nigrostriatal dopamine (DA) activity rats will circle contralateral to the more active nigrostriatal system. However, more recently it has been shown that the nigrostriatal system may not be functionally homogeneous with respect to circling behavior. It has been reported that contralateral circling can be evoked in rats following systemic apomorphine if lesions were restricted to the medial parts of the substantia nigra(SN). However, when the lesions were restricted to the more lateral components of the SN rats circled ipsilaterally (Thal et al., Brain Research 170:381, 1979).

The present experiments confirm and extend the differences found between medial and lateral SN mechanisms mediating circling behavior. Rats were tested for their circling response to imposed continuous electrical stimulation (100 Hz, .2 msec pulses) of either the medial or lateral SN. The results showed that the direction of circling depended on the medial-lateral position of the electrode in the SN. That is, stimulation of the medial SN produced contralateral circling and stimulation of the lateral SN produced ipsilateral circling. In addition, rats were tested for their circling response to d-amphetamine (2 mg/kg) following lesions restricted to either the medial or lateral SN. It was found that d-amphetamine induced ipsilateral circling following medial SN damage and contralateral circling following lateral SN damage.

A second experiment showed that both ipsilateral and contralateral circling induced by electrical stimulation (.2 sec trains of 100 Hz, .2 msec pulses delivered at the rate of 4 trains per sec) of the lateral and medial SN sites, respectively, depended on the proximity of SN DA cells to the locus of stimulation.

Also, pimozide, a DA antagonist, dose dependently blocked both ipsilateral and contralateral stimulation induced circling elicited from the SN but did not affect circling elicited from the cerebral peduncle.

The results of these experiments suggest that the lateral SN is functionally different and possibly antagonistic to medial SN mechanisms involved in circling behavior. Furthermore, both medial and lateral mechanisms appear to be DA dependent.

149.3 SNOUT CONTACT FIXATION IS AN INVARIANT FEATURE OF STEREOTYPED BEHAVIOR INDUCED BY APOMORPHINE. H. Szechtman[1], K. Ornstein[*2], P. Teitelbaum[3] and I. Golani[*4]. 1. Dept. of Neurosciences, McMaster Univ., Hamilton, Ont., L8N 3Z5. 2. Dept. of Isotope Research, Weizmann Inst. Science, Rehovot, Israel. 3. Dept. of Psychology, Univ. of Illinois, Champaign, Il. 61820. 4. Dept. of Zoology, Tel Aviv., Ramat Aviv, Israel.

The stereotypy of rats injected with apomorphine (APO) is strikingly different in a small walled enclosure and in an open field without walls. After 2.5 to 20 mg/kg of APO, rats (N=28) assume an upright posture in a walled cage and remain elevated, as if attempting to climb up the wall, for as long as 36 to 42 min during a 60 min test. In an open field, however, rats (N=7) show no rearing during the same time period but locomote with the snout close to the horizontal surface, as if sniffing. Inspite of its different appearance, there is an invariance in the stereotypy exhibited in the two environments. Using the Eshkol-Wachmann movement notation to describe and analyze the relationship between the rat's snout and the environment, it became evident that in either environment rats establish close snout contact with a surface within 2-3 min after drug injection. Once contact is established, it is not released until 50 to 90 min later, when the drug seems to wear off. This suggests that on a horizontal surface APO-injected rats do not rear because that would entail breaking snout contact. Indeed, with a hood over the head to provide pressure on the snout, rats did not maintain uninterrupted snout contact and 3 of 6 animals reared in the open field during a 10 min test. Thus, the maintenance of snout contact may be a fundamental behavioral process induced by APO, accounting in part for environmental molding of the drug's behavioral manifestations.

149.4 6-OHDA LESIONS OF THE NAS REDUCE THE FACILITATORY EFFECTS OF D-AMPHETAMINE ON LOCOMOTOR ACTIVITY BUT NOT ON SELF-STIMULATION. A. Robertson and K.B.J. Franklin* (SPON: R. Hirsh). Dept. of Psychol., McGill Univ., Montreal, Quebec.

We examined the effects of 6-hydroxydopamine (6-OHDA) lesions of the dopamine-containing nucleus accumbens (NAS) on the facilitatory actions of D-amphetamine on self-stimulation response rates and on locomotor activity. Forty-two rats received bilateral infusions of 6-OHDA (2 µl of 4 µg/µl) or 0.1% ascorbic acid vehicle into the NAS. Twenty six (14 6-OHDA lesioned and 12 controls) of these rats were also implanted with a bipolar electrode aimed at the lateral hypothalamus and were then trained to self-stimulate through this electrode. Starting three weeks later, they were tested with 0.3, 0.8 and 2.0 mg/kg D-amphetamine sulfate, injected at three day intervals. In the second group of 16 rats (8 6-OHDA lesioned and 8 control), the locomotor stimulant effects of the same three doses of D-amphetamine were measured in a box equipped with photocell beams and a tilt floor. Extent of dopamine denervation of the NAS in both groups, determined by glyoxylic acid histofluorescence, ranged from 22% to 97% of the structure. Correlated to this, there was damage to adjacent structures including the olfactory tubercle and anterior caudate-putamen. In the locomotor group, amphetamine increased activity in a dose-related manner, but more so in controls than in experimentals. In contrast, none of the 14 self-stimulating rats treated with 6-OHDA showed any change in their responsiveness to D-amphetamine compared to controls, regardless of the extent of damage. The results suggest that a functional dissociation can be made between the effects of D-amphetamine on locomotor activity and on self-stimulation. The former but not the latter seems to critically depend upon a substrate involving the mesolimbic dopamine system terminating in the NAS.

149.5 CYCLES OF DEMAND FOR ELECTRICAL BRAIN STIMULATION OF RATS. L.H. Schneider[#], E.E. Coons[#], R.B. Murphy[%], A.V. Reed[*@] and D.Lewart[*$]. [#]Department of Psychology, and [%]Department of Chemistry, New York University, New York, NY 10003, [@]Graduate Faculty, The New School for Social Research, New York, NY 10011, and [$] M.I.T. , Cambridge, MA

The temporal pattern of responses was recorded under precisely controled self-administration of constant current stimuli into the Ventral Tegmental Area of Tsai in male Sprague-Dawley rats. The duration and amplitude of each pulse were fixed at 0.1 msec and for the individual rats at 75, 100, 125, 140, and 200 μA. The separation (10 msec) and the number (50) of pulses per train, trains per trial (20), and trials per session (25) were held constant throughout the experiment. The distribution of interresponse times, measured to the nearest millisecond, was analyzed; periodicities were found under autocorrelation. These were of similar duration (8±1 daily experimental sessions) in all five animals. Observation of behaviors suggests the involvement of opiate as well as monaminergic neurotransmitter pathways. We speculate that these effects arise as a result of the functional modulation of catecholamine receptor density and/or affinity. Preliminary examination of dopamine receptor density as reported by the in vitro binding of the ligand [3H]-spiroperidol to striatal homogenates from stimulated animals is used to assess the functional significance of these changes. (This work was supported in part under NIMH predoctoral fellowship 5F31 MH05787-02)

149.6 SPATIAL LEARNING ABILITY IS RELATED TO AN ENDOGENOUS ASYMMETRY IN THE NIGROSTRIATAL DOPAMINE SYSTEM IN RATS. Dianne M. Camp*, Barbara A. Therrien, and Terry E. Robinson (SPON: E.S. Valenstein). Psychology Department and Neuroscience Laboratory, University of Michigan, Ann Arbor, MI, 48109.

A wide variety of behaviors, including rotational behavior, side preferences and learning a T-maze, have been related to an endogenous asymmetry in the nigrostriatal dopamine (DA) system of rodents. However, the adaptive advantage of this asymmetry is not well understood. It has been suggested that some degree of asymmetry allows an animal to distinguish left from right, and thus may aid in spatial localization (Glick, 1977). To test this hypothesis we have examined the ability of lateralized and non-lateralized rats to perform a spatial task which required learning the position of a concealed goal using only distal cues.

Adult female Holtzman rats were individually placed in a 1.52 m diameter tank filled to a depth of 16 cm with 20°C opaque water. To escape from the water each rat had to swim to a 10.2 cm diameter platform which was concealed under 1 cm of water. A trial consisted of placing an animal in 1 of 4 randomly determined locations and recording a) the time taken to find the goal, b) the path taken to reach the goal and c) the initial direction the rat swam. Every rat received 4 trials/day for 4 days (initial learning). On the 5th day the goal was moved to a new location, and the animals received an additional 4 consecutive days of testing (reversal learning). Each animal was allowed to remain on the platform for 30 sec during which time its behavior was recorded. There were at least 2 min between each trial. During this training it was not known which animals were lateralized and which were not. This was estimated post-hoc by testing every rat for amphetamine (AMPH)-induced rotational behavior (1.25 mg/kg, i.p.). On the basis of their rotational behavior the animals were divided into 2 groups: 1) a lateralized group which made more than 10 net rotations/hr after AMPH, and 2) a non-lateralized group which made 10 or fewer net rotations.

In the initial task all animals learned the position of the goal, and there was no difference between lateralized and non-lateralized rats on any of the measures taken. However, when the error in initial direction was calculated (the angle between a line drawn directly to the goal and the direction the animal initially swam) it was apparent that lateralized rats learned the reversal task significantly faster than non-lateralized rats ($p < .05$). In addition, degree of lateralization was negatively correlated with the error in initial direction ($p < .05$). These results support the idea that some degree of asymmetry in the nigrostriatal DA system facilitates spatial learning.

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149.7 MEASUREMENT OF 3-METHOXY-4-HYDROXYPHENYLGLYCOL IN RAT BRAIN USING HPLC WITH ELECTROCHEMICAL DETECTION. Ann L. Acheson and Richard W. Keller, Jr. Dept. Biological Sciences, University of Pittsburgh, Pittsburgh, PA 15260.

3-Methoxy-4-hydroxyphenylglycol (MHPG) is a major deaminated metabolite of norepinephrine (NE) in rat brain, and, as such, can be useful as an index of the rate of NE utilization. We now report a method for measuring MHPG using high pressure liquid chromatography (HPLC) with electrochemical (EC) detection. The chromatographic system consists of a μBondapak C-18 column with a mobile phase containing 14.2 g monochloroacetic acid, 1.0 ml 0.1 M Na_2EDTA, 20 ml methanol and 2.0 ml 0.2 M octyl sulfate per liter, adjusted to pH 3.0. The EC detector is set at an applied potential of +0.92 V vs Ag/AgCl. Tissue is homogenized in 0.1 N $HClO_4$ containing 0.2 mM bisulfite and centrifuged at 39,000 X g for 15 min. Since most of the MHPG in rat brain exists in a sulfate-conjugated form which is not electrochemically active, an aliquot of the supernatant is first acid hydrolyzed at 100° C for 15 min to convert conjugated MHPG to free MHPG. This hydrolysate is then extracted with chloroform. The aqueous layer is applied to a Sephadex G10 column at pH 6.0. MHPG is eluted with 0.01 N $HClO_4$ in a 600 μl fraction and extracted with ethyl acetate at pH 6.0. Ethyl acetate is then evaporated to dryness under N_2, and the sample is reconstituted in 0.1 N $HClO_4$. This preparative phase of the procedure takes about 40 minutes and can be carried out on 4-6 samples simultaneously. Finally, the sample is injected into the chromatographic system. With a flow rate of 1.2 ml/min, MHPG elutes at 6 minutes, and is well separated from the solvent front and from other unidentified peaks. Recovery of MHPG through the entire procedure is 65-80% as determined by the addition of known amounts of MHPG to tissue.

We have used this procedure to measure MHPG in specific regions of rat brain. For example, the MHPG content of hippocampus was observed to be approximately 90 ng/g and of cerebellum, 60 ng/g. These values were decreased to 43% of control 30 minutes after the administration of the MAO inhibitor, pargyline (75 mg/kg, i.p.), illustrating the role of deamination in the formation of MHPG.

This method has several advantages: (1) It is sufficiently sensitive to detect 50 pg of MHPG. (2) It is highly reproducible (12% variation among 5 replicates). (3) NE can be measured in a separate aliquot of the tissue supernatant using the same mobile phase together with a much simplified preparatory step which has been described previously (Acheson et al., Life Sciences 28:1407-1420, 1981).

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149.8 IN VIVO VOLTAMMETRY IN RHESUS MONKEY STRIATUM. J. G. Herndon*, W. S. Lindsay*, R. D. Blakely*, J. B. Justice* and D. B. Neill* (SPON: J. Tigges). Division of Neurobiology of the Yerkes Primate Center, and Departments of Chemistry and Psychology, Emory University, Atlanta, GA 30322.

Recently, voltammetric techniques have been employed to measure release of neurochemical substances from the brains of rodents. The use of this technology in the primate offers several theoretical and practical advantages. Theoretically, the richer behavioral repertoire of the primate may permit the acquisition of more information than can be obtained from lower species. On a practical level, the larger brain of the macaque offers the possibility of implanting a larger number of detecting electrodes in a single animal, and may permit significant signal changes to be characterized more discretely with regard to anatomical locus.

In this preliminary study, we implanted carbon-paste electrodes into the caudate and putamen of a chair-restrained, adult (6 kg) female rhesus monkey. Voltammetric measurements were made by a computer-controlled system, in which 0.6 V pulses were applied and current was measured 1 sec later (Lindsay et al., Chem., Biomed., and Environ. Instrumentation 10:311, 1980). When the electrochemical signal had stabilized, the animal was given food or exposed to a visual stimulus (a leather capture glove) which caused obvious behavioral arousal. Both stimuli resulted in significant ($p<0.05$) increases in the magnitude of the voltammetric signal. These results are comparable to those obtained following behavioral arousal and eating in rats.

The present study confirms in the monkey several earlier observations made in rodents, and demonstrates the feasibility of applying voltammetric methods to primates.

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149.9 HALOPERIDOL-INDUCED INCREASE IN BRAIN DOPAC IS CORRELATED WITH INCREASED TYROSINE HYDROXYLASE ACTIVITY. Linda Toth Kennedy and Michael J. Zigmond (SPON: B. Dixit). University of Pittsburgh, Pittsburgh, PA 15260.

Activation of tyrosine hydroxylase (TH) is believed to underlie the sustained increase in dopamine (DA) turnover which occurs in DA neurons during increased impulse flow. However, we have observed that the increased DA turnover in frontal cortex produced by restraint stress is not accompanied by increased TH activity in that structure (Kennedy and Zigmond, Neurosci. Abs., 1980). This could be the result of the unique nature of mesocortical DA neurons, the type of stimulus used, or the relatively small increase in DA turnover produced. To distinguish among these alternatives we have examined dihydroxyphenylacetic acid (DOPAC), an index of DA turnover, and TH activity after systemic administration of haloperidol, a DA receptor antagonist known to increase impulse flow in DA neurons. One hr after administration of 1 mg/kg haloperidol to adult, male Sprague-Dawley rats, an increase was observed in both DOPAC content (+285%) and TH activity (+67%) in striatum. In contrast, while DOPAC was also increased in frontal cortex (+30%), there was no significant change in TH activity. The following lines of evidence suggest to us that this apparent dissociation results from the relative insensitivity of mesocortical DA neurons to haloperidol and from the difficulty of detecting small changes in TH activity: First, we compared the haloperidol-induced increase in DOPAC content with the changes in TH activity in individual samples of striatum, frontal cortex, and two other DA-rich areas, olfactory tubercle and hypothalamus. We observed a strong correlation between these two variables ($r=0.78$, $p<.01$) which was independent of brain region. Second, we examined the effect on TH activity in striatum of low doses of haloperidol (10-100 ug/kg) which produced increases in DOPAC comparable to those observed in frontal cortex after higher drug doses. Again, the change in TH activity was correlated with that in DOPAC level, although this was only observable when the enzyme was assayed at pH 7.0, a pH well above its pH optimum, permitting the expression of small increases in TH activation. (The specific activity of the enzyme in frontal cortex is too low to be assayed under these conditions.) Third, a similar correlation was observed when the striatal DOPAC response was attenuated by using shorter post-injection intervals (10-30 min). Thus, our observations support the hypothesis that activation of TH accompanies and may be causally related to increases in DA turnover during increased impulse flow. However, they also indicate that in structures containing relatively little TH, small but significant changes in enzyme activity may not be easily detected. (Supported by USPHS grants NSMH-16359 and MH-29670.)

149.10 A BEHAVIORAL PARADIGM TO ASSESS THE RELATIVE DOPAMINERGIC AND SEROTONERGIC ANTAGONISTIC PROPERTIES OF SELECTED DRUGS. W.J. Heinze*, R.F. Schlemmer, Jr., J.I. Javaid, and J.M. Davis (SPON: L.F. Eastman). Illinois State Psychiatric Institute Chicago, IL 60612 and National College, Lombard, IL 60148

A behavioral paradigm is proposed for assessing the relative dopaminergic (DA) and serotonergic (5-HT) antagonist properties of drugs using rats. The paradigm employs two well studied drug-induced behaviors as tests. Antagonism of stereotyped licking & chewing (LC), induced by the DA agonist, apomorphine (APO), is used to assess DA antagonist properties of drugs. Antagonism of repetitive pawing (RP), induced by the 5-HT agonist, 5-methoxy N,N-dimethyltryptamine (5-MeODMT), is used to assess 5-HT antagonist properties of drugs. Male Sprague-Dawley rats no less than 50 days old were used throughout the study. Observation was conducted by a "blind" observer who rated the presence (behavior observed > 5 sec.) or absence & the duration of each behavior on a checklist of more than 10 behaviors described as components of stereotyped behavior & the 5-HT syndrome. During this time each rat was rated for 30 sec. once every 15 mins. for 1 hr. for the APO expt. and once every 5 mins. for 30 mins. for the 5-MeODMT expt. All drugs were administered i.p. & all doses are expressed as the base. A dose-response study of the behavioral effects of APO and 5-MeODMT revealed a dose-dependent increase in LC from 0.5 to 2.5 mg/kg APO which peaked at 15 mins. and a similar dose-dependent increase in RP from 1 to 5 mg/kg with 5-MeODMT which peaked at 5 mins. Six drugs with varying DA & 5-HT antagonist properties were then tested: haloperidol & trifluoperazine (which reportedly have greater DA than 5-HT antagonist properties), chlorpromazine & methiothepin (antagonists of both DA & 5-HT), & the 5-HT antagonists cyproheptadine & cinanserin. 4-5 doses of antagonist were administered 30 mins. prior to APO or 5-MeODMT & 4 rats were tested per dose. ID50's were determined for each drug for the presence of APO-induced LC and 5-MeODMT-induced RP using probit analysis. Haloperidol was the most potent drug in inhibiting LC, 17x greater than the ID50 for RP. Trifluoperazine was more than 4x more potent in inhibiting LC than RP. Chlorpromazine & methiothepin ID50's were approximately the same for LC and RP for each drug. Cyproheptadine was the most potent antagonist of RP, more than 20x more potent than inhibiting LC. Cinanserin was more than 7x more potent in antagonizing RP than LC. These results suggest that haloperidol and to a lesser extent trifluoperazine are more potent DA than 5-HT antagonists, cyproheptadine & cinanserin are more potent 5-HT than DA antagonists, and that chlorpromazine & methiothepin are approximately equipotent antagonists of DA & 5-HT systems. We suggest that this paradigm will be useful in determining in vivo relative DA & 5-HT antagonist properties of drugs.

149.11 INCREASED NOREPINEPHRINE TURNOVER AND INCREASED FIRING RATE IN RESIDUAL NEURONS AFTER NE-DEPLETING LESIONS. M.J. Zigmond, A.L. Acheson, L.A. Chiodo and E.M. Stricker. Depts. of Biological Sci. and Psychology, Univ. of Pittsburgh, Pittsburgh, PA 15260.

Intracerebroventricular administration of the neurotoxin 6-hydroxydopamine (6HDA; 250 µg) has little gross behavioral effect on rats under basal laboratory conditions, despite the loss of 80-90% of norepinephrine (NE)-containing nerve terminals in brain. One possible explanation is that following 6HDA, the noradrenergic cells and their residual terminals are able to provide a near-normal output of transmitter thereby compensating for the lesion. We have examined this hypothesis in two ways. First, NE turnover was assessed by measuring levels of the NE metabolite, 3-methoxy-4-hydroxyphenylglycol (MHPG), in a terminal-rich area, hippocampus, using HPLC with electrochemical detection (see Acheson and Keller, this volume). Second, the NE cells of the locus coeruleus (LC), which are not damaged by 6HDA, were identified using standard electrophysiological and pharmacological criteria, and their firing rate was measured under chloral hydrate anesthesia (400 mg/kg, i.p.). Thirty-six hr after administration of 6HDA to adult, male Sprague-Dawley rats, hippocampal NE content had decreased to 10% of control. In contrast, MHPG levels were virtually unaffected, resulting in a 10-fold increase in the ratio of MHPG to NE, a possible index of NE turnover in residual terminals. Consistent with this, firing rate in LC cells had increased 4-fold. These effects persisted for 21-28 days post-lesion.

	Firing Rate in LC (Hz)	Hippocampal NE ug/g	%	Hippocampal MHPG ng/g	%	MHPG/NE
SHAM	2.7±0.3	0.49 ±.04	100	91.0 ±2.0	100	1.0
6HDA						
36 hr	13.5±2.0	0.043±.002	10	86.6±14.0	95	9.5
21-28 d	11.6±1.6	0.047±.008	9	70.4 ±4.4	78	8.7

Mean ± S.E.M. of 4-10 animals.

NE released from recurrent collaterals exerts an inhibitory influence on LC firing rate. To determine whether increased firing of LC cells in lesioned animals was accompanied by subsensitivity of cells to NE, we measured the inhibitory effect of iontophoretically applied NE (0.1 M, pH 4.0, 5-40 nA). At both 36 hr and 21-28 days post-lesion, the effect of NE on LC firing was markedly reduced, as evidenced by a 4-fold shift in the dose-response curve. These changes may underlie an adaptive increase in NE release from residual terminals, thus leading to a functional reinnervation of target cells.

150.1 CORRELATION BETWEEN CYTOCHROME OXIDASE ACTIVITY AND NEURONAL ACTIVITY IN THE HIPPOCAMPUS. G. H. Kageyama* and M. Wong-Riley (SPON: R. Harris). Dept. of Anat., U. of Calif. San Fran. CA94143

Studies on normal and physiologically altered neuronal systems have indicated that there is a close relationship between physiological activity, glucose utilization and oxidative metabolism in the brain (Reivich et al.,'75; Wong-Riley,'79). If such a relationship exists in individual neurons, one would expect that cells with a higher level of spontaneous or synaptic activity would have a higher level of oxidative enzymes as well. In the hippocampal formation, several distinct cell types are present with known levels of spontaneous activity (Vinogradova,'75; Schwartzkroin and Mathers,'78). This study was aimed at determining whether there is any regional, cellular and subcellular differences in the level of cytochrome oxidase (C.O.) staining that could be correlated with the levels of neuronal activity there.

Hippocampal sections from bat, mouse, rat, woodrat, gerbil, cat and squirrel monkey were reacted for C.O. histochemistry. Four patterns of enzymatic distribution were discerned in all species. (1) Layers: stratum moleculare/lacunosum of the hippocampus and the outer molecular layer of the dentate were the most reactive layers, while stratum oriens was moderately reactive and stratum radiatum was the least reactive. (2) Regional: CA3 was the most reactive region followed by CA1 and the dentate. (3) Cellular: presumed short-axon and basket interneurons (resembling in shape and distribution GAD-positive cells described by Ribak et al.'78) had the highest C.O. activity in their somata and varicose dendrites. These cells also have the highest spontaneous activity in the hippocampus. CA1 pyramidal cells were more reactive than granule cells but less reactive than CA3 pyramidal cells, again correlated with reported levels of spontaneous activity.(4) Subcellular: the most darkly-reactive mitochondria were found in the somata, dendrites and axon terminals of presumed interneurons. Moderate to highly reactive mitochondria were found in high concentrations in the distal apical dendrites (region of entorhinal input) of granule and pyramidal cells, while much lower concentrations of reactive mitochondria were found in the proximal apical dendrites of the same cells. CA3 pyramidal cells had more reactive mitochondria than CA1 cells, while granule cell bodies, axons and axon terminals generally were nonreactive.

These findings are consistent with our hypothesis that neurons with higher levels of spontaneous and synaptic activity (e.g. CA3 pyramidal cells and presumed GABAergic neurons) also have higher levels of C.O. activity. Moreover, the distribution of C.O. activity can vary on a laminar, regional, cellular and subcellular level, presumably reflecting differential or localized concentrations of synaptic or metabolic activity.

150.2 MAKING MEMORIES OF REWARDS IN THE ENVIRONMENT METABOLIC: RADIAL MAZE LEARNING ALTERS PYRUVATE DEHYDROGENASE IN VIVO PHOSPHORYLATION. S. Caine*, R. Akers*, I. J. Collier*, and A. Routtenberg Cresap Neuroscience Laboratory, Northwestern University, Evanston, Ill. 60201.

Recent evidence from our laboratory indicates that brain phosphorylation studied in vitro is influenced by the behavioral state of the animal (Routtenberg, A., Prog. Neurobiology, 12:85-113, 1979). This is particularly true for band F-2 (MW~41K) which is altered by an aversive experience (Routtenberg, A.and Benson, G., Behav. Neural Biol., 29:168-175, 1980). We have identified band F-2 as the alpha-subunit of pyruvate dehydrogenase (PDH; Morgan, D. G. and Routtenberg, A., Biochem. Biophys. Res. Comm., 95:569-576, NO. 2, 1980). Moreover, PDH activity, which is inversely related to PDH phosphorylation, is elevated following aversive experience.

We now report the effects on PDH phosphorylation of an appetitive task where animals have eight chances to find food in eight arms of a radial maze environment. The three groups of animals compared were a food-deprived control group, a group sacrificed immediately following completion of the task and a group sacrificed 24 hrs after completion of the task. Band F-2 phosphorylation in the dorsal hippocampus was significantly reduced ($p<.05$) in homogenate tissue from animals sacrificed immediately following the task. Band F-2 phosphorylation in a crude synaptosomal preparation (P-2) from the same tissue was also reduced. No differences in band F-2 phosphorylation were observed in neostriatal homogenate tissue.

These results indicate that PDH phosphorylation in specific brain locations can be altered by behavioral manipulations which are known to require the participation of these specific structures. In addition, these results may be taken to suggest that memory formation processes involve regulation of key intermediary metabolic enzymes, perhaps at specific synaptic junctions. This metabolic mechanism is not simply based on increased energy need as both increments and decrements have been observed following learning. Rather, PDH activity may be altered in relation to the type of task and brain location studied. Supported by N.I.M.H. 25281 to A. R.

150.3 HIPPOCAMPAL RECURRENT INHIBITION: DECREASED PYRAMIDAL CELL FIRING WITH INCREASED METABOLISM IN THE PYRAMIDAL CELL LAYER DEMONSTRATED BY THE 2-DEOXYGLUCOSE AUTORADIOGRAPHIC TECHNIQUE. Robert F. Ackermann, David M. Finch, Thomas L. Babb, and Jerome Engel, Jr. Reed Neurological Research Center, and Brain Research Institute, UCLA, Los Angeles, CA 90024.

Postsynaptic inhibition is often invoked to account for subnormal brain metabolism as measured by the Sokoloff 2-deoxyglucose (2DG) method. However, IPSPs are themselves a product of neuronal firing and synaptic processes, both of which require energy to sustain. The present experiments were performed in order to provide more direct information concerning the metabolic demands of neuronal inhibition. Electrical stimulation of the fornix antidromically activates hippocampal pyramidal cells and initiates a profound short-latency, long-lasting recurrent postsynaptic inhibition of pyramidal cell firing. The inhibition is mediated by increased firing of internuncial basket cells. In the present experiments, anesthetized rats were stimulated in the fornix for 60 min under 14C-2DG administration. Multiple unit activity was recorded bilaterally from the CA3 region of the hippocampus throughout the experiment. In 7 of 8 animals the expected recurrent inhibition of CA3 pyramidal cells was accompanied by a distinct increase of hippocampal 2DG uptake; increased uptake was particularly apparent in the pyramidal cell body layer, which is the locus of the dense basket-cell plexus of inhibitory synaptic terminals. Both inhibition and increased 2DG uptake were bilateral, but greater ipsilateral to the stimulating electrode. In 3 of the 7 animals, the hippocampal commissures and the fornix were cut several weeks prior to the 2DG experiments to allow afferent fibers to degenerate; excitatory influences from the septum and contralateral hippocampus were thus removed. Inhibition and increased 2DG uptake in these 3 animals were the same as in intact animals, except that they were restricted to the ipsilateral hippocampus. The single animal in which 2DG uptake did not accompany stimulation had its 'fornix' electrode misplaced caudally to the anterior hippocampus; stimulation in this animal produced only short-duration inhibition and no unusual 2DG uptake. These results indicate that hippocampal postsynaptic inhibition, when it is powerfully evoked as in the fornix stimulation paradigm, requires more metabolic energy than it saves. Although it is possible that regions of hypometabolism observed in other paradigms do in fact represent active inhibition, other mechanisms such as disfacilitation must also be considered.

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150.4 PERFORANT PATH AFFERENTS TO THE HIPPOCAMPUS: HIPPOCAMPAL PYRAMIDAL CELL FIRING AND REGIONAL METABOLISM DEMONSTRATED BY THE 2-DEOXYGLUCOSE AUTORADIOGRAPHIC TECHNIQUE. David M. Finch, Robert F. Ackermann, Thomas L. Babb, and Jerome Engel, Jr. Reed Neurological Research Center, and Brain Research Institute, UCLA, Los Angeles, CA 90024.

Experiments were performed to determine the distribution of glucose utilization within the hippocampus during repetitive activation of an excitatory input to the hippocampus, the perforant path (PP). PP axons from the entorhinal cortex via the angular bundle excite dendrites of granule and pyramidal cells; the terminal zone appears as a distinct band (the PP band) in 2-deoxyglucose (2DG) autoradiographs. Anesthetized rats were stimulated in the entorhinal cortex or angular bundle for 60 min under 14C-2DG administration. Frequency of stimulation was varied from 2-9/sec in different experiments. Multiple unit activity was recorded bilaterally from the CA3 region of the hippocampus. Three of the animals had received prior sections of the fornix and hippocampal commissures so that experimental effects could be restricted to the ipsilateral side. In two control animals in which electrodes were implanted but not stimulated, no asymmetries in the PP band 2DG uptake were seen. Entorhinal stimulation evoked a brief burst of action potentials followed by recurrent inhibition lasting from 33 msec to more than 150 msec. The results were frequency-dependent. At higher frequencies (7-9/sec) the 2DG autoradiographs showed, in 4 out of 6 cases, evidence of both PP excitation and intrinsic inhibition. Increased 2DG uptake in the PP band reflected the excitatory input, and increased uptake in the pyramidal cell body layer reflected the recurrent inhibition (see Ackermann et al., 1981, this volume). One case with mild epileptiform activity showed similar but greater 2DG uptake. By contrast, low frequency stimulation (2-4/sec) decreased 2DG uptake in ipsilateral hippocampus in 4 out of 4 animals; this decrease was most evident in the PP band. The reduced 2DG uptake in the ipsilateral hippocampus could be due to: 1) reduced net PP excitation of hippocampal pyramids (disfacilitation) secondary to recurrent inhibition in the entorhinal cortex and 2) entrainment by low frequency stimulation of hippocampal pyramidal cells and inhibitory interneurons to lower than baseline net firing rates.

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150.5 METABOLIC CHANGES AND IRREVERSIBLE LOSS OF SYNAPTIC TRANSMISSION DUE TO SEVERE HYPOXIA IN THE RAT HIPPOCAMPAL SLICE. Ira S. Kass and Peter Lipton. Dept. of Physiology, Sch. Med., Univ. of Wisconsin, Madison, WI 53706.

Hippocampal slices from adult (110-150 day old) and young (30-40 day old) rats were subjected to severe hypoxia for 10 minutes and allowed to recover for one hour. The perforant path was stimulated once every 15 seconds and the evoked population spike was recorded from the dentate granule cell layer.

The population spike from adult slices recovers to only 10% of control values one hour after the 10 minutes of hypoxia. ATP declines from (all metabolites expressed as nM/mg protein) 13.9 to 3.6 during the hypoxia and then recovers to 9.7 one hour later. Thus while ATP recovered, it did not reach its prehypoxic level.

Previous studies by Whittingham and Lipton (Neurosci Abst. 6, 1980) showed that adding 25 mM creatine to the medium bathing guinea pig hippocampal slices protects against ATP loss during acute hypoxia. We preincubated hippocampal slices from adult rats in buffer containing 25 mM Cr for 2 hours. When these slices were exposed to 10 minutes of hypoxia and allowed to recover for one hour the population spike recovered to 80% of its control value. ATP dropped from 14.6 to 7.9 during the hypoxic period and recovered to 11.9 one hour later. Thus the preincubation with Cr protected the fall in ATP during hypoxia and allowed greater recovery of the ATP. Using the creatine kinase equilibrium reaction to determine intracellular pH we found that the pH fell to the same level during hypoxia in the Cr treated and the normal slices. These results indicate that it is the level to which ATP falls during hypoxia and/or the level it attains during recovery that determines the extent of irreversible electrophysiological damage in the hippocampal slice.

Hippocampal slices from young rats showed a much better electrophysiolgocal recovery from hypoxia than did the slices from adult rats. However, both ATP and pH fell to the same level during hypoxia. ATP levels following recovery from hypoxia reached a higher level in the slices from young rats than in those from the adults (13.2 vs. 9.7).

Thus, adult hippocampal slices show irreversible loss of function after 10 minutes of severe hypoxia; they can be protected against this loss by a treatment that maintains ATP levels. This suggests that the fall in ATP is at least partially responsible for the irreversible damage. However, young rats show as great a decrease in ATP and pH as the adult animals yet they show much better electrophysiological recovery. This indicates that young animals are protected from irreversible damage in spite of the fall in high energy metabolites. (Supp. by NINCDS)

150.6 CHANGES IN THE ENERGY PROFILE AND ELECTRICAL RESPONSE OF HIPPOCAMPAL SLICES DURING DECAPITATION ISCHEMIA AND RECOVERY IN VITRO. T.S. Whittingham, W.D. Lust, H. Arai*, A.B. Wheaton* and J.V. Passonneau. Lab. of Neurochem. NINCDS, NIH, Bethesda, MD 20205

The neurophysiological characteristics of the hippocampal (H) slice have been extensively studied. However, the biochemical status of the slice under similar conditions is less well defined. In this study, the levels of the adenylates and P-creatine (P-cr) were measured in guinea pig H slices during either 7 or 15 min of decapitation induced ischemia and during a 30 min recovery superfusion in Krebs-Ringer Bicarbonate medium containing glucose and oxgyen. In another series, the slices were exposed to a second 7 min period of ischemia in vitro and then allowed to recover. The trans-synaptic evoked activity was monitored from the perforant path-dentate granule cell region of one slice to assess function. The P-cr levels were essentially depleted by 2 min of ischemia, while the fall in ATP was biphasic; the half-times were 1.7 and 7.6 min for the fast and slow phases, respectively. The AMP concentrations increased to a peak at 7 min of ischemia and then decreased thereafter. A similar response occurred during the second period of ischemia. Total AXP (ATP + ADP + AMP) decreased during the ischemic period to 40% of the 2 min value (30 nmole/mg prot). The AXP levels were maintained during the second ischemic insult. During the recovery period, the metabolites reached a similar steady-state concentration in all ischemic groups. P-cr and ATP increased to about 20 and 9 nmole/mg prot., respectively and AMP decreased to 2 nmole/mg prot. within 30 min of reperfusion. The recovery values for AXP in all groups was approximately 14 nmole/mg prot. Following the changes in AXP during the initial decapitation-induced ischemic insult, the levels remained unchanged during recovery and any subsequent ischemic periods. The depressed energy charge (ATP + 0.5 ADP/AXP) during ischemia was partially restored within 30 min of recovery. The evoked EPSP returned within 2 to 9 min of recovery in the 7 min ischemic group, while the response was delayed or absent in the 15 min ischemic group. Thus, the original ischemia induced by decapitation irreversibly alters the metabolic profile of the in vitro hippocampus, even during recovery. Subseqent ischemic insults in vitro produced a transient change in the metabolites which were reversible to the new steady-state. In spite of the uniformity of the biochemical responses, there were significant differences between the return of the electrical response in the 7 and 15 min ischemic groups.

150.7 METABOLIC ALTERATIONS ASSOCIATED WITH ISCHEMIA AND RECOVERY IN THE HIPPOCAMPAL SLICE. W.D. Lust, T.S. Whittingham, H. Arai*, A.B. Wheaton* and J.V. Passonneau. NINCDS, NIH, Bethesda, MD 20205

The hippocampal slice has been used extensively as a model for the investigation of a variety of neural functions. However, there is little or no information about the metabolic status of the hippocampus in vitro. Therefore, the concentrations of glucose, lactate, creatine, GABA and cyclic AMP were measured in slices both during and for 35 min following an ischemic insult. Since all hippocampal slices are subjected to varying periods of decapitation-induced ischemia, the effect of ischemia on hippocampal metabolism was examined in three different groups. The first two groups represent those slices deprived of oxygen and glucose for either 7 or 15 min following decapitation. At the appropriate times, the slices were perfused with oxygenated Krebs-Ringer Bicarbonate containing 4 mM glucose (pH 7.45, 37°) for 35 min. The third group included slices that were exposed to an additional "second ischemia" in vitro by perfusing the slices with a medium devoid of glucose and equilibrated with 95%N_2-5%CO_2. The intracellular pH was calculated from the creatine kinase equilibrium. The glucose levels decreased to a minimum within 2 min of decapitation and within 5 min following the onset of the second ischemia. The lactate concentrations increased rapidly to a peak (125 nmole/mg prot) at 7 min in the decapitation groups and decreased to 40 nmole/mg prot during 30 min of recovery. In contrast, the lactate levels remained unchanged in the slices undergoing a second ischemia. In all three groups, the intracellular pH decreased to about 6.5 during the first five minutes of ischemia. Upon the restoration of oxygen and glucose, the intracellular pH rapidly increased to 7.2 and then gradually increased to 7.5 at 15 min of recovery. The creatine concentration increased to a maximum (90 nmoles/mg prot) at 2 min following decapitation and then gradually decreased (half-time = 30 min) during the ensuing 25 min of ischemia and recovery. The initial rise in creatine reflects the rapid loss of P-creatine during this period, whereas the creatine decrease represents a net loss from the tissue. The GABA levels were essentially unchanged during ischemia, but increased approximately 50% during the first 15 min of recovery and then remained constant. While the concentrations of cyclic AMP did not vary substantially from 2 to 15 min of ischemia, there were large increases of up to 1000 pmole/mg prot during the early stages of recovery. Further, the magnitude of the response increased with longer periods of ischemia. While the changes in the metabolites reported here suggest that the metabolic profile of the hippocampal slice is quite distinct from the hippocampus in vivo, these marked changes are compatible with neural function.

151.1 DENDRITIC AND AXONAL MORPHOLOGY IN THE DORSAL LATERAL GENICULATE NUCLEUS OF MACAQUE MONKEYS. J.A. Robson. Dept. of Anatomy, Upstate Med. Center, Syracuse, NY 13210.

In the dorsal lateral geniculate nucleus of primates distinct functional cell types are segregated into the parvocellular laminae (3-6) and magnocellular laminae (1-2). In the present study these different laminar regions are examined for their dendritic and axonal morphology. Dendrites were revealed by Golgi impregnation and retino-geniculate axons were filled with horseradish peroxidase. The results of the Golgi studies are similar to those of Wilson and Hendrickson (1981) and indicate that the main distinctions between neurons in the magnocellular and parvocellular laminae are the size of their somata and the distribution of their dendrites. That is, neurons contributing dendrites to more than one lamina are mostly in the magnocellular laminae. There is tremendous variation in dendritic patterns throughout the nucleus and, with one exception (see below), separate classes cannot be defined. Dendrites frequently enter the interlaminar zones and in some cases are confined predominately to these zones. The one clearly distinct cell type that is particularly striking in our material is found in all laminae. These neurons have small to medium sized somata, few primary dendrites (usually 2-4), and correspond to the type B cell of Wilson and Hendrickson. The striking feature of these cells is the complexity of their dendritic endings which usually give rise to many short branches and appendages. In most respects these dendrites resemble axons in their branching patterns and terminal morphology. Retino-geniculate axons in macaque monkeys are also similar in the magnocellular and parvocellular laminae. Large diameter axons (>4μm) are primarily in the magnocellular laminae but they are quite rare. Most retino-geniculate axons are of medium diameter (1.5-2.5μm) and give rise to numerous branches. Compared with our findings in the cat (Mason and Robson, 1979) retino-geniculate axons in macaque monkeys give rise to more fine diameter, beaded branches and more terminals in the interlaminar zones. Retinal innervation of the interlaminar zones is particularly common adjacent to magnocellular laminae in the 1-2 and 2-3 zones. However, terminals can also be found in the zones between parvocellular laminae. Compared with the cat terminal swellings on macaque retino-geniculate axons show less variation in size and shape. Very few have the large crenulated appearance indicative of glomerular synaptic relationships in cats. Correspondingly, electron microscopic studies of HRP-filled axons indicate that complex glomerular synaptic arrangements are relatively rare in macaque monkeys. (Supported by the Research Foundation of SUNY and USPHS Grants EY03490, NS14283).

151.2 TWO TYPES OF PRESYNAPTIC DENDRITES IN THE DORSAL LATERAL GENICULATE NUCLEUS (LGNd) OF MONKEYS. Tauba Pasik, Pedro Pasik and József Hámori. Dept. Neurol., Mount Sinai Sch. Med., CUNY, New York, N.Y. 10029, and 1st Dept. Anat., Semmelweis Univ. Med. Sch., 1450 Budapest, Hungary.

The LGNd of four normal monkeys (M. mulatta) and of two other such animals with total ablation of the visual cortices (4-6 days survival) were examined in serial thin sections with the electron microscope. As in previous demonstrations, the neuropil contains at least two types of dendritic profiles and four types of axonal endings. The dendrites belonging to projective or principal neurons (P-cells) are exclusively postsynaptic to various vesicle-containing profiles, and exhibit large, dark mitochondria; smooth endoplasmic cisterns which appear in cross-sections as vesicles exceeding 100 nm in diameter; and non-synaptic attachment plaques with retinal terminals and other P-cell dendrites. The dendrites of interneurons (I-cells) show small dense mitochondria, and also small pleomorphic or ovoid synaptic vesicles. These profiles, designated as presynaptic dendrites, have both postsynaptic and presynaptic sites. The latter synapse is of the symmetric type. The axon terminals of retinal and cortical origin contain spheroid vesicles, whereas those from I-cell axons and other endings of extrinsic nature are pleomorphic or flat respectively.

In the present study we have observed a new component of the LGNd neuropil. It has all the above mentioned cytological characteristics of P-cell dendrites but, in addition, it contains large spheroid synaptic vesicles. This profile can be seen in a postsynaptic position to any of the four types of axon terminals, but it is presynaptic only to I-cell presynaptic dendrites. The latter synaptic contact is always of the asymmetric type and, occasionally, a reciprocal synapse is formed between the two profiles. The novel elements are seen more frequently in the specimens with cortical ablations although their number is still very much lower than that of the other classic components of the neuropil. Measurements made on 80,000X electron micrographs of at least 300 spheroid vesicles contained in retinal terminals, cortical endings and the new profile hereby described, resulted in mean diameters of 38.6 nm, 33.3 nm and 44.3 nm respectively. The differences between the means are statistically significant.

Although the profile with large dense mitochondria and large spheroid vesicles may represent a dendrite of a different I-cell type, or a recurrent axon collateral of a P-cell, it appears more probable that it is a presynaptic dendrite of a P-cell. The infrequent but consistent occurrence of these elements suggests that at least some P-cells have the potential to develop presynaptic sites on their dendrites, a property which contributes to the synaptic complexity of the LGNd.

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151.3 IDENTIFICATION OF RETINAL SYNAPSE TYPES IN THE CAT DORSAL LATERAL GENICULATE NUCLEUS USING QUANTITATIVE ELECTRON MICROSCOPY. R. Ranney Mize, Department of Anatomy, University of Tennessee Center for the Health Sciences, Memphis, TN 38163.

Three physiological classes of retinal input have been identified in the dorsal lateral geniculate nucleus (dLGN). We have studied the retinal synapses of the cat dLGN quantitatively to determine whether different types can be distinguished morphologically. Retinal synapses were identified by their characteristic pale mitochondria and were systematically sampled at 4 rostral-caudal planes through the dLGN. The laminar position of each terminal was measured using a computerized e.m. plotter. The cross-sectional area, perimeter, number of presynaptic dendrite contacts, and total synapse contact density were measured using a computer-based digitizer.

Retinal glomerular synapses were easily identified as central terminals with scalloped contours. They were virtually surrounded by dendritic and axonal elements, some of which contained flattened vesicles. The glomerular synapses were medium-sized (mean= 3.35 um^2) and had high contact densities (mean= 0.24 per um surface area) and a high percentage of presynaptic dendrite contacts (35%). They were numerous within the rostral dLGN, accounting for 46% of all pale mitochondria terminals. They were found in fewer numbers more caudally, comprising only 23% in the most caudal dLGN. They were found throughout laminae A and A_1, but were almost never encountered within the C laminae.

The remaining population of pale mitochondria terminals fell into two peak size distributions. The first group had a peak cross-sectional area of 0.80 um^2. These terminals had a low contact density. They were most densely distributed within the C laminae. Their size range resembled that of small retinal terminals located in the upper superficial gray layer of the cat superior colliculus.

A second group had a peak cross-sectional area of 3.8 um^2. They also had a low contact density. They were commonly found within both laminae A and A_1, but were seen infrequently within the C laminae. Many had a bulbous shape, similar to large retinal terminals in the deep superficial gray layer of the cat colliculus.

Although there is substantial overlap in size, our analysis suggests there are at least three separate populations of retinal synapse in the cat dLGN. One class is easily distinguished by its glomerular organization, elaborate synaptic relationships, and laminar location. The other two types have simpler synaptic arrangements and differ dramatically in size and laminar position (Supported by NIH Grant EY-02973-02).

151.4 FINE STRUCTURE AND SYNAPTIC ORGANIZATION OF CAT PERIGENICULATE NUCLEUS. Linda S. Ide. Dept. of Pharmacol. and Physiol. Sci., The University of Chicago, Chicago, IL 60637.

The perigeniculate nucleus (PGN) is a layer of scattered cells apposed to the dorsal surface of the dorsal lateral geniculate nucleus (dLGN). Based mainly on electrophysiological data, it has been suggested (e.g., Dubin & Cleland, 1977) that these cells mediate a recurrent inhibition of dLGN relay cells. Most PGN cells are multipolar or fusiform cells with perikaryal diameters of 15-35μm. They have postsynaptic spines on both proximal and distal dendritic segments, and on their perikarya (in contrast to cells of dLGN, which lack somatic spines).

The majority of synaptic terminals in PGN fit into five major classes: 1) RSD terminals. These are small profiles, densely packed with uniform round vesicles, that make asymmetric synaptic contacts, mainly onto small and medium-diameter dendritic profiles. These terminals resemble terminals in other thalamic nuclei identified as having a cortical origin. 2) RLD terminals. These are frequently large (up to 2.5 x 6μm), contain large round vesicles, clusters of dark mitochondria, a few profiles of smooth ER, and occasionally, bundles of neurofilaments. Individual terminals make multiple asymmetric synaptic contacts onto somatic or dendritic spines, and in some cases also onto adjoining portions of the perikaryal or dendritic surface. A floccular dense material is found in expanded extracellular spaces next to many of these terminals. 3) FD1 terminals. These contain small ovoid and flat vesicles and make symmetric synaptic contacts onto dendrites, dendritic spines, somatic spines, and rarely, the perikaryal surface. They resemble the F1 profiles in dLGN. 4) FD2 terminals. These are pale profiles which contain small numbers of predominantly ovoid vesicles, and in which ribosomes occur occasionally. They are postsynaptic to other terminals, most commonly to RLDs, and presynaptic (at symmetric contacts) to dendrites and spines. They resemble the F2 profiles (P boutons) in dLGN. 5) FP terminals. These contain large polymorphic vesicles, pale mitochondria and scattered glycogen granules. They make symmetric synaptic contacts onto dendritic shafts and spines, and perikaryal surfaces and spines. In addition to these major classes of terminals, a few profiles resembling retinal terminals in dLGN (RLP terminals) occur in PGN.

Small numbers of PGN-like RLD and FP profiles occur in dLGN (cf. Famiglietti & Peters, 1972). Further, we have found a few cells in dLGN that have spines on their perikarya and receive synaptic contacts from such terminals. These rare dLGN cells bear a striking resemblance to cells in PGN.

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151.5 BRAINSTEM PROJECTIONS TO THE LATERAL GENICULATE NUCLEUS IN THE CAT. H. C. Hughes, Dept. of Psychology, Dartmouth College, Hanover, N.H. 03755.

Ascending connections of structures within the brainstem to the lateral geniculate nucleus (LGN) have been considered by many to be the anatomical substrate for a variety of non-visual influences on visual processing (for example, corollary discharge prior to saccades and the effects of attention). In order to reveal these projections in their totality, 3 cats were prepared with injections of horse-radish peroxidase into the laminated portion of the LGN. The injections (0.05-0.1μL) were delivered hydrolically via a glass micropipette which also served as a recording electrode. Placement of the injection sites was guided by electrophysiological criteria. Following a 24 hour post-injection survival period, alternate sections through the thalamus and brainstem were processed with O-Dianisidine using standard procedures.

Comparable results were obtained in all 3 cases. Labeled cells were observed in 1) the nucleus of the optic tract, 2) lamina II1, II2 and to a lesser extent II3 of the superior colliculus, 3) the parabigeminal nucleus, 4) the mesencephalic reticular nucleus and 5) nucleus locus coeruleus and subcoeruleus. The projections from the mesencephalic reticular nucleus, n. locus coeruleus, n. subcoeruleus, and the parabigeminal nucleus were all of bilateral origin, although the ipsilateral projections were dominant.

Scattered labeling was also observed in the periaquaductal grey, nucleus raphe magnus, and in the vicinity of the interpeduncular nucleus and the substania nigre.

The results emphasize the diversity of brainstem-geniculate projections and indicate that these projections convey both visual and non-visual information.

151.6 LATERAL GENICULATE CELL POPULATIONS ARE DIFFERENTLY AFFECTED BY MONOCULAR DEPRIVATION (MD) IN THE CAT. M.L. Schmidt. Dept.of Anat., Sch.of Med., U.of Penn., Phila., Pa 19104

LeVay and Ferster (J.Comp.Neurol.,172(1977)563-584)proposed that X-cells contain cytoplasmic laminated bodies (CLB) and that small and large geniculate neurons lacking CLBs are interneurons and Y-cells respectively.

In the present study neurons in the LGN A-laminae of normal and MD cats were measured from paraffin sections stained with a modified Kluver-Barrera procedure.The results show that medium sized neurons containing CLBs, most of which might be X-cells, hypertrophy in non-deprived laminae but show little or no decrease in size in deprived laminae. Large neurons lacking CLBs, which might be Y-cells, are decreased in size in deprived laminae. These cells also hypertrophy in non-deprived laminae with the exception of the largest ones. The smallest cell population was the same size in normal and deprived cats. The number of small to medium sized nerve cells that do not contain CLBs exceeds previous estimates of interneurons and thus it is concluded that at least some of these cells are relay cells.

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151.7 OBSERVATIONS ON ANTEROGRADE TRANSNEURONAL DEGENERATION IN THE CHICK ECTOMAMILLARY NUCLEUS. J. D. Peduzzi and W. J. Crossland, Department of Anatomy, Wayne State University, School of Medicine, Detroit, Michigan 48201

While making a detailed qualitative and quantitative study of anterograde transneuronal degeneration in the chick ectomamillary nucleus (EMN) following eye removal at hatching, two questions arose: Is the observed neuronal cell death (20-40%) real or do some neurons atrophy to a point where they are misidentified as glia? Second, in regard to the observed neuron atrophy (20% decrease in mean soma cross-sectional area), do all types of neurons atrophy to the same extent?

To answer these questions, 0.6 μl (3mg/10μl) of horseradish peroxidase (HRP) was injected into two of the terminal fields of the EMN (vestibulocerebellum and inferior olive) in three month old chickens enucleated at hatching. In one animal in which over 1/3 of the cells were retrogradely filled with HRP, every labeled EMN neuron in every 15th section was drawn using a camera lucida then counterstained with cresyl violet. Each cell which had been drawn was relocated in the section. Every retrogradely labeled cell was examined to determine whether it was a glial cell or a neuron. Only neurons were retrogradely labeled suggesting that either glia-like neurons are not present or they no longer project to these targets. In some neurons, the dendrites were sufficiently backfilled to determine the type of neuron labeled. Previous Golgi studies have revealed four types of neurons in the EMN. Type I neurons (large, >250 μm^2, multipolar) underwent the greatest shrinkage (47%), while type II neurons (fusiform soma, >150 μm^2, frequently branching dendrites) decreased by 12.3% and type III (fusiform soma, 150-250 μm^2, rarely branching dendrites) by 31.7%. No type IV neurons (small round soma, <150 μm^2, issuing intrinsic axon collaterals) neurons were labeled. Unlabeled Nissl stained neurons below the size ranges for types I, II and III were assumed to be type IV. These neurons were reduced in mean cross-sectional area by 33.1%. Furthermore, if a distribution of soma areas from the unaffected EMN is plotted so that the atrophy of each neuron type is considered, and compared with the affected EMN, there is no massive cell loss from any one category.

We conclude from this data: 1) it is doubtful that atrophic neurons are misidentified as glia, 2) the type IV neurons may not project outside the EMN, 3) all four types of EMN neurons undergo transneuronal atrophy and transneuronal cell loss.

(Supported by NIH grant EY-01796.)

151.8 RELATION OF THE RETINA AND OPTIC TECTUM TO THE LATERAL GENICULATE COMPLEX IN GARTER SNAKES (THAMNOPHIS SIRTALIS). S. du Lac* and D. M. Dacey* (SPON: S. P. Grossman). Dept. Anatomy, Univ. of Chicago, Chicago, IL. 60637.

Both the retina and optic tectum project to several nuclear groups in the dorsal thalamus of snakes, but the precise relationship of tectal and retinal terminal fields within the geniculate complex is unknown. This study used orthograde and retrograde tracing techniques to determine the relation of retina and tectum to cytoarchitecturally defined fields in the thalamus of Thamnophis.

The geniculate comprises a neuropile, a dorsal segment and a ventral segment. The neuropile lies medial to the optic tract and contains scattered cell bodies. The dorsal segment contains densely packed cells and is continuous rostrally with nucleus ovalis. At intermediate levels, it includes a pars dorsomedialis, pars dorsalis and pars ventralis, but only the pars ventralis continues to its caudal pole. The ventral segment is situated ventromedial to the dorsal segment and contains neurons whose dendrites extend ventrolaterally into the retinorecipient neuropile.

Retinal input, as determined autoradiographically, extends contralaterally over the entire neuropile and partes dorsalis and dorsomedialis of the dorsal segment, and ipsilaterally over the dorsal tip of the ovalis cell plate and pars dorsomedialis. Injections of horseradish peroxidase into tectum reveal that it projects to the dorsal and ventral segments of the geniculate complex via two pathways. One pathway travels ipsilaterally via the marginal optic tract and issues a topographically organized projection to the dorsal and ventral segments. The second travels via the tectothalamic tract to terminate bilaterally in the ventral segment and in a cell poor zone medial to the dorsal segment. It consists of diffusely organized, fine caliber terminal collaterals that are not topographically organized. The crossed projection to the dorsal segment is via the habenular commissure; that to the ventral segment is via the ventral supraoptic decussation.

Thus, retinal and tectal projections to the geniculate present a complex pattern in which there is some spatial segregation of retinal and tectal input, but the dendritic fields of geniculate cells are positioned so that these systems might converge on different parts of the same geniculate cells.

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151.9 NUCLEUS ROTUNDUS IN A SNAKE (THAMNOPHIS SIRTALIS). D.M. Dacey* (SPON: W.T. Rainey). Dept. Anatomy, Univ. of Chicago, Chicago, IL.

One major feature of visual pathway organization in reptiles and birds is an ascending tectal projection to the large dorsal thalamic structure, nucleus rotundus (Ro). Previous work has shown that Ro is characterized by, 1) a massive, bilateral input from the tectothalamic tract that is not retinotopically organized, 2) a lack of retinal input, due to the closed nature of its dendritic structure and 3) an ascending projection to a subdivision of the pallial dorsal ventricular ridge (DVR). Surprisingly, this major visual structure has not heretofore been identifiable in snakes. Since the tectum projects heavily to all geniculate subdivisions in snakes it has been suggested that Ro may be incorporated in the massive geniculate complex. However, a well developed nucleus rotundus in Thamnophis is clearly identified with orthograde and retrograde horseradish peroxidase tracing techniques in the present study.

HRP injections into DVR retrogradely fill a large population of cells positioned caudomedial to the geniculate complex and ventrolateral to the thalamic lentiform nucleus. The nucleus is encapsulated by a cell poor zone and can be clearly identified in Nissl material. Solid filled cells vary in size but share a characteristic morphology: they are multipolar with long, sparsely branched dendrites that reach the borders of Ro but do not extend beyond it. Dendrites of cells at the periphery of Ro are directed inward to form a "closed" nucleus. All dendrites are spine free but do bear occasional short protrusions and longer lobulated appendages.

HRP injections into the optic tectum solid filled tectorotundal axons. They join the tectothalamic tract at the lateral and ventral margin of the tectal roof, coursing rostrally to pretectal levels, where they turn medially beneath the caudal pole of the geniculate. Here they form a large, spherical mass of terminal collaterals, filling the nuclear boundaries of Ro. A lighter, crossed input reaches this region via the ventral supraoptic decussation. Solid filled tectal axons do not form restricted terminal arbors in Ro but issue fine diameter, beaded collaterals that distribute diffusely throughout the nucleus.

Using the same techniques a strikingly similar pattern of Ro intrinsic organization has been described in the pond turtle (Rainey,'80, Neurosci. Abst. 6:748). Thus, both connections and intrinsic morphology establish the identity in Thamnophis of Ro as part of an ascending tectal pathway, distinct from the retinogeniculate system. (Supported by PHS grant NS-12518).

151.10 MOTION SENSITIVITY IN TURTLE ANTERIOR DORSAL VENTRICULAR RIDGE. K. R. Dünser*, J.H. Maxwell and A. M. Granda, Institute for Neuroscience, University of Delaware, Newark, DE 19711.

Dorsal ventricular ridge (DVR) is a subcortical structure in the reptilian forebrain receiving visual input from thalamus. We studied the anterior portion (ADVR) in Pseudemys for general functional properties using extracellular, single unit recording techniques. Cells were stimulated and receptive fields were mapped with moving light stimuli projected onto a hemispheric screen centered on the turtle's left eye.

Whereas all cells revealed motion sensitivity,none were found showing clear directional selectivity. Vector analyses of data from cells stimulated by light bars moved in eight directions consistently gave even circular distributions of response strength. Vectors did not exceed 0.24 and were usually less than 0.1 (Max.: 1.0; Min.: 0.0). While there was a general lack of directional selectivity, the majority of cells processed vertical movement differently from any other directional axis when scanned with stimuli distributed across the visible spectrum.

Spectral response plots to equal quantal input revealed mirror-imaged curves for up versus down directions of movement. All other directions tested were undifferentiated in this manner: Opposite directions of movement produced essentially parallel spectral response curves.

A simple scheme might explain this phenomenon: Information from down-selective and up-selective retinal cells is processed into two channels with similar spectral sensitivities. An ADVR cell receives its inputs from one channel (e.g., down-selective information) via excitatory synapses and from the opposite channel (in this example, up-selective information) via inhibitory synapses. Such a mechanism would account for the mirror-imaging seen here for vertical movement. The data suggest that these cells receive non-vertical information via inputs within the same mode; input concerning opposite directions of movement (e.g., left versus right) arrives via synapses that are either only excitatory or only inhibitory.

151.11 THE PRETECTAL COMPLEX OF THE SQUIRREL MONKEY: A REINVESTIGATION OF THE CYTOARCHITECTURE AND RETINAL CONNECTIONS. Hutchins, Bob and Joseph T. Weber, Dept. of Anatomy, Tulane Univ. Med. Sch., New Orleans, La. 70112.

The cytoarchitecture of the pretectal complex of the squirrel monkey was examined in Nissl and fiber stained sections in the coronal, horizontal and sagittal plane. The pretectum of this primate is best seen in horizontal sections and our results are derived primarily from tissue cut in this plane. Five different pretectal subdivisions can be identified on the basis of their nuclear morphology. The general orientation and cytoarchitecture of the pretectal nuclei are similar to that described for non-primate mammals (See Scalia, J.C.N. 145:223-258, 1972). Thus, the nomenclature used to designate the pretectal nuclei in other species can now be applied to the squirrel monkey. According to this standard terminology, the pretectal complex of Saimiri consists of the nucleus of the optic tract, the pretectal olivary nucleus and the medial, anterior and posterior pretectal nuclei.

In Nissl and fiber stained sections, the pretectal olivary nucleus appears laminated. That is, in all planes of section three distinct cellular zones and two cell poor zones are apparent. This particular morphology of the olivary nucleus has never been described for the squirrel monkey.

The pattern of retinal innervation to the pretectum was determined by placing an intraocular injection of ^{3}H-proline into one eye and processing the tissue according to standard autoradiographic techniques. The pattern of transported label is more dense over the contralateral nuclei as compared with the ipsilateral side. In particular, dense transported label overlies the pretectal olivary nucleus and the nucleus of the optic tract with sparse label over the posterior pretectal nucleus, bilaterally. Additionally, sparse label overlies the contralateral medial pretectal nucleus. The pattern of transported label over both the contra- and ipsilateral pretectal olivary nucleus appears laminated in the sagittal and horizontal sections. Moreover, contralateral to the injected eye the silver grains are located primarily over cell poor zones with some sparse label over cellular zones. Ipsilateral to the injected eye the transported label is located primarily over cellular zones with some sparse label over cell poor zones. Such a distribution of label would suggest a segregation of ocular input within the pretectal olivary nucleus.

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151.12 PROJECTIONS OF MEDIAL TERMINAL NUCLEUS OF ACCESSORY OPTIC SYSTEM TO PRETECTUM IN PIGMENTED RAT. Roland A. Giolli, Robert H.I. Blanks and Sang Pham*, Depts. of Anatomy and Surgery, Coll. of Med., Univ. of Calif., Irvine, CA 92717.

Both the medial terminal nucleus (MTN) of the accessory optic system (AOS) and the nucleus of the optic tract (NOT) have been implicated as important structures in the vestibulo-ocular and optokinetic pathways, yet except for a demonstrated NOT-MTN projection, the synaptic interconnections between pretectal nuclei and AOS are poorly understood. In order to examine this problem, small amounts of H^{3}-leucine were injected unilaterally into the MTN of pigmented rats and the tissue processed autoradiographically for light microscopic studies. It was found that the MTN projects by separate pathways upon two pretectal nuclei in a highly specific fashion. One bundle courses with the superior fasciculus of the AOS to terminate densely throughout the ipsilateral dorsal terminal nucleus (DTN) of the AOS. A second, larger bundle runs dorsolaterally through the ipsilateral midbrain tegmentum to terminate as a dense patch within the lateral portion of the NOT and sparse, smaller patches located within the dorsomedial part of this nucleus. The MTN could be shown to project neither to other subdivisions of the pretectal complex nor to the superior colliculus. Control injections into adjacent structures, e.g., substantia nigra (2 cases), subcuneiform nucleus (2 cases) or mammillary nuclei (2 cases), failed to provide evidence for nigral, reticular or hypothalamic afferents to DTN or NOT.

The MTN-NOT projection was also confirmed by using the method of retrograde transport of horseradish peroxidase (HRP). Following HRP injections into the pretectum in 3 rats, a substantial number (clearly 40-50%) of the neurons in the ventral division of the MTN were HRP-filled, whereas only a few of the neurons of the dorsal division were labeled. The present demonstration of a strong, strictly ipsilateral MTN-NOT projection suggests that the MTN must play a significant role in modifying the receptive field properties of NOT neurons.

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151.13 AN ANATOMICAL SUBSTRATE FOR THE PUPILLARY LIGHT REFLEX IN THE PIGEON (COLUMBA LIVIA). Paul D.R. Gamlin, Jonathan T. Erichsen, David H. Cohen and Harvey J. Karten. Dept. of Neurobiology and Behavior, SUNY, Stony Brook, N.Y. 11794.

The Edinger-Westphal nucleus (EW) is the preganglionic source of the parasympathetic pathway controlling pupil diameter. In this study we investigated the visual pathway to EW in the pigeon.

Transneuronal autoradiographic studies of the retinal projections in the pigeon (Streit, Stella & Cuenod, Neurosci., 5:763, 1980) indicate that EW receives a major input from the ipsilateral retina, presumably via a contralateral retinorecipient area.

Horseradish peroxidase (HRP, Boehringer-Mannheim, 50%) was injected into EW. Only one contralateral retinorecipient area exhibited labeled cells: the nucleus Area Pretectalis (AP), a dorsomedial pretectal nucleus located anterolateral to the posterior commissure. These cells are scattered throughout the nucleus and do not appear to be of a single morphological type.

The role of AP in the pupillary light reflex was assessed following its unilateral destruction. Such lesions were found to abolish the pupillary light reflex and resulted in a permanent dilation of the pupil in the contralateral eye.

In order to characterize the distribution and morphology of the retinal ganglion cells projecting to AP, HRP was injected into this nucleus. Preliminary studies indicate a differential distribution of retinal ganglion cells with an apparent absence in the fovea and Red Field. At least half the labeled cells have a soma diameter of 15-25μm and are classified as "giant" ganglion cells. They are at least 500μm apart, and their total adjusted number is estimated to be 500-1000 cells.

Hodological and anatomical considerations strongly suggest that AP may be comparable to the mammalian olivary pretectal nucleus, and thus the avian visual pathway to EW is organized similarly to that reported in mammals (Steiger & Buttner-Ennever, Brain Res., 160:1, 1979). Furthermore, many of the retinal ganglion cells contributing to this pathway in birds are large, presumably with broad dendritic fields. These cells are therefore well suited to detect the changes in whole field illumination which elicit the pupillary light reflex.

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151.14 BILATERAL TECTAL AND PRETECTAL PROJECTIONS TO THE CENTRAL LATERAL NUCLEUS IN THE CAT. J. Graham and N. Berman. Depts. of Anatomy and Biochemistry/Physiology, Medical College of Pennsylvania, Philadelphia, PA 19129.

In order to study the origins of the projections to the central lateral nucleus (CL) from the superior colliculus (SC) and the pretectum (PT), unilateral injections of horseradish peroxidase (0.15-0.20 μl of a saturated solution in 2% dimethyl sulfoxide) were made in the CL in three adult cats. Neurons, containing retrogradely transported horseradish peroxidase, were visualized using various methods: cobalt-diaminobenzidine, Hanker-Yates and tetramethyl benzidine. In the SC, the majority of retrogradely labeled neurons were found in the ipsilateral stratum griseum intermediale, where neurons of all sizes of somata were labeled. In addition, a few labeled neurons were located in the contralateral stratum griseum intermediale and bilaterally in the stratum griseum profundum.

In the PT, labeled neurons were scattered throughout all subdivisions, bilaterally. As in the SC, the majority of labeled neurons were ipsilateral to the injection site. The densest concentrations of labeled neurons were in the medial pretectal nucleus, the nucleus of the posterior commissure and the anterior pretectal nucleus, in both the pars compacta and the pars reticulata. There was no evidence of a topographic organization of the projections from the SC or the PT to the CL.

In summary, the CL receives bilateral projections from both the SC and the PT. The majority of these tectal and pretectal relay neurons are not in the retinal recipient regions, but rather, at least in the SC, in the regions that are associated with eye movements (Stein, B.E., S.J. Goldberg and H.P. Clamann, Brain Res., 118:469-474, 1976). This correlates with the report of Schlag and Schlag-Rey (J. Neurophysiol., 40:156-173, 1977) that CL plays a role in visually initiated eye movements. (Supported by EY 02088).

151.15 CELLS OF ORIGIN OF TECTOFUGAL PATHWAYS; A HORSERADISH PEROXIDASE ANALYSIS. M.F.Huerta and J.K. Harting*, Department of Anatomy, University of Wisconsin, Madison, Wisconsin 53706

We have used the horseradish peroxidase method to analyze the specific cells of origin of various ascending and descending pathways of the cat's superior colliculus. Our preliminary studies have focused upon the connections of the superior colliculus with the following regions: the inferior olivary complex, the pontine reticular formation, the sensory trigeminal complex, the zona incerta, the ventral lateral geniculate nucleus, the dorsal lateral geniculate nucleus, the lateral posterior nucleus and the parafascicular-central lateral complex.

Our most interesting finding is that following HRP injections into many of these areas, retrogradely labeled collicular neurons occur in groups or clumps. Such neuronal groupings are apparent in most of our experiments and occur in the superficial, intermediate, and deep subdivisions of the superior colliculus.

The significance of this clumping of tectofugal neurons may be related to patchy patterns of afferents within the superior colliculus. Thus, in experiments where HRP-WGA was injected into the region of the pons adjacent to and including the nucleus oralis of the spinal trigeminal nucleus, aggregates of collicular (i.e., tectofugal) neurons were intimately associated with puffs of anterogradely labeled trigeminocollicular axons. This suggests a modular arrangement of the afferent and efferent connections between the sensory trigeminal complex and the superior colliculus.

In addition to our finding of neuronal clumping of efferent collicular neurons, our data reveal that cells within the intermediate grey project to extremely wide areas of the neuraxis. At present, we are attempting to correlate the size, the precise laminar position, the topography, and the clumping patterns of collicular neurons with particular axonal distributions.

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151.16 CONNECTIONS OF THE SUPERFICIAL LAMINAE OF THE GROUND SQUIRREL SUPERIOR COLLICULUS. N. Lugo-García and E. Kicliter. Lab. of Neurobiology and Dept. of Anatomy, Univ. of Puerto Rico Sch. of Med., San Juan, P.R. 00936.

The superior colliculus of the ground squirrel (Spermophilus tridecemlineatus) is interconnected with several di- and mesencephalic nuclei known to be involved in vision. Among these are the dorsal and ventral lateral geniculate nuclei and the parabigeminal nucleus. It is not known 1)whether these interconnections are restricted to specific tectal laminae nor 2)is it known whether visual field topology is maintained in these connections. In order to study the first question horseradish peroxidase (HRP) was injected into either deep (three animals) or superficial (three animals) tectal laminae. After survival periods of 48 or 72 hrs. the animals were killed and the locations of HRP were determined histochemically. Only the superficial laminae were found to be interconnected with the visual system nuclei and these connections appeared to be reciprocal. The issue of topology was investigated in the three subjects with superficial HRP injections plus three additional squirrels in which the enzyme was injected into both deep and superficial layers. Visual field topology was found to be maintained by connections between the superior colliculus and the dorsal lateral geniculate and parabigeminal nuclei. Topology between the superior colliculus and lateralis posterior was not so obvious, if present at all. These findings demonstrate that the ground squirrel superior colliculus is interconnected in a very specific fashion with other visual system nuclei. (Supported by PHS grant NS-07464).

151.17 RETINAL PROJECTIONS TO THE HUMAN SUPERIOR COLLICULUS: A PRELIMINARY REPORT. Lois E. Smith*, Alfredo A. Sadun, Walle J.H. Nauta, and Gerald E. Schneider (SPON: S. Wray). Massachusetts Eye and Ear Infirmary, Harvard Medical School, Boston, MA 02114. and Department of Psychology, Massachusetts Institute of Technology, Cambridge, MA 02139.

The superior colliculus (SC) has been shown to be a major primary visual structure in many animals and to play an important role in orienting eye movements. In man, however, a retinal projection has not been described until recently (Smith et al., ARVO 1981).

Previous visual neuroanatomical studies in man were largely limited to gross dissections, since the silver staining of degenerating fibers was technically unsatisfactory in human brain tissue. On the basis of classical techniques, early investigations indicated that in man most retinal ganglion cell fibers project to the lateral geniculate nucleus. A smaller primary projection to the human pretectum has also been proposed to explain the pupillary reflex. These descriptions of the retinal projections in man never included the SC.

We obtained brain specimens from human cases in which prior ocular damage had been documented. Employing a new technique for staining and histologically following degenerating axons (Sadun et al., Soc. Neurosci. 1981), we were able to trace retinofugal fibers.

The retinal projections to the lateral geniculate nucleus and pretectum in man were confirmed. More important, we were able to establish, in man, a retinotectal pathway and to map the pattern of its fiber distribution. Fibers were seen, apparently terminating, in a superficial band just below the surface of the SC. In this stratum, there was a suggestion of alternating clusters and gaps of degeneration. A second layer of larger degenerating axons and terminals was seen in the SC deeper than expected from animal studies. It is not surprising that the SC, shown to be an important visual nucleus in animals, also receives a direct input from the retina in man. However, details of this projection and the histoarchitecture of the superior colliculus have some unusual features requiring further analysis.

151.18 THE LATERAL SPIRIFORM NUCLEUS OF BIRDS HAS AN ENKEPHALINERGIC PROJECTION TO LAYERS 8-13 OF THE AVIAN TECTUM. Anton Reiner, Harvey J. Karten and Nicholas C. Brecha. Dept. of Neurobiology and Behavior, SUNY, Stony Brook, N.Y. 11794.

Using immunohistochemical techniques with antisera directed against either leucine-enkephalin or methionine-enkephalin (generously supplied by K.-J. Chang), four distinct bands of fibers showing enkephalin-like immunoreactivity were seen in the pigeon tectum: a) a thin band of thick fibers and tightly clustered bulbous swellings in layer 3, b) a broader band of fibers with less tightly clustered bulbous swellings in layer 5, c) a broad band of numerous obliquely- and radially-oriented fibers that spanned layers 8-13, and d) a band of sinuous fibers in layer 15. In addition, numerous enkephalinergic cell bodies with radially-ascending processes were seen in layers 8-10.

Since the neurons of the avian lateral spiriform nucleus (SpL) of the pretectum are known to contain enkephalin (Davis et al., Neurosci. Abs., '80) and project to the tectum (Brecha et al., Neurosci. Abs., '76), unilateral electrolytic lesions were made of SpL. In birds with unilateral lesions of SpL, layers 8-13 of the ipsilateral tectum were nearly devoid of enkephalinergic fibers, but no alterations were seen in layers 3,5 and 15. Since no other neurons in the vicinity of SpL are enkephalinergic and project to the tectum, the loss of enkephalin-immunoreactive fibers in the ipsilateral tectal layers 8-13 seems attributable to the destruction of SpL. Although the source of the enkephalinergic fibers in tectal layers 3,5 and 15 is unclear, part of the enkephalin pattern in layers 3 and 5 may derive from the ascending processes of the enkephalinergic neurons of layers 8-10.

The present results thus indicate that SpL has an enkephalinergic projection to layers 8-13 of the ipsilateral tectum. Since SpL receives its major input from the ipsilateral basal ganglia (Karten and Dubbeldam, JCN, '73) and projects to the tectal layers 8-13, the layers of origin of the major tectal efferent projections, the enkephalinergic fibers in layers 8-13 may have some influence upon the motor output functions of the avian tectum. This research was supported by NS 12078 and EY 02164 to H.J.K. and NS 16857 to A.R.

151.19 LAMINAR ORGANIZATION OF PEPTIDE-LIKE IMMUNOREACTIVITY IN THE ANURAN OPTIC TECTUM. Rodrigo O. Kuljis and Harvey J. Karten, Dept. Neurobiology and Behavior, S.U.N.Y. at Stony Brook, New York 11790.

Pedro Ramón described (1890-1946) an orderly laminar pattern of fifteen fibrous and cellular layers within the frog's optic tectum. Most subsequent authors have agreed on the presence of eight deeper strata (layers 1-8) in this animal's optic tectum. Considerable controversy remains, however, regarding Ramón's description of the stratification in the superficial region of this structure (layers 9-15, stratum fibrosum et griseum superficiale), the major zone of retinal termination.

Peptide-like [leucine-enkephalin (LENK), substance P (SP), cholecystokinin octapeptide (CCK8), bombesin (BOM)] and 5-hydroxytryptamine (5HT)- and tyrosine hydroxylase (TOH)-like immunoreactivity were assessed in the optic tectum of 27 specimens of Rana Pipiens by means of peroxidase-antiperoxidase and indirect fluorescent double-labeling methods.

Both 5HT- and TOH-like immunoreactivity disclosed a diffuse plexus of processes throughout the entire tectum. LENK, SP, CCK8 and BOM antisera, however, revealed a specific laminar pattern of distribution characteristic for each of these substances. Within layers 11 to 13 of Ramón, in an internal to external sequence, four independent and non-overlapping bands display SP-, LENK-, BOM- and SP-like reactivity. A CCK8 band partially overlaps the LENK and BOM bands. These specific bands have rather sharp boundaries, unlike Ramón's superficial layers, and seem to be formed in part by the level-specific horizontal expansions of the superficially directed processes of cells located in layers 2,4 and 6 that contain these substances. Since no retinal ganglion cells have been found to contain any of these peptides (Brecha and Karten, unpublished observations), none of these processes seem to be of retinal origin.

The present study shows that exquisite lamination exists in the superficial layers of the optic tectum of the frog. A feature that can be made evident through the use of cytochemical techniques while escaping the capabilities of all other available anatomical methods.

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151.20 DOES THE FROG VISUAL SYSTEM HAVE FREQUENCY CHANNEL ANALYZERS? By Evangelia Tzanakou, Dept. of Electrical Engineering, RUTGERS UNIV. P.O. BOX 909, Piscataway, N.J. 08854

In the mammalian visual system there is evidence about about the existence of frequency channel analyzers.

I tested the same hypothesis in the frog visual system. Last year we presented a new method of analyzing the intensity profiles of visual receptive fields, with a two-dimenstional Fourier transform. There, the power spectrum was calculated and the peak frequencies were used to reconstruct intensity patterns with an inverse Fourier transform.

In this paper I am going to present data recorded from retinal fibers in the frog's visual tectum. In these experiments, the receptive field was mapped and its intensity profile was analyzed with the method described above. The peak frequency pairs from the two-dimensional power spectrum were used to reconstruct patterns which in turn were used as stimuli.

The response of the neuron was recorded for each of the frequency components and their combinations.

Most of the cells recorded show maximal response to the reconstruction of the pattern corresponding to the fundamental frequency components.

The results of this study and their implications will be discussed.

152.1 DEVELOPMENT OF THE FROG CUTANEOUS PECTORIS NERVE AND MUSCLE. D. Card Linden & M. Letinsky UCLA School of Med., Dept. of Physiol., Ahmanson Lab. of Neurobiology, and Jerry Lewis Neuro-Muscular Research Center, Los Angeles, CA. 90024.

The development of the bull frog (Rana catesbeiana) cutaneous pectoris nerve and muscle has been assessed in young tadpoles through adult-aged frogs. We have determined the numbers of motor, sensory and autonomic axons, and the maturity and degree of myelination of these axons at various stages of development using light and electron microscopic techniques. Muscle fiber development was also correlated with the nerves' maturity.

In tadpoles as young as stage XVIII (metamorphosis occurs at stage XXV) the adult number of myelinated motor and sensory axons was present in the cutaneous pectoris nerve, although many axons appeared to be relatively immature and in the process of developing myelin sheaths. The total number of myelinated axons increased only slightly from stage XVIII (24 ± 5) to adult (33 ± 6). In contrast to the myelinated axon development, the number of unmyelinated axons dropped dramatically from 143 ± 38 (stage XVIII) to 80 ± 40 (17 days post metamorphosis and adult). In young animals unmyelinated, catecholamine containing axons are diffusely distributed throughout the muscle. At later stages and in adult animals, the catecholamine containing axons are primarily associated with blood vessels.

The cutaneous pectoris muscle at stage XVIII is quite immature; the number of muscle fibers is less than half that found in the adult (stage XVIII, 280 ± 56; adult, 778 ± 67). Electron microscopic analysis revealed that at stage XVIII about 40% of the muscle cells are undifferentiated and devoid of myofilaments, 8% are undifferentiated, but contain a few myofilaments, and about 53% are myotubes packed with myofilaments. Thus, at early stages of development, the muscle is composed of cells in a variety of stages of differentiation. In addition, the maturity of the muscle fibers on the muscle's lateral border is obviously greater than that of the muscle fibers on the medial border. This regional difference in maturation is also reflected in the neuromuscular contacts, as reported by Morrison-Graham (Soc. for Neurosci. Abstracts 6: 567, 1980), and Letinsky and Morrison-Graham (J. Neurocytol. 9: 321, 1980).

Even at the relatively young developmental stage XVIII, the adult complement of myelinated axons is present, although only half the adult number of muscle fibers is present. Since the nerve appears much more mature than the muscle, this may mean that, as reported by others, the nerve does indeed have an important inductive influence in muscle development.

This research was supported by USPHS grant NS13470.

152.2 DEVELOPMENT OF THE SEGMENTAL PATTERN OF SKIN SENSORY INNERVATION IN THE CHICK HINDLIMB. S.A. Scott, Dept. of Neurobiology and Behavior, SUNY at Stony Brook, Stony Brook, NY 11974.

In adult vertebrates sensory innervation of skin is organized segmentally; axons from each dorsal root ganglion (DRG) innervate skin in a characteristic location, termed a dermatome. Honig (Neurosci. Abst. '79) has shown that from their earliest outgrowth skin afferents from the three most rostral hindlimb DRGs project through the limb via precise pathways. I have confirmed and extended her observations by studying the development of the dermatomes of all eight hindlimb DRGs to determine the precision with which skin sensory innervation per se is established.

The "mature" pattern of dermatomes was mapped in St37-38 embryos, after the peak of cell death, by recording from each dorsal root while stimulating the skin electrically or with a fine bristle. These maps showed that lumbosacral DRGs 1-3 innervate the anterior thigh and knee, DRGs 4-6 the distal shank and foot, and DRGs 7-8 the posterior thigh and shank. The location and amount of overlap of dermatomes mapped in embryos as young as St29, when skin innervation is clearly functional, were essentially similar to the mature pattern. To study younger embryos in which physiological mapping is difficult or impossible each DRG was labeled with horseradish peroxidase in embryos from St26, before cell death, to St32. Reconstructions of axonal pathways within the limb showed that axons from each DRG grow to their dermatomes via characteristic sets of nerves. Axons were rarely found in "aberrant" pathways, even at early stages before they reached the skin. Whole mounts of skin from limbs with a labeled DRG showed that axons first reach the skin at about St27, invading the skin at one or more characteristic spots. The dermatomes then enlarge, adding fine axonal branches. Dermatomes seen morphologically were about the same size, shape and location as those mapped physiologically at each stage.

Together these findings rule out several possible mechanisms by which dermatomes might develop. Axons do not simply grow to the nearest available skin, nor are axons "towed" to their final location by skin movements; moreover dermatomes are not shaped by cell death and elimination of random or excessive axonal projections in the limb or the skin. Rather it appears that skin afferents from each DRG grow directly to their target skin along a defined set of pathways and establish their dermatome precisely at its characteristic location. (Supported by NIH grant NS16067).

152.3 RECIPROCAL CONNECTIONS BETWEEN INDIVIDUAL THALAMIC NUCLEI AND NEOCORTICAL FIELDS IN PERINATAL MICE. J.E. Crandall and V.S. Caviness, Jr. E.K. Shriver Center for Mental Retardation, Inc., Waltham, MA 02154

Thalamocortical axons are known from analyses of orthograde degeneration and Golgi impregnations to reach the subplate of developing neocortex of mice and rats, future layers V and VI, during the third week of gestation. The axons ascend to terminate upon their principal cellular targets, stellate cells of layer IV and pyramidal cells of layer III, only later during the first postnatal week. The present study is an analysis of connections between individual thalamic nuclei and neocortical fields in mice ages embryonic day 17 to postnatal day 1. This interval comes after thalamocortical axons arrive in the subplate but before they invade the suprajacent layers. Horseradish peroxidase (HRP), impregnated in polyacrylamide gels or recrystallized as micropellets, was applied via trochar to limited sectors of various frontal, parietal and occipital neocortical fields. Descending projections were demonstrated by this method only if the injected HRP extended into the subplate and not if it penetrated no deeper than the marginal zone and cortical plate. Within the thalamus, orthogradely labeled fibers were found to be distributed congruently with retrogradely labeled neuronal somata. The reciprocal connections between individual neocortical fields and thalamic nuclei demonstrated in this fashion in developing mice correspond well with those of the adult: parietal fields 3 and 1, 40 & 40a with VB, Po & Pom, respectively; occipital fields 17 & 18b with LGN & L; and frontal fields 4 & 6 with VL (Caviness and Frost, J. Comp. Neurol., 194:335,1980). These observations indicate that reciprocal connections become definitively established between individual neocortical fields and thalamic nuclei at a time in development when thalamocortical axons are confined to the subplate and before they are distributed to their principal target cell classes in layers IV and III.

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152.4 INNERVATION PATTERN OF CHICK WINGS WITH A PROXIMAL-DISTAL DUPLICATION: ELECTROPHYSIOLOGICAL STUDY. C. Muniak* and M. Neset (SPON: ,C. Edwards). Neurobiology Research Center, SUNY Albany, Albany, N.Y. 12222.

Motor nerves are known to form specific connections with the limb muscles during development. In the present study, electrophysiological methods were used to examine the motor innervation pattern of the normal and surgically altered chick wing. Particular attention was paid to which spinal segments innervate the flexor carpi ulnaris (f.c.u.). Donor grafts were added to 4 day old embryos such that the distal part of the operated wing was duplicated along the proximo-distal axis. This was done at day 4, before the limb was innervated. Therefore, the choices of muscles to be innervated by the motor nerves growing into the limb was greater than normal. In normal embryos (day 12 or older), the largest electrical response from the f.c.u. was recorded when spinal root 16 was stimulated. A response approximately one-third as large was obtained when root 15 was stimulated. Stimulation of root 14 gave no response. In the experimental embryos where the host f.c.u.'s were well developed, stimulation of the spinal roots elicited a robust response in the host but only a small or no response in the donor f.c.u. However, in cases where the host f.c.u. was small or absent, the donor f.c.u. responded to spinal root stimulation. When the donor f.c.u. was innervated, it was innervated the same way as the control, that is, predominantly by spinal segment 16. Supported by grants from the Muscular Dystrophy Association and the NIH (NS07681).

152.5 INNERVATION OF CHICK WINGS WITH A PROXIMO-DISTAL DUPLICATION; A MORPHOLOGICAL STUDY. M. Neset and C. Edwards. Neurobiology Research Center, SUNY Albany, Albany, N.Y. 12222

To learn more about the origin of nerve-muscle specificity we have studied the innervation of the flexor carpi ulnaris muscle (f.c.u.) in 12 day embryonic chick wings having limb segments serially duplicated along the proximo-distal axis. Duplications were produced microsurgically by grafting whole day 4 (st. 19/20) wing buds to the stumps of partially truncated host wing buds (st. 23). Innervation is absent at this stage. The embryos were sacrificed at day 12, at which time the adult nerve pattern has been laid down and the main period of cell death is over. The innervation of the grafted limbs was studied in whole mounts and in sections.

The innervation of the f.c.u.-muscle of the graft was found to vary with the extent of development of the f.c.u.-muscle of the host. In limbs in which the host f.c.u.-muscle was lacking, the f.c.u.-muscle of the graft was well innervated. In limbs in which the f.c.u.-muscle of the host was present, the innervation of the muscle of the graft was sparse or absent. The density of innervation, as measured by cross-sectional area of the nerve, seemed to be a function of the relative mass of the host and donor muscle.

The f.c.u.-muscle in operated wings was injected with HRP at 12 days to localize, in the cord, the motor neurons innervating the muscle. The number of labelled cells and the localization in the cord varied with the type of graft. Labelled cells were always present in spinal segment 16, which contributes the main innervation of the normal chick wing, and in segment 15 in some animals. These data are supported by electrophysiological recording. The simplest model to explain the results is to assume that a certain number of nerve fibers are destined to connect with the f.c.u.-muscle.

Supported by the Muscular Dystrophy Association.

152.6 THE CONSTANT NUMBER OF GRANULE CELLS PER UNIT SURFACE AREA OF THE FASCIAE DENTATAE OF THREE DIFFERENT SPECIES. Mark J. West. Institute of Anatomy B, University of Aarhus, 8000 Aarhus C, Denmark.

A comparison of the number of granule cells per unit surface area (isomorphic plane) of the fasciae dentatae of three species (European hedgehog, Insectivora, erinaceus europaeus; Wistar albino rat; DBA-2J mouse) that vary significantly in absolute size and evolutionary development reveals no significant differences in the number of neurons per unit surface area of this archicortical structure in these three species. The number of granule cells was determined from estimates of neuron density in the stratum granulosum (stereological) and from estimates of the volume of the stratum granulosum. The volume and surface area of the stratum granulosum of four to five adults of each species were computed from the volume and area at each level of a series of sections passing through the entire hippocampal region (West and Andersen, Brain Res. Rev. 2: 317-348, 1980). With coefficients of variation of about 15% for species, the number of granule cells per mm^2 of the isomorphic plane was found to be:

mouse	1.25×10^5 cells/mm^2
rat	1.04×10^5 cells/mm^2
hedgehog	1.14×10^5 cells/mm^2.

The similarity between these three area densities and those estimated from the data from different neocortical areas of a number of species, including mouse, rat, monkey and man (Bok, S.T., Histonomy of the Cerebral Cortex, Elsevier, 1959; Rockel, A.J. et al., Brain 103: 221-244, 1980) indicates that the number of neurons per unit surface area of cortex is the same in the neocortex and allocortex of a wide range of mammalian species and that differences in the volumetric density of neurons in the different cortical areas of these animals is proportional to differences in the thickness of the cortex. This common feature of cortical organization facilitates inter- and intra-species comparisons of cortical structure and function.

152.7 RETZIUS-CAJAL CELLS: AN ULTRASTRUCTURAL STUDY IN THE DEVELOPING VISUAL CORTEX OF THE RAT. S.M. Edmunds* and J.G. Parnavelas (SPON: D.C. German). Dept. of Cell Biology, The Univ. of Texas Health Science Center, Dallas, TX 75235.

Retzius-Cajal cells are a unique feature of developing cortical layer I in a variety of mammalian species. Autoradiographic studies have demonstrated that they are the first cells of the cerebral cortex to be generated, and their maturation is extremely precocious. Although these cells have received much attention for nearly a century, their life history remains enigmatic. In this ultrastructural study we sought to investigate the ontogenesis of Retzius-Cajal cells in the visual cortex of a closely spaced time series of rats between 17 days of gestation and adulthood.

Sprague-Dawley albino rats of both sexes were perfused with mixtures of aldehydes, and tissue from the visual cortex was processed for electron microscopy. Serial sections cut in both coronal and tangential orientations were used to identify and study these cells at various stages of development. At 17 days of gestation, Retzius-Cajal cells begin to acquire the characteristic appearance and cytoplasmic organelles by which they are identified in the perinatal period. At birth they are recognized in layer I by their large size, long processes, dark cytoplasmic ground substance and abundance of tightly packed organelles. One feature which is most typical of these cells is the presence in the cytoplasm of numerous wide cisterns of rough endoplasmic reticulum filled with electron-opaque material. Synapses are rarely seen on the perikarya and processes during the first week of postnatal life but become more frequent later in development. A pattern of modifications becomes noticeable in the morphology of these cells during the first postnatal week with the appearance of growth cones and new processes of varying sizes. Furthermore, their cytoplasm slowly acquires a lighter appearance, organelles become more organized, and the size of the cell body and characteristically long processes diminish. The frequency of Retzius-Cajal cells decreases with age and at postnatal day 18 only very few can be recognized with certainty. Careful examination of a large series of sections at the subsequent days revealed that the morphological characteristics of Retzius-Cajal cells eventually resemble and become virtually indistinguishable from classical cortical non-pyramidal neurons.

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152.8 DISPLACED PHOTORECEPTOR CELLS IN THE DEVELOPING RAT RETINA. A.W. Spira, M. Patten*, and R. Hannah*, Lions Sight Centre, Dept. Anat., Faculty of Medicine, Univ. of Calgary, Calgary, Alberta, Canada T2N 1N4.

Differentiation of the neural retina involves a migration and segration of neuroblasts into three layers of perikarya separated by two synaptic zones. Although neuroblast cells normally differentiate only after they reach their definitive layer, maturation may proceed before migration has ceased (e.g. ganglion cells). Several authors have noted that during early postnatal stages in the rat and mouse retina there exists a group of cells located in the inner nuclear layer (INL) whose nuclei resemble photoreceptor (PR) cells in the outer nuclear layer (ONL) (Sanyal and Bal, Z. Anat. Entwickl-Gesch. 142: 219, 1973; Raedler and Sievers, The development of the visual system in the albino rat, Springer-Verlag, 1975; Blanks and Bok, J. Comp. Neur. 174: 317, 1977). These cells have been considered to be either differentiating bipolar cells destined to remain in the INL, or PR cells on the wrong side of the outer synaptic layer (OSL) which subsequently migrate back to the ONL. Our light and electron microscopic study of Sprague Dawley (albino) and Long Evans Hooded (pigmented) rats has supported the view that these cells are the precursors of photoreceptor cells. Their nuclei resembled closely those of the photoreceptor cells in the ONL even as the latter changed their chromatin pattern during differentiation. They were located in the OSL and outer region of the INL during the first two postnatal weeks and scattered through the middle and outer regions of the INL during the third p.n. week. Their numbers decreased with increasing postnatal age. PR-like cells were evident as late as the third postnatal month. The maximum decrease in their number occurred coincident with the peak period of cell death in the INL. Ultrastructural cytoplasmic features attested to their PR cell identity. These included myoid (Golgi complex) and ellipsoid (mitochondrial) regions. Long cilia associated with a basal apparatus extended from the perikarya in a scleral direction. Outer segments were occasionally seen filled with closely packed membranous lamellae. Some cells contained a synaptic ribbon. Some of these displaced cells likely comprised a part of the degenerating population of cells in the INL, containing some characteristics of degenerating neurones. Those cells remaining in the INL in later stages were often found adjacent to capillaries, some extending processes to the basal lamina of endothelial cells. These observations confirm that not only are these cells displaced PR cells but also that they possess a substantial capacity to differentiate in vivo at a considerable distance from the retinal pigmented epithelium.

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152.9 THE REPRESENTATION OF AGE IN THE GOLDFISH OPTIC NERVE. Anne C. Rusoff. Oklahoma State University, Stillwater, OK 74078.

Retinal ganglion cell axons in the goldfish optic nerve are organized near the retina according to two patterns: 1) Axons from dorsal retinal ganglion cells are separated from axons from ventral retinal ganglion cells. Near the retina the x-section of the optic nerve is shaped like a trapezoid. The axons from dorsal cells fill a central band perpendicular to the two parallel sides of the trapezoid. Axons from ventral retinal cells fill the two flanking bands. 2) Axons from annuli of retinal ganglion cells, that is, axons of the same age, are clustered together.

The obvious question is how do these two patterns fit together in the nerve? The cluster of axons from the newest ganglion cells is easily identifiable in 1 um thick x-sections of the nerve because its axons are not yet myelinated. In x-sections of the nerve this cluster of axons is found at the narrow end of the trapezoid where the bands of axons from dorsal and ventral retinal cells come together. This position of the newest axons suggests that age may be plotted in a line across the nerve with the newest axons at one end (the narrow end of the trapezoid) and the oldest axons (from central retinal cells) at the other end (the base of the trapezoid). I have tested this suggestion by tracing axons at different positions in the x-section of the nerve back to their cell bodies in the retina. To accomplish this, the optic nerve was hemisected mid-orbitally, and the cut axons were coated with horseradish peroxidase (HRP). Fish survived 1-2 days; then the fish were killed and the retinas and optic nerves removed. Retinas were prepared as whole mounts and the optic nerves were cut into serial 40 um sections. The tissue was reacted with o-dianisidine and H_2O_2 to reveal the cells and axons filled with HRP. Alternate sections of the optic nerve were embedded in epon and sectioned at 1 um. These sections were used to determine the relationship between the axons filled with HRP-reaction product and the non-myelinated (newest) axons. The retinal whole mounts were traced with a drawing tube and the areas containing cells filled with HRP-reaction product were noted on the tracings. In all cases the pattern of HRP-reaction product in the retina and in the optic nerve was compatible with the suggestion that axons are arranged by age across the diameter of the nerve with the oldest axons (from most central cells) at one side and the newest axons being continually added at the opposite edge of the nerve. Thus one may think of the newest axons as a slab which adds onto one edge of the nerve with new axons from each retinal region in register with slightly older axons from the same retinal region. (Supported by PHS Grant # EY03666.)

152.10 MONOCLONAL ANTIBODIES TO RAT CEREBELLAR ANTIGENS. Richard Hawkes* and Evelyn Niday (SPON: A. Matus). Friedrich Miescher-Institut, P.O.Box 273, CH-4002 Basel, Switzerland.

We are studying the neurogenesis of the rat cerebellum using a panel of monoclonal antibodies developed for this purpose. Starting with a rat cerebellum synaptosomal plasma membrane fraction as antigen, several hundred hybridoma cell lines were derived which secrete antibodies against cerebellar antigens. We routinely characterise the antigens in three ways. Firstly, using immunohistochemistry at both light and electron microscope levels, the cellular distribution of the antigen is determined. Secondly, we identify the specific polypeptide antigens by gel electrophoresis and immunoblotting. Thirdly, we use a solid phase dot immunobinding assay to establish the antibody titer and class and to study the tissue and species distribution of the antigen. This poster describes the results of one experiment, which yielded 176 hybridoma lines secreting anti-cerebellar antibodies of more than 20 different specificities.

152.11 HISTOGENESIS OF THE NEURONS OF THE EXTRA-PYRAMIDAL SYSTEM OF THE RAT BRAIN. R.G. Marchand and L.J. Poirier. Lab. de Neurobiologie, Hôpital de l'Enfant-Jésus, Québec, Canada, G1J 1Z4.

This study provides basic data on the time of origin as well as on the spatio-temporal setting patterns of the neurons of the neostriatum, the globus pallidus, the basal nucleus complex, the entopeduncular nucleus, the subthalamic nucleus, the small parvocellular group of neurons embedded in the fibers of the cerebral peduncle immediately rostral to the substantia nigra, and the substantia nigra. Twelve gestating rats of 10, 11, 12... 21 days and 3 pups, aged 1, 2, 3 days respectively were injected with ^{3}H-thymidine. The brain of 40 and 125 day old pups were studied. The nucleus of every neuron which contained 25 silver grains or more (heavily labeled) was considered to represent a neuron that entered its final mitotic cycle shortly after the injection of ^{3}H-thymidine during gestation. The small parvocellular group of neurons embedded in the cerebral peduncle display heavily labeled nuclei on day 11 and 12. The neurons of the entopeduncular nucleus and globus pallidus are generated from days 11 to 14 and days 11 to 15, respectively. In the pallidum the first neurons to form on day 11 settle caudally and while on day 15 neurogenesis has ceased in this area, it is still intense in the rostral extremity of the pallidum. The neurogenesis of the small to medium sized neurons of the neostriatum extends from day 12 to at least postnatal day 3. Neurogenesis of the neostriatum follows a moderate caudo-rostral gradient. The heavily labeled neurons are located at the caudal extremity of the neostriatum on day 12 and in the rostromedial areas on postnatal days. Three modes of development have been observed in the neostriatum of the rat. In the caudal neostriatum (putamen), at the level of the entopeduncular nucleus, the neurons display an inside-out spatio-temporal gradient in which earlier generated neurons are located along the globus pallidus and later formed neurons along the external capsule. More rostrally the neurons of the neostriatum do not exhibit any clear spatio-temporal gradients in their distribution. However, the first neurons generated for this area on days 13 and 14 settle according to two patterns. Many neurons settle rather densely along the external capsule occupying more rostral sites on day 14. Other neurons settling in the body of the neostriatum are grouped in clusters more or less uniformly distributed on these days. In this area, neurogenesis reaches its peak on days 15 and 16. Large chromophilic neurons of the neostriatum appear exclusively in the earlier period, that is on days 12-14. Similarly, the large neurons of the substantia innominata-basal nucleus complex which are mixed with the neurons of the globus pallidus in the rat also appear on days 12 to 14. The neurons of the subthalamic nucleus and of the substantia nigra are generated on days 13-15 and days 12-15, respectively.

152.12 TRANSPLANTATION OF FETAL RETINA, SUPERIOR COLLICULUS AND LATERAL GENICULATE NUCLEUS TO HOST VISUAL CORTEX. A LIGHT AND ELECTRON MICROSCOPIC ANALYSIS. M. A. Matthews. Dept. Anat., LSU Med. Ctr. New Orleans, LA. 70119.

It is now well established that a variety of CNS tissues, including cerebellum, brainstem, hippocampus and visual neuronal elements can be transplanted to the brain of a host animal. Such transplants have been shown to survive and to establish reciprocal connections with the host. The purpose of our experiments were: 1) to examine neuronal and synaptic development within the transplant; 2) to determine if axonal sprouts from the transplant grow into appropriate targets. Following injection with ^{3}H Thymidine on gestation days 12-15 to label transplant neurons, pregnant female albino rats were laparotomized on gestation days 15-17 and samples of fetal retina, superior colliculus and dorsolateral thalamus containing the lateral geniculate nucleus were removed from the fetuses and transplanted to the visual cortex of neonatal host rats of the same strain.

Analysis of retinal transplants reveals a partial retention of histotypic organization with the appearance of ganglion, bipolar and sensory cells which become arranged in rosettes. Apicolateral junctions are readily identified near the center of a rosette to form a rudimentary limiting membrane. Formation of photoreceptor elements and synapses of the plexiform layers is stunted. Examination of the periphery of the transplant demonstrated concentrations of axons corresponding to the optic fiber layer of the normal retina. Transplants of retina together with superior colliculus were analyzed with silver methods for axons and this procedure reveals numerous fibers arising from the retinal ganglion cells, coursing into the adjacent transplant of superior colliculus and ramifying among these cells. Very few axons arose from the portion of transplanted retina in contact with the cortex. Transplants of thalamic tissue containing the lateral geniculate nucleus exhibit normal neuronal and synaptic development with the notable exception of the encapsulated glomerular complex of the retino-fugal terminal. Such transplants also display projections which enter the host visual cortex and the numbers of such axons appear to increase substantially if axon transport inhibitors are injected into the contralateral eye at the time of transplantation.

These preliminary studies show that some degree of specificity exists in transplanted mammalian CNS and should lead to an important model for further study of plasticity in host-transplant reciprocal projections.

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152.13 HYDROCEPHALUS PRODUCED BY PRENATAL IRRADIATION. C. J. D'Amato and S. P. Hicks. Department of Pathology, University of Michigan Medical Center, Ann Arbor, MI 48109.

Hydrocephalus with greatly enlarged ventricles has been produced in rats by early prenatal irradiation, and some animals have grown to maturity, but the morphogenesis of the condition has not been worked out. Rats pregnant 11 days, fetal stage of about 16 to 24 somite pairs, were exposed to 200 R (250 kvcp x-rays, 60 R/min) and fetuses were removed surgically at intervals afterward for serial histologic sections to study the development of the hydrocephalus in stepwise fashion. Enlargement of the head owing to dilation of the lateral and third ventricles became evident between 18 and 20 days. It corresponded to a gradual narrowing of the lumen of the aqueduct to closure, owing to disorderly overgrowth of the rostral tectum (superior colliculus) and pretectal region. The ependyma of the stenosed aqueduct remained intact. The posterior and superior colliculus commissures, and usually the subcommissural organ, did not form. The pituitary was displaced into the nasal mucosa through the sphenoid bone. Most animals had eye defects. Abnormality of the tectal-pretectal region was first seen about the 15th day when the commissures failed to develop. By 18 days the affected midbrain and pretectal region were dorsoventrally thickened, and the dorsal convexity of the midbrain was flattened. Some animals deviated from this developmental constellation showing severely malformed faces and others showed a dorsal midbrain encephalocoele or malformed forebrain. The initial injury was a destruction of many primitive neural and mesenchymal cells, chiefly G1 and G2, as we have described in detail. By 48 hours regulative regeneration by residual primitive cells had substantially restituted the damage except in the regions noted. Other litters exposed to 150 R on the 11th day showed no hydrocephalus.

This radiation induced development of hydrocephalus resembled that caused by a recessive mutation in which early overgrowth of the commissural fibers and cells in the tectal-pretectal region led to obstruction of the aqueduct around the 19th day (D'Amato, Hicks 1981). The latter period seems to be critical for the development of the proper caliber of the aqueduct. (USPHS NS 10531)

152.14 THE CENTRAL CANAL AREA OF THE IMMATURE RAT: NORMAL DEVELOPMENT AND RADIATION SENSITIVITY. S. A. Gilmore, J. E. Leiting* and T. J. Sims, Depts. of Anatomy and Pathology, Univ. Arkansas Med. Sciences, Little Rock, AR 72205.

The central canal area of the spinal cord in the immature rat differs in appearance from that in the adult. During the early postnatal period, particularly the first two weeks, the cellular arrangement at the dorsal and ventral aspects of the walls of the central canal is markedly different from that present in the mature state. Dorsally, cells extend upward into the median septum giving this portion of the central canal the appearance of having an apex. Ventrally, a cluster of cells extends from the central canal; these cells are closely packed in contrast to neighboring neurons and neuroglia. Cursory observations in previous studies suggest that these cells at the ventral aspect may be extremely sensitive to ionizing radiation. The present study was undertaken to evaluate the effects of x-rays on the central canal area, particularly its ventral aspect, and to characterize the cells ultrastructurally.

A 5-mm length of lumbosacral spinal cord was x-irradiated (4000R) in Charles River CD rats. Groups of rats were perfuse-fixed from 2 hours to 2 days post-irradiation (P-I). The vertebral columns containing the irradiated and the adjacent non-irradiated regions of spinal cord were removed, and interrupted serial sections were prepared for light microscopic observation. Spinal cords from normal 3-day-old rats were prepared for ultrastructural studies.

As early as 2 hours P-I, an occasional cell in the ventral cluster is pyknotic, and by 6 hours pyknosis is a prominent feature of this area. Within 12 hours, karyorrhexis is occurring, and at this time most of the cells remaining in this cluster are either pyknotic or karyorrhectic. Some cells apparently have disappeared because there are clear areas lacking any cells. By 20 to 24 hours, this ventral aspect of the central canal is essentially acellular, containing only an occasional pyknotic cell. A similar situation persists at 2 days P-I. During this 2-day interval, some pyknotic cells occur in the lateral walls and at the dorsum of the canal, but they are much less frequent than at the ventral aspect.

These data indicate that the population of cells at the ventral aspect of the central canal is extremely sensitive to ionizing radiation. Ultrastructural studies are in progress to identify these cells, and other studies are planned to evaluate whether or not this population is reconstituted following irradiation.

(Supported by NIH Grant NS 04761.)

152.15 NEURONAL MORPHOLOGY IN ECTOPIC CORTEX FOLLOWING GESTATIONAL X-IRRADIATION. J.A. Donoso and S. Norton.* Ralph L. Smith Research Center and Pharmacology Department, University of Kansas Medical Center, Kansas City, Ks. 66103.

When dividing cells of the ventricular ependymal layer are exposed to antimitotic agents breakage of the layer occurs. Double breaks in the layer result in rosettes containing dividing matrix cells. Neuroblasts forming in these rosettes from subsequent divisions do not migrate normally to form the cerebral cortical layers, but form cortical ectopias below the corpus callosum. About one third of the total cortical mass developed ectopically. The present study was conducted to describe the ectopic cortex with respect to neuronal cell type, orientation of neurons and synaptic arrangement on the neurons using light and electron microscopy. Rats were irradiated with 125 R on gestational day 15 and examined at 4 weeks and 4 months postnatally. No specific layers were identified in the ectopic cortex. With maturation, the rosettes which are clearly demonstrated at birth tend to fuse. At 4 weeks of age many ectopic pyramidal cells have few spines and short branches with many varicosities, resembling immature pyramidal cells. At 4 months, many of the immature looking cells have disappeared. The total number of spines in the apical dendrite of ectopic cortex pyramidal cells does not change with age and is significantly less than those present in the corresponding irradiated cortex located above the corpus callosum. Electron microscopic examination of ectopic and irradiated cortex as well as control cortex shows axodendritic and axosomatic synapses. The synapses in control cortex and irradiated cortex above or below (ectopic) the corpus callosum are morphologically indistinguishable. Therefore, the pyramidal cells in the ectopic cortex have synaptic contacts which present the structural basis necessary to participate in neuronal interaction with other brain areas. This research was supported by a NIH Grant NS 16694-01 and U.S.P.H.S. Grant HD-02528.

152.16 EMBRYOGENESIS OF THE CORTICAL PLATE: A COMPARATIVE STUDY IN THE THREE REPTILIAN ORDERS. A.M. GOFFINET(SPON: M. David-Remacle). Unité de Neurologie du Développement, Université de Louvain, Bruxelles, Belgium.

Radial presentation is a general characteristic of the neurons of the mammalian cortical plate. In order to know wether this property is general and necessary for the formation of the cerebral cortex, the embryogenesis of the CP has been studied in reptiles, the vertebrates which have the most primitive cerebral cortex.

The observations were made on embryological series of a turtle (E. orbicularis), a squamate (L. viridis) and a crocodile (C. niloticus), and confirmed by the examination of at least another species in each order.

In all species observed, the CP develops according to the same sequence : it appears in the dorsal part of the hemispheres and extends medially and laterally; during the last third of incubation the three architectonic areas of the cortex are recognized, namely the general pallium, brachetted between the medial (hippocampal) and the lateral (pyriform) cortices.

However, there is a constant difference in the cytology of the CP, between turtles and crocodiles on one end, and squamates on the other end. In the latter the CP cells, from their earliest stage of differentiation, manifest the property of radial presentation: the neurons are arranged radially, closely packed into a well defined plate. At later stages, the CP remains compact, the molecular zone (MZ) increases considerably, presumably reflecting the development of the apical dendrites; both the MZ and the white matter are clearly delimited. In turtles and crocodiles, the organization of the early bipolar CP cells within the CP is less regular than in squamates; the cells are more obliquely oriented and the plate appears as a loose network. At later stages, the neurons increase in size and the apical dendrites develop predominantly towards the pial surface; the spatial definition of the cortex, the MZ and the white matter improves but remains imperfect.

This study shows that the property of radial presentation of CP cells is present in squamates but lacking in turtles and crocodiles, and provides support to the theory that the evolution of the cortex can be dissected into discrete steps. The biological basis for this phenomenon is unknown but it might reflect species differences in the interaction between neurons and radial glial guides during embryogenesis.

153.1 NEUROPHYSIOLOGICAL STUDIES ON REINNERVATED MECHANORECEPTORS IN THE GLABROUS SKIN IN MAN. R.MACKEL* AND A.STRUPPLER* (SPON: B. Peterson)Department of Neurology, Technical University of Munich, Munich, FRG

Percutaneous microneurographic recordings from single regenerated afferent fibers were performed in 12 patients following complete traumatic transsection of the median (5 patients) or ulnar (7 patients) nerves. The cut ends of the nerves were bridged with a piece of the sural nerve and 39 mechanoreceptors were studied by recording their discharge characteristics proximal to the site of injury, following peripheral mechanical skin stimulation. The time elapsed between injury and nerve repair ranged between 2 and 24 months between nerve repair and examination 1 to 10 years. The age of the patients ranged between 16 and 59 years.

The discharge characteristics of identified mechanoreceptors were found to be comparable to those observed in normal human glabrous skin. They could be classified as Rapidly Adapting (RA,n=10) and Slowly Adapting (SA I,n=9 and SA II,n=18). Two receptors were classified as joint receptors. High threshold mechanical stimulation of the skin was usually required to activate the receptors, although some receptors responded to very low stimulation. Most reinnervated receptors were located in deeper tissues and more proximally in the extremity. Almost all mechano-receptors had only one circumscribed receptive field, which was usually located in the representative projection zones of the ulnar or median nerves. Receptive field location, whether distal or proximal in the extremity, and receptive field distribution, either singly or multiply represented, could be attributed to factors such as the time elapsed between the injury and the nerve repair, the age of the patients and the location of the injury i.e. distal at the wrist or more proximal towards the elbow.

It is concluded that these factors are responsible for the distribution and location of receptive fields, but not for the shaping of the functional discharge characteristics of the reinnervated mechanoreceptors.

153.2 ^{3}H 2 DEOXYGLUCOSE UPTAKE IN THE HYPOGLOSSAL NUCLEUS AFTER NERVE TRANSECTION. Philip Singer and Sharon Mehler*. Neurology Histochemistry Laboratory, Veterans Administration Medical Center, Kansas City, MO 64128

We have demonstrated (Singer, P. and Mehler, S., Neuroscience Absts. 5:683, 1979 and Exp. Neurol. 69:611-626, 1980) and others have confirmed (Kreutzberg, G. and Emmert, H., Exp. Neurol. 70: 712-716, 1980) that there is a marked increase in glucose utilization in the hypoglossal nucleus beginning 24 hours after hypoglossal nerve transection. These results were obtained with the ^{14}C 2 deoxyglucose technique. This technique is not capable of cellular resolution because of the problem of diffusion of the glucose when the sections are applied to slides. Therefore, the question of cellular location of the glucose uptake remains unresolved. We used the ^{3}H 2 deoxyglucose technique as described by Sharp (Sharp, F.R., Brain Res. 110:127-139, 1976) and modified by Steward (Steward, O. and Smith, L., Exp. Neurol. 65:513-527, 1980) to attempt to obtain cellular resolution.

150 gm male Sprague-Dawley rats underwent left hypoglossal nerve transection under nembutal (50 mg/kg IP) anesthesia. They were sacrificed at 1, 3, 7 or 14 days after axotomy. At the time of sacrifice 67 μCi/100 gm ^{3}H 2 deoxyglucose was injected IV via the tail vein while they were awake. Forty-five minutes later they were again anesethized with pentobarbital and the brainstem was removed and frozen in Freon cooled to -70°C. Slides were cleaned and coated with Kodak NTB emulsion and stored with drierite in light proof boxes at 5°C until use. Twenty micron sections were cut in a cryostat at -18°C under a safelight and applied to the precoated slides. These were exposed at room temperature for three weeks and then processed in D-19 developer and fixer and lightly stained with cresyl violet.

Some sections showed obvious evidence of diffusion of the isotope with smearing of surrounding high grain density into the 4th ventricle, central canal and tears in sections. Other sections, however, showed good localization of increased grain densities not impinging on to these structures and were selected for further analysis. In these sections there was a higher grain density in the nucleus on the side of nerve transection compared to the control side but there was a uniform distribution of grains over the neuropil and cell bodies with no definite localization in either cell bodies or neuropil. This suggests that increased glucose use is not only confined to the cell body but includes the neuropil as well. Thus, in addition to cell synthetic activity increased glial glucose use or dendritic electrical activity are possible pathways. However, because of the possibility of diffusion firm conclusions must await substantiation with other techniques.

153.3 EFFECTS OF VARIOUS POTENTIAL DIFFERENCES ON TRIGEMINAL GANGLIA NERVE REGENERATION IN VITRO. Betty F. Sisken and Philippe Sechaud.* Wenner-Gren Research Lab, and Department of Anatomy, University of Kentucky, Lexington, KY, 40536. Low levels of direct current have been used to augment bone healing, increase DNA synthesis in chondrocytes and to stimulate limb regeneration in non-regenerating vertebrate forms. We have also reported that the administration of nA levels of direct current (10nA total) by tantalum electrodes act to increase neurite outgrowth in sensory and sympathetic ganglia of the chick embryo; this outgrowth is oriented to the cathode. Additionally, after 3 days of constant exposure to the current, neuronal survival is increased in both explant and dissociated cultures. We have used this system to stimulate neurite outgrowth and maintain the neuronal population in studies to ascertain the interactions of trigeminal ganglia co-cultured with developing cornea.

In order to determine the electrochemical contributions to this system we have completed voltage/current experiments and found that 10nA, the current routinely used, corresponds to an electrode potential of -400mV/SCE. Therefore, we decided to look at the effects of imposing different levels of electric current by maintaining fixed electrode potentials on the neurite outgrowth and cellular morphology of cultured trigeminal ganglia. Potentials of 200,400,600 and 800mV were imposed on our culture dishes containing either platinum or tantalum electrodes by employing an agar bridge to a saturated calomel reference electrode connected to a potentiostat.

The results obtained in these experiments depended on the metal used for the electrodes and the current imposed. When platinum was used, the greatest neurite outgrowth was obtained between 200-400mV (20-40nA). No effects were obtained at 600mV (100nA), and tissue destruction occurred at 800mV. When tantalum electrodes were used, no effects were seen at 200mV (2nA), stimulation of neurite outgrowth occurred at 400-600mV (10-20nA) and no effects were seen at 800mV (40nA).

These experiments illustrate that a direct relationship exists between current and voltage levels in term of their biological effects. They also serve to define specific "windows" within which electric current/voltage reflect maximum stimulation.

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153.4 RELATIONSHIP BETWEEN AXONAL ELONGATION AND DEGENERATION AND SLOW FLOW. P. Cancalon. Dept. of Biological Science, Florida State University, Tallahassee, FL 32306.

Previous studies of the olfactory nerve have indicated that axonal injury causes total degeneration of the mature neurones followed by their replacement by new neuronal cells arising from undifferentiated mucosal cells. A constant turnover of the olfactory neurones has also been demonstrated in the intact olfactory nerve.

The olfactory nerve of the garfish was crushed 1.5 cm from the mucosa. Degeneration and regeneration were followed by measuring the proximo-distal decrease or increase in nerve weight in successive 3 mm segments. Regeneration was also followed by measuring the accumulation in the growing nerve endings, of labeled proteins transported by fast intra-axonal transport. As shown previously three distinct population of regenerating fibers were characterized. Each of the first two phases represent 3 to 5% of the original axonal population and at 21°C the leading fibers in each group progress at 5.8 ± 0.3 mm/d and 2.1 ± 0.1 mm/d. The third population contains 50 to 70% of the original axonal population and grows at a velocity of 0.8 ± 0.2 mm/d. At 31°C significantly higher regenerating velocities were measured: 10.3 ± 0.7 mm/d for the first phase fibers and 6.3 ± 0.4 for the third phase axons.

Rates of axonal transport were measured at different temperatures in these various phases of regenerating axons. The rate of fast transport is temperature dependent but is unaffected by regeneration. Slow flow velocity is also temperature dependent but is greatly affected by regeneration. Similar rates of slow flow were measured in the three populations of regenerating fibers. In the first phase fibers the base front of the slow peak moves at 5.2 ± 0.6 mm/d at 21°C and 9.8 ± 0.8 mm/d at 31°C. Slow flow appears to be the factor limiting the growth of the most rapidly regenerating fibers. Since these first phase fibers represent the axons already growing in the nerve before the crush the fastest rate of regeneration may represent the actual rate of nerve elongation during neuronal turnover occurring in intact nerves. Other limiting factors are preventing the fibers in the other phases to grow at the maximum speed (A lack of conditioning effect has been postulated).

After nerve crush, degeneration progresses along the nerve at 5.7 ± 0.3 mm/d at 21°C and 11.4 ± 0.5 mm/d at 31°C, values again similar to the rate of slow flow. It can be hypothesized that the extensive degeneration of the nerve is triggered by the lack of molecules provided by slow flow. From these results, it appears that slow flow plays a major role in the mechanisms of nerve degeneration and regeneration. (NIH 17198).

153.5 ELECTROPHYSIOLOGICAL, MORPHOLOGICAL AND BIOCHEMICAL CORRELATES OF NEUROMUSCULAR JUNCTION REFORMATION IN RAT PERIPHERAL NERVE REGENERATION. A. Gorio, G. Carmignoto, *M. Finesso, *L. Di Giamberardino,°* J. Y. Couraud°* and M. G. Nunzi *Fidia Research Laboratories, Department of Cytopharmacology, Abano Terme, Italy and °Centre d'Etudes Nucleaires, Commissariat a l'Energie Atomique de Saclay, Departement de Biologie, 91190 Gif-sur-Yvette, France.

Rat EDL muscles are denervated by crushing the sciatic nerve at the last gluteal branch. Reinnervation of the muscle starts 2 weeks after. Unmyelinated axons penetrate the muscle and make the synaptic contact at the old post-synaptic site. At this stage the ultrastructure of the nerve endings is not well differentiated yet, they are small, contain very few vesicles and seem to be changing from growth cone to synaptic terminals. The neuromuscular transmission is however recovered rapidly and in less than 24 hours the end-plate potential is capable of inducing muscle contraction. On the other hand, m.e.p.p.'s are rare at this stage and recover their frequency and distribution only two months after denervation. Electrophysiologically and histochemically we have shown that polyneuronal innervation begins immediately and reaches its maximum 7-10 days after reinnervation. The end-plate is invaded by flat nerve endings which gradually grow in size and differentiate in synaptic terminals. The correlation between the repression of the redundant innervation and the ultrastructural features of the terminals will be discussed. The flow-rates of the AChE forms drops after nerve crush and it recovers quite rapidly, beginning from the 2nd week post-crush.

The recovery is complete by 30 days post-crush for A_{12} and $G_1 + G_2$ while G_4 shows a slower rise. The concentration of the AChE forms in the denervated EDL also shows a decline and recovery pattern after nerve crush. The form G_4 reaches its lowest concentration 2 weeks post-crush and recovers afterward, yet without reaching the control value, at least until 60 days post-crush.The form A_{12} exhibit an extremely low concentration at 7 days, it starts rising at 15 days and is maximal at 30 days, with a kinetic almost superimposable to that of the flow-rate of A_{12} in the nerve. The recovery rate of A_{12} form in the muscle is similar to the recovery of the muscle membrane resting potential suggesting that mechanical activity may be responsible for both of them. Our results are suggesting that the complete restoration of synaptic transmission may be related to the recovery of axonal components very slowly transported.

153.6 Axon Regeneration Is Initiated But Not Maintained In The Absence of a Cell Body Response. Richard C. Carlsen, Jane E. Kiff, Karen Ryugo. Departments of Human Physiology and Zoology, University of California, Davis, CA 95616.

Frog spinal neurons, in animals maintained at environmental temperatures above 20°C, show all of the classic features of the cell body response following peripheral axotomy. In contrast, axotomized spinal neurons in frogs held at an ambient temperture of 15°C show no apparent cell body response to injury. The 15°C neurons retain their normal morphology, continue to synthesize and transport normal levels of acetylcholinesterase activity, and sustain their normal reflex connections. However, despite the apparent absence of a cell body response, the proximal nerve stump begins to regenerate. The dissociation of cell body changes and peripheral axon regeneration may offer some insight into the mechanisms responsible for both phenomena. The initiation of the cell body response, for example, does not appear to be due to the elimination of a normal peripherally-derived axonal constituent. Injured axons at 15°C were still able to acquire and transport horseradish peroxidase from the point of transection to the cell body. This suggests that normal retrogradely-transported material should also be drained from the injured 15°C axons over time, yet a cell body response does not appear. Similarly, the initiation of axonal sprouting and regeneration may not depend on alterations in the cell body. The 15°C proximal nerve stump begins to regenerate after a post-axotomy latent period of approximately 4.5 days. Regeneration of the most rapidly growing axons proceeds at a rate of 0.6 mm/day for at least the first 21 days. Subsequently, regeneration slows and may cease. Reinnervation of skeletal muscles 3cm distal to the point of injury (using either transection or crush) has not been observed after recovery intervals as long as 92 days. We propose that changes in the local environment surrounding the injured axon, e.g. increased vascular or axonal permeability to exogenous protein, may serve as a trigger for the initiation of axonal growth. Subsequently, the local influences are supplanted by intraaxonal contributions from the cell body which maintain continued axonal regeneration. In the absence of cell body support regeneration slows and may stop. (Supported by NIH NS 15065).

153.7 NERVE REGENERATION AND MUSCLE REINNERVATION. P.K. Law and T.E. Bertorini*. Dept. Neurol., Univ. of Tennessee, Memphis, TN 38163.

Reinnervation of denervated muscles depends on nerve regeneration and the presence of denervation changes in the muscle. This study tests if nerve regeneration is triggered by the muscle changes. We tested if earlier development of denervation changes in the muscle would lead to earlier reinnervation, and possibly, better recovery of muscle function. The sciatic nerve of 1-month-old C57BL/6 mice was cut *in situ* in both legs just distal to the bifurcation with the femoral nerve. The right soleus nerve was then cut where it entered the soleus. Whereas reinnervation of right and left solei required regeneration of similar lengths of nerves, the right soleus should develop denervation changes sooner because its shorter nerve stump provided less trophic support. Conversely, because the left soleus was trophically maintained longer by a longer nerve stump, its fibers should become less atrophic and generate greater tensions, provided that both solei were reinnervated simultaneously. The earliest detected reinnervation occurred at 3-4 weeks after surgery when supramaxial nerve stimulations of any soleus elicited twitch tensions of variable amplitudes. Both solei of the mouse responded to nerve stimulation indicating that the right soleus was not reinnervated earlier than the left. Due to incomplete reinnervation and inconsistence of immature motor unit responses, the tensions at this stage were not quantified. At 5-6 weeks and at 14 weeks after surgery, the left solei generated greater twitch (Po) and tetanus (Pt) tensions than the right. Although Pt increased from 5-6 weeks to 14 weeks, Po remained unchanged. Mean contraction time (CT) and half-relaxation time (½RT) were longer for the right solei than the left at 5-6 weeks. These time differences disappeared at 14 weeks. Fiber atrophy was more pronounced in the right than the left.

	5-6 Weeks		14 Weeks	
	Right	Left	Right	Left
Po(g)	1.80±0.55	2.66±0.99	1.79±0.31	2.73±0.68
Pt(g)	4.20±1.23	9.90±1.37	9.13±3.98	15.13±4.61
CT(msec)	24.20±4.70	13.70±2.21	10.00±1.31	9.75±2.05*
1/2RT(msec)	41.90±11.84	15.90±3.51	12.44±3.88	12.33±6.91*

In vivo measurements at 37°c; $\bar{x} \pm$ SD shown; all differences significant at P<0.005 by paired-t tests except *, not significant; 10 mice at 5-6 weeks, 8 mice at 14 weeks.

We conclude that the nerve regenerates primarily in response to injury. Unlike axonal sprouting, such regeneration is not initiated by denervation changes in the muscle. In addition, the muscle function recovered following denervation is dependent on the neurotrophic support prior to reinnervation. (Supported by MDA and NSF PCM 7921008 awarded to P.K.L.)

153.8 INDUCTION OF CHEMOSENSITIVITY IN A MUSCLE NERVE AFTER GRAFTING THE CAROTID BODY INTO THE MUSCLE. L. Monti-Bloch*, L.J. Stensaas and C. Eyzaguirre. Dept. Physiol., Univ. Utah Col. Med., Salt Lake City, UT 84108.

An important problem in neurobiology is to determine whether or not the periphery can influence the specificity of sensory fibers. To study this, the carotid body of six cats was unilaterally removed under anesthesia, grafted into the pelvic end of the tenuissimus muscle and the pelvic branch of its nerve sutured to the graft. A small piece of fat tissue served as a control implant in two other animals. Five months later the cats were again anesthetized and the graft implantation site exposed by opening the skin and making a paraffin-filled pool. The knee branch of the tenuissimus nerve was cut as was the main trunk of the nerve near the sciatic. Its distal end was then placed on electrodes to record afferent discharges. The cats were allowed to spontaneously breathe room air through a tracheal cannula. The grafted carotid bodies were stimulated by delivering special gas mixtures through the cannula or by drugs topically applied to the surface of the graft. In five animals the grafted carotid body survived and was reinnervated by tenuissimus nerve fibers. It did not survive in one animal where only a neuroma was observed. In the five successfully implanted cats, inhalation of 100% N_2 or 5% CO_2 in O_2 and applications of NaCN (1-10 µg) increased the discharge frequency of 9 units while 2 were unaffected or depressed. ACh (25-200 µg) increased the activity of 6 units, depressed 2 and was without effect in 3. Nicotine (0.5-10 µg) increased the afferent activity of all (11) fibers. Bethanechol (50-100 µg) and dopamine (40-200 µg) had variable effects. These drugs alternately increased or depressed the discharge of several fibers. All afferent fibers connected to the grafts responded with an increased discharge to mechanical tapping and muscle stretch. The conduction velocity of the active fibers was 12.5-20 m/sec at 31-35° pool temperature. The normal tenuissimus muscle and preparations containing transplanted fat tissue or the neuroma responded to tapping and to applications of ACh or nicotine as a consequence of muscular and, presumably, intrafusal contractions. These preparations did not respond to N_2, CO_2, NaCN or dopamine. Electron microscopy of grafts with a chemosensitivie response revealed numerous glomus (type I) cells--some of them innervated--and sustentacular (type II) cells. It is concluded that carotid body cells can induce chemosensitivity in muscle nerve fibers which also have mechanosensory properties. Supported by grants NS 05666, NS 07938 and GM 24487.

153.9 THE EFFECT OF TESTOSTERONE ON THE REGENERATION OF THE HYPOGLOSSAL NERVE IN MALE RATS. W.H.A. Yu. Dept. of Anatomy, Mount Sinai Sch.of Med. New York, N.Y. 10029.

Administration of testosterone proprionate (TP) to female rats after hypoglossectomy accelerated axonal outgrowth (Yu & Srinivasan, Exp.Neurol. 71:431,1981). The present study was designed to determine whether or not TP promotes nerve regeneration in male rats and to trace the regenerating fibers as they travel to their targets. In 6 weeks old rats the hypoglossal nerve was transected unilaterally in the neck region proximal to its bifurcations. The cut ends of the nerve were opposed without suturing. After surgery, the rats were divided into two groups and treated as follows: 5mg TP in 0.1 ml sesame oil, 3 times per week; or oil vehicle alone. Seven rats from each group were killed 1,2,3, and 4 weeks post-operatively (PO). 24h prior to killing, 50 ul of a 10% horseradish peroxidase (HRP) solution was injected into the midline of the tongue. The presence of HRP-labeled neurons in the hypoglossal nucleus ipsilateral to the side of axotomy indicates that the regenerating fibers reached the tongue. No HRP-labeled neurons were seen in the side of lesion 1 week PO. However, labeled neurons were present at 2 weeks PO; their number was significantly higher in the TP group. By 3 and 4 weeks PO, there was no difference in the degree of regeneration between the two groups. Four weeks PO, another 7 rats each from both groups were subjected to bilateral resection of the medial branch of the hypoglossal nerve prior to the HRP injection. This procedure blocked HRP transport in the medial division but allowed the neurons whose axons reached the tongue via the lateral division to be labeled by HRP. A comparison of the patterns of labeled neurons in the hypoglossal nuclei between ipsilateral and contralateral to the side of the first lesion indicated that regenerating fibers entered the two divisions at random. These findings suggest that TP promotes nerve regeneration also in the male rat; however, the accelerated regeneration does not facilitate the reestablishment of the specificity of innervation. The somatotopic organizations were altered after regeneration similarly in both groups. Supported by a grant from the Organon Pharmaceuticals.

153.10

WITHDRAWN

153.11 IN VIVO MODEL FOR NERVE REGENERATION: PRESENCE OF NEURONOTROPHIC FACTORS. Frank L. Longo*, Göran Lundborg*, Marston Manthorpe, Stephen D. Skaper and Silvio Varon. Dept. Biol., Sch. Med., Univ. Calif. San Diego, La Jolla, CA 92093

Lundborg and coworkers have developed an in vivo model, where pre-formed cylindrical chambers are implanted into adult rats in such a way that proximal and distal stumps of a resected nerve are inserted into the opposite ends of the chamber, allowing up to a 1 cm gap between them. Within 1-3 months, a fully organized nerve structure develops across the gap culminating in some motor and sensory function (Lundborg & Hansson, Brain Res. 178, 153, 1979; J. Hand Surg. 5, 35, 1980). A silicone tube can replace the previously used "mesothelial" chamber, with similar results. Within this silicone chamber, however, the regenerating nerve structure is surrounded by fluid, which can be collected and tested in vitro for neuronotrophic activities. We have examined fluid collected from chambers implanted in the rat which has received both proximal and distal stumps (P-D), only one stump (P-O or O-D, respectively, with the empty end ligated), or neither (both ends open). Miniturized test cultures included sensory neurons from newborn mice, and sensory, sympathetic and spinal cord neurons from embryonic chick (Varon et al, Dev. Brain Res. 1, 73, 1981; Longo et al, Trans. Am. Soc. Neurochem. 12, 188, 1981). All in vivo-derived fluids displayed neuronotrophic activity for all the neurons tested, and exhibited much higher titers than observed from in vitro cell conditioned media. Activity was present at least as early as 6 days after chamber implantation. The results indicate that such neuronotrophic factors i) are not solely produced in vitro, and ii) can occur in the immediate environment of a regenerating nerve in vivo. Their direct relevance to the regeneration process is currently being examined.

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153.12 EFFECT OF PARTIAL TARGET AREA ROTATION ON THE REGENERATION OF SPECIFIC CRAYFISH NEUROMUSCULAR CONNECTIONS. James F. Clement*, William P. Hunt and Samuel J. Velez. (SPON: M. Marin-Padilla). Department of Biological Sciences, Dartmouth College, Hanover, New Hampshire 03755.

The six axons that innervate the superficial flexor muscles of the crayfish can regenerate their connections with a high degree of specificity (Ely & Velez, Soc. Neurosci. Abstr., Vol. 5, p. 677, 1979). In previous work we have reported that if the target area of the nerve is changed, some of the neurons are capable of forming normal maps while others do not form normal patterns (Hunt & Velez, Soc. Neurosci. Abstr., Vol. 5, p. 679, 1979). When transplanted to the contralateral muscle, axons 2 and 3 form normal connections but axons 1 and 4 are unable to do so. In control animals, and in contralateral transplants, the nerve approaches the muscle field from a medial-to-lateral direction; the first fibers receiving connections are always on the medial edge of the muscle. We are presently studying the changes in the connectivity maps that might occur as a result of changes in muscle fiber spatial arrangements. The superficial flexor nerve was transplanted to its contralateral muscle and was secured in place at the medial-lateral junction, approximately at the mid-point of the muscle field. The medial-lateral junction separates the field of innervation for most of the axons in this system. The nerve was allowed to regenerate for 10 weeks, the time period for complete regeneration, and the animals were analyzed by recording junction potentials from all the muscle fibers and closely matching them with the spikes of identified axons. Preliminary results indicate that the regenerated maps of all axons studied are not affected by the new entry point of the nerve into the muscle field. The connectivity patterns were similar for axons 1, 2, 3 and 4 when the nerve grew into the contralateral muscle following the normal medial-to-lateral growth path and when the nerve grew following a central-to-edges growth path. This suggest to us that this nerve is capable of following a spatial gradient on the surface of the muscle.

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153.13 EFFECT OF INTERSEGMENTAL TRANSPLANTS ON THE REGENERATION OF SPECIFIC CRAYFISH NEUROMUSCULAR CONNECTIONS. Ji-Chuu Hwang*, William P. Hunt and Samuel J. Velez. Department of Biological Sciences, Dartmouth College, Hanover, New Hampshire 03755.

The superficial flexor muscle of the third abdominal segment of the crayfish Procambarus clarkii is innervated by six axons which distribute their innervation in a very specific pattern over the muscle surface (Velez & Wyman, J. Neurophysiol. 41: 75-96). Axon 3 innervates all the medial fibers and most of the laterals; the probability of lateral muscle fibers receiving connections from this axon decreases as the lateral edge of the muscle is approached. Axon 4 innervates all the lateral fibers and some of the medials; the probability of medial muscle fibers receiving connections from this axon increases as the lateral fibers are approached. The connections to the superficial flexor muscles of the second abdominal segment were examined and a specific connectivity map was also found. The maps of homologous axons 3 and 4 on the second segment were the reverse of the maps of axons 3 and 4 on the third segment. We investigated the effect of changing the target area of the third segment nerve on the connectivity patterns of axons 3 and 4. The third segment nerve was transplanted into the second segment of the animal, and analysis was performed at regular intervals after the operation. Analysis consisted on recording junction potentials from the muscle fibers and matching them with the spikes recorded from the spontaneous activity of the nerve in isolated abdomens. Preliminary results indicate that during the initial stages of regeneration, axon 3 growing into the second segment forms the pattern of connections that correspond to the third segment. However, axon 4 growing into the second segment forms the pattern of connections that correspond to the second segment. Thus, the connectivity maps of the two axons are the same. This suggest to us that the growth pattern of axon 3 is not being affected by changes in the target, whereas the growth pattern of axon 4 is more sensitive to alterations in its environment.
(Supported by NIH Grant NS 13800 to SJV)

153.14 THE DISTRIBUTION OF REGENERATED FIBER TRACTS IN THE GOLDFISH SPINAL CORD STUDIED BY USE OF HORSERADISH PEROXIDASE (HRP). P. F. Moebs* and S. M. Bunt. Dept. of Anatomy, Med. Univ. of S.C., Charleston, S.C. 29425.

Because of renewed interest in the possibility of obtaining regeneration in the mammalian spinal cord, we have examined the ability of regenerating fiber tracts to maintain or regain their normal pathways following a spinal cord transection. This study also investigates whether preservation of normal fiber pathways is essential for formation of appropriate connections in the regenerated goldfish spinal cord.

Spinal cords were transected in 70 goldfish. Verification of complete transection in randomly chosen fish was obtained using the Holmes' silver technique to demonstrate nerve fibers. Three months later, regeneration was observed both by histological examination and by return of sensation and voluntary movement in caudal segments.

Descending and ascending tracts were identified in normal goldfish by transecting the spinal cord and examining sections for degeneration at both light and electron microscopic levels. Comparison of the above with sections of the regenerated spinal cord revealed that although a great disruption of the normal fiber tracts was seen at the transection site, the morphology of spinal cord segments more distal to this site appeared normal with little evidence of displaced fiber tracts.

HRP labeling of the regenerated spinal cord either rostral or caudal to the original transection demonstrated regenerated ascending fibers terminating in the first few segments rostral to the transection site, while regenerated descending fibers could be labeled up to 1 cm caudal to the site.

To investigate the fine ordering of regenerated fiber tracts, partial HRP applications were made either rostral or caudal to the transection site in the regenerated cord and the distribution of the labeled fibers examined in 2 μm plastic sections. Although displaced fiber bundles were observed in some sections of regenerated spinal cord, the sections more distal to the transection site appeared grossly normal.

We conclude, therefore, that maintenance or restitution of normal fiber ordering at the site of the spinal cord injury may not be necessary for return of fibers to their normal pathways. (Supported by NIH grant EY03414)

154.1 SPROUTING OF CATECHOLAMINERGIC AXON TERMINALS IN THE PARTIALLY DEAFFERENTED INTERPEDUNCULAR NUCLEUS. Z. Gottesfeld. Department of Neurobiology and Anatomy, The University of Texas Medical School, Houston, Texas 77030

The interpeduncular nucleus (IPN), a mesencephalic tegmental structure, receives a massive fiber input, especially from the habenular complex (Hb), via the fasciculus retroflexus (FR). Some of these fibers have been characterized as cholinergic, while others have been shown to contain substance P. However, the noradrenergic and dopaminergic innervation of the IPN project to this nucleus via pathways other than the FR. Thus, by removing the FR projections from the IPN, the catecholaminergic innervation will remain intact in this nucleus. The question addressed by this work is whether or not these spared axon terminals will grow new sprouts in the partially deafferented IPN. Sprouting was assessed both chemically and by fluorescent histochemistry.

Radiofrequency lesions were produced in the Hb of male Sprague Dawley rats (200g). Sham-treated control animals underwent similar surgical procedures except lesioning current was not passed. After survival periods of 1 day, 3 days, 6 weeks or 6 months, the rats were killed by decapitation. Brain sections (300 μm thick) were cut in a cryostat at -8°C and the accuracy of the lesions were verified histologically in 60 μm thick sections. Two tissue pellets (750 μm id) were removed from the IPN, then homogenized in 100 μl of 0.1 N perchloric acid and aliquots were taken for catecholamine assays[1] and for protein determination[2]. No significant changes were found in norepinephrine (NE) or dopamine (DA) levels following 1 or 3 days survival after Hb lesions. However, both NE and DA increased significantly ($P<0.001$) after surviving lesions for 6 weeks or 6 months. The subsequent use of fluorescent histochemistry[3] demonstrated increased proliferation of catecholaminergic axon terminals following the long-term survival periods.

Thus, the combined use of chemical assays and morphological visualization provide evidence that NE and DA have the capacity to sprout concurrently in the partially deafferented IPN.

Supported by BRSG.

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154.2 THE EFFECT OF ACUTE AND CHRONIC DENERVATION ON THE RAT SPINAL CORD. H. Markus*, D. Krushelnycky*, B. Pomeranz. Dept. of Zoology, University of Toronto, Toronto, ONT., CANADA M5S 1A1.

Previous reports indicate that following peripheral nerve or dorsal root lesion, dorsal horn cells respond to electrical or mechanical stimulation of areas of skin outside their normal receptive fields. In this study the left sciatic nerve was lesioned and ligated in mid-thigh. 23 days later the right sciatic was acutely lesioned and extracellular unit recordings were made from those areas of the cord which normally receive input from the saphenous and sciatic nerves.

In the acutely denervated side we found the area of the cord which responded to mechanical stimulation of the leg as well as to electrical stimulation of the saphenous nerve. Caudal to this area there was a 2nd zone which responded to electrical stimulation of the saphenous nerve but not to mechanical stimulation of the leg in regions normally served by the sciatic afferents. Caudal to this there was a 3rd zone which responded to mechanical stimulation of the leg and tail in regions above the site of the sciatic lesion but not to electrical stimulation of the saphenous.

In the chronically denervated side there was a rostral zone which responded to electrical stimulation of the saphenous nerve as well as mechanical stimulation of the leg. Caudal to this zone was a 2nd zone which responded to mechanical stimulation of the leg and tail but not to electrical stimulation of the saphenous. The border of the electrically and mechanically responsive zone was more caudal on the chronically denervated than on the acutely denervated side of the cord suggesting that chronic denervation caused the saphenous inputs to spread caudally several mm into the sciatic zone of the cord. In the acutely denervated side these inputs may exist but are weak and therefore only activated electrically. In the chronically denervated side the inputs are strengthened and therefore respond to mechanical stimulation.

154.3 ACUTE DEXAMETHASONE TREATMENT ALTERS AXON SPROUTING IN THE DENTATE GYRUS OF THE HIPPOCAMPAL FORMATION. Stephen W. Scheff and Steven T. DeKosky. Depts. Anatomy & Neurology, Univ. of Kentucky, Lexington, Kentucky, 40536.

The dentate gyrus of the hippocampal formation has been utilized extensively to study reactive synaptogenesis (axon sprouting) in the mammalian CNS. Previous experiments have shown that animals treated continuously with hydrocortisone or corticosterone, two naturally occuring glucocorticoids, manifest reduced axon sprouting in the dentate gyrus. The present study was designed to test whether or not a synthetic glucocorticoid, dexamethasone could be effective in altering the same growth response. Dexamethasone is the glucocorticoid frequently administered to neurosurgical patients post-operatively or following traumatic brain injury.

Adult male Sprague-Dawley rats, 75-90 days of age, received a unilateral electrolytic lesion of te entorhinal cortex. This lesion partially denervates the dentate gyrus ipsilaterally. At the same time of surgery the animals received glucocorticoid treatment which continued for eight consecutive post-operative days. Treatment consisted of a single daily injection of dexamethasone (0.2mg/kg) subcutaneously, a dosage similar to that administered to human patients. Control animals were injected with the vehicle alone. Following the eight days of treatment all animals were allowed an additional seven days of convalescence.

The brains were examined for changes in hippocampal circuitry as a result of axon sprouting elicited by partial denervation of the dentate gyrus. The cholinergic projections were monitored with AChE histochemistry and revealed little qualitative difference between steroid treated animals and controls. Changes in the commissural-associational fibers, as monitored by the Holmes' fiber stain, revealed a statistically significant difference between the two groups. Control animals showed a normal sprouting response in agreement with previous results. Dexamethasone treated rats showed a significant decrease in the sprouting response as compared to controls. Finally, sections from each brain were stained with Cajal's gold sublimate for astrocytes. Dexamethasone treated animals showed hypertrophy of astrocytes throughout the hippocampal formation.

Dexamethasone, a synthetic glucocorticoid has thus been shown to depress the sprouting reaction in the dentate gyrus. The mechanism underlying this effect may relate either to its specific effects in the hippocampal system, which contains numerous glucocorticoid receptors, or as a result of a generalized effect on CNS inflammatory and/or immune response.

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154.4 NEONATAL SEPTAL LESIONS RESULT IN SYMPATHOHIPPOCAMPAL INNERVATION. K.A.Crutcher.Department of Anatomy, University of Utah College of Medicine, Salt Lake City, Utah 84132.

Sympathetic axons,arising in the superior cervical ganglion, appear in the rat hippocampal formation and neocortex following cholinergic denervation of these brain regions. Such sympathetic sprouting appears to occur specifically in response to loss of central cholinergic fibers in the adult since denervation of other cortical afferent fibers does not elicit this response. The purpose of the present study was to determine whether the transmitter identity, i.e., the "cholinergicity", of the lost fibers, is related to subsequent sympathetic sprouting. This was accomplished by destroying the septum before evidence of cholinergic innervation to the hippocampal formation develops e.g., high-affinity choline uptake, acetylcholine synthesis, choline acetyltransferase activity, and acetylcholinesterase activity. The septal region was aspirated on postnatal day 2 or 3. At 2 months of age the animals were killed and sections through the hippocampal formation were examined with acetylcholinesterase (AChE) and fluorescence histochemistry for the presence of cholinergic and sympathetic fibers, respectively.

In all cases in which the medial septum had been destroyed there was a tremendous loss of hippocampal AChE staining compared to control littermates. Adjacent sections stained for noradrenergic fluorescence revealed the presence of sympathetic axons within the hippocampal formation with a distribution and extent indistinguishable from that obtained following adult septal lesions. In those cases in which the cingulum had also been interrupted sympathetic fibers were present within the cingulate cortex posterior to the level of the lesion. Sympathetic fibers were not present within the hippocampal formation or cingulate cortex ipsilateral to a superior cervical ganglionectomy. These results indicate that the "cholinergicity" of the lost septohippocampal pathway is not related to sympathohippocampal sprouting. However, it is not possible to rule out a potential role for non-cholinergic or pre-cholinergic axons in eliciting sympathetic sprouting since recent retrograde tracing studies with the fluorescent marker bisbenzimide demonstrate the presence of numerous septohippocampal axons as early as the day of birth.

Based upon the specificity of this neuronal rearrangement and the early age at which it can be elicited, it is hypothesized that the same tropic factor accounts for the normal development of the septohippocampal innervation and sympathetic sprouting following septal lesions. The normal lack of sympathohippocampal fibers may be accounted for by their later arrival compared to septal fibers. SUPPORTED BY NS 17131-01.

154.5 SYMPATHETIC INNERVATION OF PINEAL AND HABENULA INCREASES AFTER SEPTAL LESIONS: COVARIATION WITH SYMPATHOHIPPOCAMPAL SPROUTING. F.H. Gage and M.D. Chafetz (SPON:J. Flynn). Chemistry of Behavior Program, Psychology Department, Texas Christian University, Fort Worth, TX 76129.

Sympathetic nerve endings innervate the pineal body (Axelrod, 1974) and habenula (Bjorklund, Owman, & West, 1972). During the course of a quantitative study of sympathohippocampal sprouting, measurements of the sympathetic innervation of pineal and habenula were obtained. If a cell body response to distal axotomy occurred during the growth of sympathohippocampal fibers, central sites of normal sympathetic innervation might be affected.

The measurement procedure used was developed by Chafetz and Gage (1981). Two specific and independent indices of the field of fluorescence are obtained with this procedure: fiber intensity (normalized for fading) and areal density. The animals were killed for preparation of fluorescence histochemistry (de la Torre and Surgeon, 1976) between 1 and 39 days after septal lesions. Statistically significant positive correlations were found for areal density in pineal, fiber intensity in habenula, and areal density in hippocampus. These correlations indexed an increase in the innervation of these areas over time. The results were discussed as relating to a retrograde response of the cell body to distal axotomy. The implications for functional changes as a result of septal lesions were also discussed.

154.6 REPEATED ADMINISTRATION OF HIGH DOSES OF ETHANOL MAY INHIBIT LESION-INDUCED AXONAL SPROUTING IN THE RAT HIPPOCAMPUS. M.D. Lind*, J.R. West, R.M. Demuth*[1], E.S. Parker*[2], R.L. Alkana[3], and A.C. Black, Jr. Dept. Anatomy, Univ. Iowa Coll. Med., Iowa City, IA, [1]Dept. Psychol., Univ. N. Illinois, De Kalb, IL, [2]Lab. Clin. Studies, N.I.A.A.A., Bethesda, MD, and [3]School of Pharmacy, USC, Los Angeles, CA.

Axonal sprouting by several different inputs produces alterations in the characteristic width of the laminated afferent zones of the molecular layer of rat dentate gyrus following unilateral electrolytic lesions of the entorhinal cortex (EC). Adult Sprague-Dawley rats were given 6 gm/kg-day ethanol by gastric intubation (n=5) or 11.6 ± 0.5 gm/kg-day ethanol in a liquid diet[1] (n=8) for 2 weeks. They were then given a unilateral electrolytic EC lesion under ether anesthesia and continued on the ethanol regimen for an additional 9 days after lesion. Rats were sacrificed and their brains processed for acetylcholinesterase (AChE) histochemistry according to a modification of the method of Geneser-Jensen and Blackstad[2]. Results were compared to normal controls (n=5), and controls which had received unilateral EC lesions without ethanol (n=5). Intensified AChE staining in the outer molecular layer is the result of a proliferation of AChE-containing terminals (presumably septal in origin) and is normally observed as soon as 4-5 days post-lesion. Although all ethanol-treated rats exhibited increased AChE staining in the outer molecular layer, the increase was less obvious in the liquid diet group. Moreover, the commissural/associational (C/A) zone usually exhibits increased width and diminished AChE staining between 5 and 12 days after lesion[3]. This change in staining is believed to denote the sprouting of C/A fibers into a more distal region of the molecular layer. The width of the pale staining C/A zone was significantly increased in all lesioned rats except those receiving the higher dose of ethanol by liquid diet. Interpretation: Sprouting occurs in the C/A zone following EC lesion, but such sprouting is reduced in animals receiving large daily doses of ethanol. Thus high concentrations of ethanol may have deleterious effects on axonal sprouting occurring in response to brain injury. [1]Ethanol was added to a liquid diet (Bio-Serv 711-PR) so that it represented 35% of the total calories available to the rats as the sole source of food and water. [2]Z. Zellforsch. 114:460-481, 1971. [3]Nadler et al., J. Comp. Neurol. 171:561-588, 1977. (Supported by grant AA-03884 to J.R.W. from the N.I.A.A.A., and by grants from the National Council on Alcoholism to JR.W. and A.C.B.).

154.7 AXONAL SPROUTING AT THE NEUROMUSCULAR JUNCTION OF ADULT AND AGED RATS. G. E. Fagg*, S. W. Scheff and C. W. Cotman (SPON: V. Vijayan). Dept. of Psychobiology, University of California, Irvine, CA 92717.

Partial denervation of a skeletal muscle in the adult animal is followed within a few days by a characteristic repair response, involving the growth of fine processes (sprouts) from the remaining intramuscular nerves. These sprouts, derived either from the end-plate region (terminal sprouting--TS) or from nodes of Ranvier (collateral sprouts--CS), eventually reinnervate the denervated muscle fibers. In the present study, we have compared the response to unilateral partial denervation (L4 transection) of the soleus muscle in adult (3 month-old) and aged (27 month-old) Sprague-Dawley rats. Two weeks postoperatively, muscles were stained using the zinc iodide/osmium tetroxide procedure, and sprouting was assessed by measuring the percentage of end-plates with TS (%TS), the percentage of pre-terminal axons with CS (%CS), the end-plate length and the length of TS. In adult control animals, end-plates were small (43 ± 1 um), %TS and %CS were low (6 ± 2 and 7 ± 2, respectively) and TS were short (23 ± 2 um). Partial denervation significantly (P 0.01) increased these parameters by 1.2-fold (end-plate length), 3.8-fold (%TS), 2.6-fold (%CS) and 1.7-fold (TS length); there were no differences between contralateral and control muscles. In aged controls, end-plates were much larger (65 ± 4 um) and more complex, frequently comprising 2-3 components arising from the terminal axon and 1 or 2 collateral sprouts; %TS and %CS were high (19 ± 2 and 55 ± 10, respectively) and TS were relatively long (51 ± 7 um). In this age group, partial denervation significantly increased only end-plate length and %TS (1.3- and 1.7-fold, respectively); again, there were no differences between contralateral and control muscles. Hence, relative to controls, aged rats exhibit a more limited capacity than adults to respond to nerve injury with compensatory axonal growth. Since aged animals show evidence of considerable sprouting prior to partial denervation, one explanation may be that there is a maximum limit to the amount of axonal growth for each motor neuron determined by the synthetic capacity of the cell. Alternatively, the diminished response in aged animals may be due to a longer latency or higher threshold for repair than in adults. Supported by NIA grant AG00538.

154.8 THE FATE OF DISUSE-INDUCED TERMINAL SPROUTS AT THE RAT NEUROMUSCULAR JUNCTION. Greg L. Harris* (SPON: A.R. Light). Dept. Physiol., Sch. Med., U. of N.C., Chapel Hill, N.C. 27514

The relationship between motor nerve terminal sprouting and junctional transmitter release was studied in disused rat extensor digitorum longus (EDL) muscles following an 8-9 day conduction block of the sciatic nerve with tetrodotoxin (TTX). Mean quantum content (m) and the frequency of miniature endplate potentials (m.e.p.p.s) were estimated from intracellular recordings of disused and control (contralateral EDL) muscle fibers in excised muscle-nerve preparations superfused by an oxygenated low Ca^{2+}-high Mg^{2+} saline solution. Following the electrophysiological experiments, the muscles were stained to allow visualization of the motor nerve terminals and junctional acetylcholinesterase. Endplate size was estimated by tracing the course of the nerve terminal arborization along the surface of single dissected muscle fibers.

As was the case for rat soleus muscles (Snider and Harris, Nature 281:69, 1979.), the synaptic efficacy of EDL muscles was significantly enhanced by disuse. The mean m and m.e.p.p. frequency (f) of disused junctions were ~3.5 and ~2 times the control values, respectively. Seventy percent of the disused and 5% of the control junctions were judged to possess terminal sprouts. The measured nerve terminal lengths (Ln) of disused junctions were, on the average, 1.7 times the control lengths.

The Ca^{2+} dependence of evoked release and the effects of K^{+}-induced depolarization on f were compared at junctions of disused and control muscles to determine if the increased synaptic efficacy of disused junctions may be due, in part, to alterations in the properties of the transmitter release process. There were no significant differences in the response of disused and control junctions to these tests.

Junctional morphology and synaptic efficacy were also studied 4 and 10 weeks following the resumption of muscle activity in animals permitted to recover from an 8-9 day TTX block. At 4 weeks, the incidence of sprouting at disused endplates had returned to control levels. The mean Ln of disused junctions, however, was 1.5 times the mean control length. The quantum content and f at disused junctions were ~1.7 and ~2 times the respective control values. At 10 weeks, there were no significant differences in Ln, m or f at disused and control junctions. The similarity of morphological and electrophysiological parameters at 10 weeks appeared to be due to increases in control rather than decreases in disused values.

The data support the hypothesis that terminal sprouts are able to form functional connections with disused muscle fibers.

(supported by USPHS grant NS 10319)

154.9 GROWTH OF SYMPATHOHIPPOCAMPAL FIBERS MEASURED QUANTITATIVELY USING A NEW MICROFLUOROMETRIC METHOD. M.D. Chafetz and F.H. Gage (SPON: M. Emmett-Oglesby). Chemistry of Behavior Program, Psychology Department, Texas Christian University, Fort Worth, TX 76129.

Sympathetic nerve endings innervate the hippocampal formation as a result of damage to septohippocampal fibers (Loy & Moore, 1977; Stenevi & Bjorklund, 1978). These sympathetic endings are not observed as a result of damage to entorhinal afferents or to specific monoamine projections, nor are they seen in undamaged animals. In order to study the functional significance of this sympathetic growth, a quantitative index of fiber density is needed. This index should be specific for the fluorophore, sensitive to the experimental manipulations, and easily obtained for practical use. No such index is currently available.

A method was developed using a microspectrofluorometer to sample fields of fluorescence at specific wavelengths. Multivariate analyses were used to analyze the variables obtained with several different criteria. Two independent components (n=309) were identified: fiber intensity and areal density. The indices of density were identified as the best discriminators between animals with septal lesions and sham controls. Indices of intensity, normalized for fading, exhibited a statistically significant correlation with time (days) between days 1 and 39 after septal lesions. These measures were insensitive to several of the criteria employed. This finding indicated that different investigators who might use slightly different criteria would obtain a consistent measurement of the two underlying variables.

154.10 INCREASES IN INCORPORATION OF PROTEIN PRECURSORS DURING REINNERVATION OF RAT DENTATE GYRUS ARE PARALLELED BY INCREASES IN THE INCIDENCE OF POLYRIBOSOMES ASSOCIATED WITH DENDRITIC SPINES. Oswald Steward and Barry Fass. Dept. of Neurosurgery, University of Virginia School of Medicine, Charlottesville, VA 22908.

When the dentate gyrus (DG) is denervated by destroying afferents from the ipsilateral entorhinal cortex (EC), the denervated dendrites are reinnervated by several surviving afferent systems. Recent quantitative autoradiographic studies designed to assess protein synthesis revealed an increased incorporation of protein precursors in the denervated neuropil during the reinnervation, especially 6-12 days postlesion (Fass & Steward, Anat.Rec., 199; 80A, 1981). The present study evaluates some aspects of the distribution of polyribosomes in granule cell dendrites during the period of reinnervation and increased incorporation.

Adult rats received unilateral EC lesions and were prepared for electron microscopy 2,4,6,8,10,12,14, and approximately 180 days postlesion. The incidence and location of polyribosomes in denervated dendrites (ipsilateral to the lesion) was compared with that on the contralateral (control) side and in the DG of intact rats.

In the control DG, granule cell dendrites contain polyribosomes which often are associated with dendritic spines, lying under the intersection of the spine neck and main dendritic shaft. No polyribosomal rosettes were observed in spine heads in control material. Quantitative analyses of identified neck/shaft intersections in mid proximo-distal locations along the dendrite (within EC terminal field) revealed that 10% of the intersections has associated polyribosomes. In denervated dendrites, the incidence of polyribosomes under neck/shaft intersections increased during the period of reinnervation. The increase first became apparent 6 days postlesion, peaked at about 8 days, and declined thereafter. The average incidence of polyribosomes increased almost 4-fold at 8 days postlesion, with 38% of the intersections having polyribosomes. In contrast to the control, polyribosomes were also found in spine heads in the denervated DG of 6- and 8-day cases, immediately subjacent to the postsynaptic membrane specialization.

The altered distribution of polyribosomes closely parallels the increased incorporation of protein precursors observed during the period of reinnervation. Both changes become apparent at about 6 days postlesion, peak at 8 days, and decline thereafter. Although our studies were performed on separate groups of rats, the parallels suggest that the polyribosomes might be responsible for the increased incorporation. We propose that these changes reflect the synthesis of postsynaptic protein(s) associated with the reinnervation process. Supported by NIH Grant 5 RO1 Ns12333-06 and RCDA 5 K04 NS00325 to O.S.

154.11 ATTENUATION OF LOCOMOTOR HYPERACTIVITY AFTER BILATERAL PRIMING- AND SECONDARY-LESIONS OF ENTORHINAL CORTEX. Barry Fass and Oswald Steward. Dept. of Neurosurgery, University of Virginia School of Medicine, Charlottesville, VA 22908.

Although bilateral lesions of the entorhinal cortex (EC) partially denervate the dentate gyrus, surviving afferent systems proliferate and reinnervate the vacated synaptic sites on dentate granule cell dendrites (Lynch et al., Brain Res., 110:57, 1976; ibid, 42: 311, 1972). The extent and/or rate of proliferation apparently can be increased by damaging the EC progressively;i.e. by making a priming lesion of the medial EC, followed several days later by a secondary lesion of the remaining EC (Scheff et al., Brain Res., 150: 45, 1978). The present study assesses some of the behavioral consequences of progressive EC lesions. Simultaneous bilateral lesions of the EC result in an increase of open-field locomotor activity which peaks 4-5 days postlesion and subsequently "attenuates" (returns toward control levels)(Steward et al., Brain Res. Bull., 2: 41, 1977). The parallel between attenuation of hyperactivity and sprouting implies that the two might be related. If the behavioral changes are related to sprouting, then progressive bilateral lesions of the EC might facilitate this attenuation.

Adult male albino rats were tested in an open field for 4 consecutive days prior to receiving bilateral lesions of the medial EC or control surgery. Half of the operated rats received secondary bilateral lesions of the remaining EC 11 days later. They underwent testing for 6 consecutive days beginning on day 13. The remaining operated rats were tested similarly without receiving secondary lesions. Another group of rats received simultaneous bilateral lesions and was tested similarly.

Progressive bilateral lesions resulted in virtually complete destruction of the medial and lateral EC, whereas medial lesions were selective. Despite intergroup differences in lesion size, the two operated groups exhibited comparable levels of locomotor activity between 13 and 18 days postlesion. Intact control rats showed lower and fairly stable levels of activity. By contrast, rats with simultaneous bilateral lesions displayed greater activity during the 13-18 day postlesion interval than did rats with progressive lesions of equivalent size. The present findings indicate that bilateral priming- and secondary-lesions of the EC produce increases in locomotor activity similar to those produced by bilateral lesions restricted to the medial EC, but that the increases are less than those after simultaneous bilateral lesions. These findings are consistent with the proposal that progressive lesions produce less severe behavioral changes by enhancing the extent and /or rate of sprouting.
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154.12 EVIDENCE THAT THE ASSOCIATIONAL AFFERENTS TO THE DENTATE GYRUS ARE CAPABLE OF RESPONDING BOTH TO THE ELIMINATION OF THE COMMISSURAL FIBERS AND THE LATER REMOVAL OF THE ENTORHINAL AFFERENTS. G.M. Peterson, D.D.M. O'Leary, and W.M. Cowan. The Salk Institute, La Jolla, CA 92037.

It has been known for some time that lesions of the entorhinal cortex or interruption of the perforant path through which the afferents from the entorhinal cortex reach the outer two-thirds of the molecular layer of the dentate gyrus, result in a prompt sprouting reaction in several of the other extrinsic afferents to the dentate gyrus. We have re-analyzed the response of the so-called hippocampo-dentate or ipsilateral associational afferents to lesions of the entorhinal cortex in young adult rats with the autoradiographic and Timm's methods, and have examined the capacity of this system to respond to an entorhinal lesion in animals in which they can be assumed to have previously expanded their distribution in response to the early postnatal elimination of the commissural afferents (O'Leary et al., Anat. Embryol., 1979, 156: 283-289; McWilliams and Lynch, J. Comp. Neur., 1979, 187: 191-198).

After injections of ^{3}H-proline into the caudal hilar region of the dentate gyrus in normal young adult rats, the associational afferents can be shown, in appropriate autoradiographs, to occupy a zone corresponding to the inner 25-28% of the molecular layer at more septal levels. Eight weeks after an entorhinal lesion comparable autoradiographs indicate that the zone occupied by the associational afferents has expanded to occupy approximately 40% of the molecular layer, but since the molecular layer as a whole is appreciably shrunken, the actual expansion of the associational afferents amounts to about 16-20%; this is comparable to the degree of expansion of the commissural afferents that occurs after similar lesions.

In a series of 16 week-old animals in which the commissural fibers were eliminated by the ablation of the contralateral hippocampus on the day of birth, and the fibers of the perforant path sectioned at 8 weeks of age, the associational fibers were again found to occupy about 40-45% of the molecular layer. Since the degree of shrinkage of the molecular layer was comparable to that in the previous experiments, the actual expansion of the associational afferents was at least as great as that following entorhinal lesions alone. This implies that the associational afferents are able to expand their distribution on at least two separate occasions, first to occupy all or most of the synaptic sites made available by the elimination of the commissural fibers and later, to occupy some proportion of the space vacated by the removal of the entorhinal afferents.

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154.13 INDUCED DENDRITIC SPROUTING AND SOMA EXCITABILITY ARE DETERMINED BY THE SITE OF AXON TRANSECTION. E. Roederer* and M.J. Cohen. Biol. Dept., Yale Univ., New Haven, CT 06511.

When the axon of an identified central interneuron of adult crickets (Acheta domesticus) is transected, the remaining arborization, axon stump and cell body undergo characteristic injury responses. We find that when the axon in the central connective is cut 200 μm or closer to the ganglion containing the dendritic arborization and cell body of the medial giant interneuron (MGI), the cell body becomes excitable by 24 hours and supernumerary neurites emerge from the arborization in the neuropil by 2 days.

The MGI has a well-defined, characteristic morphology and its cell body normally does not support action potentials. Previously, we had demonstrated for axon transection at distances greater than 200 μm the following response of the MGI: (i) Sprouts emerge from the proximal axon stump by 2 days. (ii) Dendritic morphology and afferent synaptic inputs are not obviously modified at any time following transection. (iii) By 40 days post-transection neuritic sprouts emerge directly from the soma which is normally rounded and smooth. (iv) The electrophysiological properties of the MGI cell body do not appear substantially altered.

However, if the axon is transected at a distance of 200 μm or closer to the ganglion, a different response is observed in the MGI: (i) As early as 18 hours after such close transections, the cell body can now generate overshooting action potentials. By 2 to 3 days, however, this induced excitability is lost. (ii) Substantial "die-back" of the proximal axon stump is observed, often retracting well into the neuropil of the ganglion. (iii) By 2 days post-axotomy, supernumerary sprouts emerge from the dendritic arborization, often bearing growth-cone-like swellings at their terminals. They continue to grow throughout the post-axotomy period and often take paths not normally followed by the MGI, such as peripheral nerve trunks and contralateral, central connectives. (iv) The dendritic arborization of the MGI remains excitable to synaptic input from the cercal sensory afferents at all times.

The contralateral, intact MGI served as the control and its morphology and electrophysiological properties remained unchanged.

The sprouting from the dendritic arborization induced by axotomy displayed two features: (i) Rather than emerging from random points along the arborization, supernumerary neurites preferentially arose from certain branch points, axon collaterals and some dendritic tips. (ii) The amount of dendritic sprouting induced in the MGI was roughly inversely proportional to the length of the proximal axon stump.

We conclude that the induction of dendritic sprouting and soma excitability by axotomy, depends on the distance of the axonal lesion from the soma. (Supported by N.I.H. Spinal Trauma Center Grant 2P50 NS10174-07.)

154.14 HIPPOCAMPAL CHOLINERGIC RECEPTOR LEVELS REMAIN CONSTANT IN RESPONSE TO ENTORHINAL LESIONS. D.A. Matthews and P.M. Salvaterra. Division of Neurosciences, City of Hope Research Institute, Duarte, CA 91010.

Alterations in the distribution and number of cholinergic terminals have been found in the molecular layer of the dentate gyrus following entorhinal lesions. The activity of the acetylcholine synthetic enzyme, cholineacetyltransferase (ChAT) and degradative enzyme, acetylcholinesterase (AChE) also increase. In an attempt to assess functional capabilities of this proliferated afferent system we have used micro-ligand binding assays to measure levels of cholinergic receptors. [^{125}I]α-bungarotoxin ([^{125}I]αBTX) and [^{3}H]quinuclidinylbenzilate ([^{3}H]QNB) were used to measure putative nicotinic and muscarinic receptors. Standard microassays were also used to measure ChAT, AChE and protein on the same samples. Homogenates were made by pooling hand dissected molecular layers of the dentate gyrus from individual adult male rats 165 days after unilateral lesion of the entorhinal cortex or from unoperated control animals. In agreement with other reports, both AChE and ChAT levels were significantly elevated in samples from the lesioned side compared to the contralateral control samples. However, only AChE levels were significantly higher (about two-fold) than those found in samples from unoperated animals. ChAT levels were generally only half that found in unoperated controls. No change was seen in the binding of either ligand in lesioned samples compared to either the contralateral or unoperated control samples. [^{125}I]αBTX and [^{3}H]QNB binding levels in the molecular layer were also about the same as we have previously reported for whole hippocampal samples. The short term effects of afferent removal and possible transient nature of alterations in the septal-hippocampal cholinergic system will also be examined by assays of samples obtained from animals which are allowed to survive 30 days after entorhinal lesion. Supported by NS 12116.

155.1 Varieties of Supraspinal Motor Control Established by Different Degrees of Spinal Cord Injury in Man . M.R. Dimitrijevic, J. Faganel*, W.B. McKay*, A.M. Sherwood Clinical Neurophysiology, The Institute for Rehabilitation and Research, Houston, Texas, 77030.

At least four distinct patterns of abnormal suprasegmental motor control can be recognized in spinal cord injury patients: 1) control of isolated, organized joint movement via a pool of motor neurones surrounded by proximal and distal pools exhibiting no motor control, 2), the presence of gross flexor and or extensor pattern movements via suprasegmental initiation; 3), the presence of restricted sub-clinical activation of single motor units; and 4), supraspinal modification of ongoing segmental reflex activity within paralyzed muscles. We have documented these types of motor control through the systematic study of segmental reflex response behaviors and their dependence on supraspinal mechanisms. Utilizing 12 pairs of surface EMG electrodes, with five pairs on the muscles of each leg, plus 1 each on the abdomen and lower back, we have examined more than 200 spinal cord injury patients by invoking a standardized series of maneuvers involving volitional attempts to activate their (paralyzed) muscles, passive movement of their legs, elicitation of reflexes such as tendon jerks, vibratory reflexes and clonus, and reinforcement maneuvers carried out in the non-paralyzed muscles of the upper trunk and extremities. The resulting EMG activity, recorded with a Mingograph 800 Hz bandwidth ink jet recorder, has been carefully analyzed to determine the patterns of EMG activity that can be categorized as described above. Illustrations of each pattern will be presented. The significance of these findings for neurocontrol of motor activity in man will be discussed with special regard for the function of the residual integrated descending motor control pathways.

Support was provided by the Bob and Vivian Smith Foundation, Houston Tx, the Rehabilitation Research and Training Center No. 4 (Rehabilitation Services Administration Grant 16-P-56813-6) and Rehabilitation Services Administration Grant 13-P-59275-6.

155.2 NEUROMUSCULAR PATTERNING OF A SEQUENTIAL MOVEMENT. M. C. CARTER* and D. C. SHAPIRO* (SPON: G. P. Moore). Dept. of Kinesiology UCLA, Los Angeles, CA 90024.

Previous evidence suggests that movement sequences are composed of invariant and variant timing characteristics. In various tasks, Terzuolo and Viviani (1980), Shapiro (1976;1979) and Shapiro, Zernicke, Gregor and Diestel (1981) have demonstrated that relative timing, the maintenance of the temporal relationship among a movement sequence regardless of actual movement time was maintained while the overall speed of the movement sequence varied. The present study examined whether relative timing is also reflected in the neuromuscular patterning underlying sequential movements.

Four subjects practiced a rapid movement sequence consisting of pronation and supination of the forearm to specified targets (90^{o}, 65^{o}, 35^{o}, & 60^{o}). In addition, subjects were to arrive at each target location in specific movement times (200, 100, 130 & 160 msec). Thus, the total time of the spatial-temporal pattern was 600 msec. Electromyographic (EMG) activity was recorded from the biceps brachii and pronator teres muscles by surface electrodes. After several days of practice, subjects were asked to ignore timing while increasing the movement velocity and maintaining target accuracy.

The results demonstrated that subjects significantly reduced movement time ($p < .01$) while maintaining target accuracy at each movement segment. However, relative timing was maintained across the two velocity conditions. The maintenance of the relative timing was also reflected in the neuromuscular patterning of the response since the proportion of time that each muscle was active remained constant across conditions. In addition, the proportion of time to reach peak velocity for each segment of the movement sequence was similar in both conditions. In general, the biceps and pronator-teres muscles were reciprocally activated with little or no cocontraction during the production of the response.

In summary, the invariance of relative timing is a characteristic of both the overt movement pattern and the neuromuscular pattern underlying the movement sequence. Velocity appears to be a variant property that can be imposed on the movement sequence to vary total movement time.

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155.3 SHORT LATENCY REACTION TIMES TO LOADING OF LIMB AND RESPIRATORY SYSTEMS: WITHIN AND ACROSS SYSTEM COMPARISONS. L. H. Meyerink* and R. Lansing. Dept. of Psychol. Univ. of Arizona, Tucson, AZ 85721.

Many investigators, attempting to identify late reflex (M2) responses to loading from computer averaged EMGs have assumed that learned reaction times (RT) must have latencies greater than 70 to 80 msec. Evarts and Granit (Prog. Brain Res, 1976) however reported "intended" biceps responses as early as 65 msec after loading, and we have found the minimal RTs for abdominal muscle responses to respiratory loads in the 45 to 60 msec range (Lansing & Meyerink, J. Physiol., in press).

The purpose of this research was 1) to compare minimal RT latencies of responses to limb and respiratory system loading, 2) to obtain a clear separation of learned RT and reflex components by loading respiratory and limb movements separately and requiring a response in the unloaded system, 3) to determine the dependence of EMG component latencies on the method of measurement. EMGs were recorded over abdominal and biceps muscles in 4 unpracticed college students. Latencies were measured for each of 160 trials per subject, and from computer averages of the rectified EMG for the same trials. Subjects maintained a pre-load isometric contraction of both abdominal and biceps muscles, holding a constant expiratory pressure and handle position. A sudden load (added pressure at the mouth or handle torque) was delivered 2 to 3 sec after a warning signal and the subject was told to respond as quickly as possible. In several series of trials a response with the loaded muscles was required, in others subjects were instructed to respond with the unloaded muscles.

Early stretch reflexes (15-30 msec) appeared only in biceps muscles, with handle torque loads. Clear separation of early stretch reflex and RT components was achieved by the experimental conditions, but for two subjects the RT response merged with the reflex activity. Learned RT latencies did not differ significantly according to the system loaded or to the muscles responding. The average RT, calculated from individually measured trials was 97 msec (subject means ranged from 78-128). RT latencies measured from the computer average of the same trials was 63 msec (subject range 52-108). Two subjects had very brief RTs regardless of the movement loaded or the muscles responding. Computer latencies were 52 and 67 msec; averages for all individual trials were 72 and 78 msec; over 20% of the trials were in the 40 to 68 msec range.

The results emphasize 1) the dangers of identifying intermediate latency reflexes (M2) on the basis of latency alone and 2) the importance of type of latency measure used to establish minimal RTs.

155.4 H REFLEX CHANGES PRECEDING A VISUALLY ELICITED MOVEMENT, Ann M. Baylor and Bruce R. Etnyre*. Neuromuscular Lab., Dept. PHE, Univ. of Texas, Austin, TX 78712.

Amplitude changes in the 50% H reflex were used as an index of motor pool excitability changes. These changes were a function of a randomized warning period preceding a visually elicited movement. The movement task was a rapid plantar flexion of the preferred foot following both an auditory warning signal and a visual stimulus to respond. Fourteen human males completed 15 practice trials followed by 75 test trials. Response speed was measured by the latency of initial onset of EMG in the soleus muscle. On each trial the H reflex stimulus was either omitted entirely (none, no H reflex) or Presented at one of three intervals with reference to the visual stimulus: 0 (simultaneous with light stimulus), 100 or 200 msec following visual stimulus. Trials were also taken before and after movement (pre and post movement).

Excitability as measured by the H reflex technique showed the highest values in the post movement trials condition (66%) followed by the movement conditions of 200 (57%), 0 (52%) and 100 (51%) and finally the premovement control (47%). The excitability changes as a function of the 2, 3, or 4 sec randomly presented warning interval showed a tendency to maximize at the median or 3 sec interval; however these differences were not statistically different.

Latency of onset of EMG varied greatly as a function of the H reflex presentation. The none (277 msec) and 200 msec (281 msec) responses were very similar to each other but different from the 100 (245 msec) and 0 (185 msec) responses. It seems that on many trials the shock stimulus to elicit the H reflex replaced the visual stimulus to respond and resulted in a much faster EMG response.

The latency of onset of EMG in the muscle was poorly predicted by the motor pool excitability index (H reflex). The onset of EMG was slower following a 2 sec warning than following the 3 or 4 sec warning -- a pattern which was not reflected in the amplitude of H reflex data. When percent maximum H amplitude values were correlated to latency of EMG response at each of the stimulus intervals, the correlations were all of low order and insignificant, indicating that this measure of motor pool excitability is not a good predictor of latency of EMG initiation. Separate mechanisms appear to be responsible for the H reflex amplitude changes preceding EMG in the muscle and the latency of EMG onset in this type of movement.

155.5 THE RELATION OF FORCE AND EMG TO THE PERCEPTION OF EFFORT. L.A. Jones and I.W. Hunter. Psychology Dept., and Biomedical Engineering Unit, McGill Univ., Montreal, Quebec, Canada H3A 1B1.

The concept of a sense of effort is typically related to a memory of the magnitude of a motor command sent to a muscle. It has been proposed by McCloskey (1978) and others that this sensation is employed in the estimation of weight and of the force achieved by muscular contraction. However, several authors have suggested that knowledge of the motor command itself is not sufficient for judgements of force and that afferent signals are important in the estimation of the force achieved (Roland and Ladegaard-Pedersen, 1977).

In this series of experiments a cross-limb matching paradigm was employed to investigate how human subjects matched the effort of an isometric muscular contraction. The matching ratio between the reference and indicator limbs was obtained for a range of forces (from 15% to 85% of maximum voluntary contraction) under fatigued and non-fatigued conditions. EMG recordings were taken from the biceps and triceps brachii muscles of each arm and the concomitant forces exerted were measured. Under non-fatigued conditions a linear relation was found between the force exerted and the matched force. The EMGs of the reference and indicator arms were similarly related. However, the frequently observed non-linear relation between the force produced and numerical estimates of magnitude was also found. The EMG was non-linearly related to magnitude estimates, but the force-EMG relation was linear. This linear relation was manipulated by fatiguing the muscles of the reference limb at different rates during constant force contractions. Both the reference arm EMGs and the matching ratio increased non-linearly with time. With larger reference forces the rate of these changes increased systematically. From these results we have developed a model relating force, EMG and duration of contraction from which the matching ratio can be predicted.

These results lend support to the notion that force matching and perhaps the sense of effort arise from motor commands to the muscles rather than from afferent signals.

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155.6 EFFECTS OF AFFERENT INPUTS ON DYNAMIC AND STATIC PERFORMANCE ERRORS IN HUMANS. J. N. Sanes & E. V. Evarts, Lab. Neurophysiol. NIMH, Bethesda, Md. 20205.

Many investigations of the role of afferent input occurring during movement have dealt with the effects of relatively large disturbances delivered during relatively large movements. However, there is evidence that both cutaneous and muscle receptors have high sensitivity for small signals. Thus, the effects of afferent signals in controlling movement may be seen most clearly if the afferent signals are generated by small perturbations delivered during precisely controlled small movements.

Normal subjects performed forearm pronation-supination movements in a visual tracking task by moving a handle coupled to a torque motor. Handle rotations of 30°, 10° or 3° were performed in separate blocks of 250 trials. At the beginning of each trial the target was displaced from the center and subjects moved the handle to realign their cursor with the target. After 1.2-2 s of realignment the target jumped back to the center and subjects moved the handle as quickly and as accurately as possible to realign the cursor. For 1/2 of the trials the visual display was blanked and on 1/6 of the trials the limb was perturbed for 100 ms; both events occurred when the handle moved beyond the hold zone. Effects of 3 perturbations were studied --(1) a stop, (2) ramp stimuli opposing movement and (3) ramp stimuli assisting movement. The ramps moved the handle forward/backward 1/3 of the total movement size in 100 ms. Error of the handle position relative to movement size was measured when handle velocity first reached zero (dynamic error) and 500 ms later (static error).

In the absence of visual feedback, relative dynamic and static error increased as movement amplitude decreased. Perturbations stopping or opposing handle movement increased percentage error and the increases were greater for smaller movements. Perturbations that assisted handle movement did not alter dynamic error but static error was changed in a manner similar to that seen following other disturbances. The final handle position for large movements tended to be overdamped whereas the handle position for small movements was typically underdamped. The underdamping of small movements was exacerbated by perturbations.

These findings demonstrate that peripheral disturbances modify movement accuracy, with small movements being more affected than large displacements. The mechanism responsible for the effects may be recruitment of a significantly greater proportion of motor units following perturbations delivered during small movements. The source of excitation may be both segmental and supraspinal since neural elements in both regions are sensitive to small afferent signals.

155.7 EMG RESPONSES TO FORCE PERTURBATIONS PRECEDING ACCURATE ARM MOVEMENTS IN HUMANS. Susan H. Brown and J. D. Cooke, Dept. of Physiology, Univ. of Western Ontario, London, Ontario, Canada.

Recent investigations in this laboratory have shown that the triphasic EMG pattern associated with rapid limb movements is also characteristic of slower, more accurate movements. It has also been shown, for fast movements, that the late phase of the initial agonist EMG burst can be modulated by force perturbations applied prior to movement onset with no change occurring during the early part. The present experiments were designed to determine the effects of perturbations applied prior to the onset of slower, accurate movements.

Experiments were performed on normal human subjects performing a visual step-tracking task involving flexion-extension movements about the elbow. Emphasis was placed on accuracy in reaching the target rather than on speed. In randomly selected trials, brief (50 msec) torque pulses were applied following the change in target position but before movement onset. Perturbations either opposed or assisted flexion (flexion load and flexion unload).

The initial agonist burst of unperturbed flexion movements often showed two components: an initial component lasting 75±12 msec duration immediately followed by a second component 74±12 msec in duration. As seen previously for fast movements, unloading prior to onset of these slower, accurate movements resulted in a reduction in the magnitude of the late component. In some cases this component was completely lacking. Conversely, loading forces increased the magnitude of this late component. In general, the magnitude of the late component was proportional to the magnitude of the force perturbation. The early component of the initial agonist burst showed a marked increase in magnitude during both loading and unloading of these accurate movements. This was in contrast to the lack of modulation of this early component during fast movements.

The results suggest that the initial agonist burst of the triphasic EMG pattern is, in fact, comprised of two separate components and that the degree to which the early component can be modulated is dependent upon movement strategy.

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155.8 MODIFICATION OF MOTOR OUTPUT TO COMPENSATE FOR UNEXPECTED LOAD CONDITIONS DURING BALLISTIC MOVEMENTS. R.G. Lee, G.E. Lucier and D.G. White*. Neuroscience Research Group, Faculty of Medicine, University of Calgary, Calgary, Alberta, Canada T2N 4N1

Twenty normal human subjects were trained to perform rapid wrist flexions to move a cursor to a specified position on an oscilloscope screen. These movements were performed repeatedly against a constant small load generated by a torque motor. When the subjects had "learned" the program to produce the required movement against the predicted load, the torque was increased prior to movement on a small percentage of randomly selected trials. Since the task was initiated with the manipulandum resting against a mechanical stop, the subjects had no cue that load conditions had changed until after the movement commenced. Wrist position, torque, and EMG activity from agonist and antagonist muscles were averaged and patterns associated with movement against each of the load conditions were compared.

When the load was greater than predicted the velocity of the movement was slightly decreased and there was a compensatory increase in the EMG output from the agonist muscle. This occurred approximately 20-25 msec. after the onset of movement or 30-40 msec. following the earliest change in the torque recordings associated with the two load conditions. At the same time there was a decrease in the EMG burst from the antagonist muscle. The magnitude of the compensatory increase in agonist EMG activity was linearly related to the amount of difference between the predicted and the actual load.

The latency of this compensatory change in EMG output is compatible with a monosynaptic reflex and suggests that it is mediated by spinal rather than long loop mechanisms. Since the increase in EMG output occurred at a time when the muscle was being shortened, it cannot be considered a stretch reflex in the true sense. However increased discharge from spindle afferents could occur as a result of a mismatch between gamma motoneuron output and the expected velocity of the movement. These observations suggest that mechanisms exist at the spinal level to allow rapid modification of motor programs when unexpected load conditions are encountered during ballistic movements.

155.9 POSTURAL ADJUSTMENTS ASSOCIATED WITH PRECISE ARM MOVEMENTS IN FREELY STANDING SUBJECTS. L. M. Nashner and P. J. Cordo. Neurol. Sci. Inst., Portland, OR 97209.

When a freely standing subject pulls upon an external object, the forces acting between the subject and the object upset the distribution of support forces which maintain equilibrium. We and a number of other investigators reported earlier that postural adjustments helping to maintain equilibrium anticipate the force perturbations caused by reaction time arm pulls. Furthermore, we reported that the linkages between voluntary and associated postural activities are "set" dependent and are mediated by rapid acting, low level motor circuits.

If a freely standing subject is asked to produce a precise force waveform while standing freely, arm and associated postural activity must both be precisely modulated. If the associated postural adjustments are imprecise, shifts in the orientation of the subject will disturb the accuracy of the arm pull. We have compared the tracking accuracy of subjects following step and ramp waveforms while standing with postural support and while standing freely in order to determine the role played by posture in the execution of precise, focal movements.

Subjects were given a compensatory visual display (force error deflected a horizontal line upward or downward on an oscilloscope screen) and asked to keep the force trace aligned with a fixed target. At random times, the error trace was deflected (step or 1 sec. ramp), and the subject was required to pull on the handle to restore it to the target position.

Despite the increased organizational complexity required to follow a precise force waveform while freely standing, subjects could perform this task as quickly and as accurately during free standing as during supported trials. While postural muscles were quiescent during supported trials, they were activated in anticipation of the arm movement. Furthermore, the torque waveform produced by postural muscles against the support surface was coincident with or slightly advanced in time and precisely mirrored the force trajectory produced by the arm.

We argue that set dependent coordination of intentional motor and associated postural activities enable the subject to execute pulls with equal precision while performing under different configurations of postural support. Linkages between postural and focal muscles combine motions of the various body parts into a fixed functional unit. By establishing the appropriate temporal and metrical relationships between focal and postural activities in advance of movement, waveform tracking can be accurately executed under freestanding conditions.

155.10 Are Motor Units Fully Activated During Strong Effort?
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It is still not known whether motor units are fully activated during maximal voluntary contractions. One approach to the problem would be to compare the force of a maximal voluntary contraction with that developed during tetanic stimulation of the same muscle. Although simple in concept, the method is fraught with technical difficulties and is uncomfortable for the subject. We have therefore preferred the following technique (Merton, P.A., J. Physiol., Lond., 123:553, 1954): during a strong effort a single maximal electrical stimulus is delivered to the appropriate motor nerve. In theory a twitch should be superimposed on the force recording only if some motor units have either not been recruited or are discharging at less than their tetanic fusion frequencies. The validity of this method has been established in various ways and tests have been conducted on the ankle plantarflexor and dorsiflexor muscles of 28 healthy men and women aged 19-45 years. Measurement of muscle contraction was made with a specially designed leg-holding device (Marsh et al, J. Applied Physiol., 1981, in press). Plantarflexor muscles (PF) were excited through the tibial nerve at the popliteal fossa whereas tibialis anterior (TA) was stimulated indirectly over its muscle belly. It was found that full activation of TA could be achieved quite easily in all subjects (27/28); in contrast, PF activation was not quite maximal in 14 subjects despite the greatest effort having been made. A possible explanation for the incomplete activation of PF muscles would be that the PF motoneurones, although receiving strong inputs from homonymous muscle spindles, have less powerful synaptic connections from volitional motor pathways.

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155.11 NEUROMUSCULAR CONTROL OF MAXIMUM HEIGHT JUMPING IN HUMANS WITH ALL JOINTS VOLUNTARILY CONSTRAINED FROM MOTION EXCEPT THE ANKLE. F.E. Zajac[1,2], W.S. Levine[3], M.R. Zomlefer[1], M. Belzer*[3]. Rehab. Eng. Res. and Dev. Ctr. (153), VA Medical Ctr., Palo Alto, CA 94304[1], Mech. Eng. Dept., Stanford Univ., Stanford, CA 94305[2], Elect. Eng. Dept., Univ. of Maryland, College Park, MD 20742[3].

This constrained jump was chosen for study in order to develop a theoretical basis for understanding muscle coordination patterns needed to jump maximally, given that the jump may start from one of many initial postures. This jump can be modeled by a 2-segment inverted pendulum controlled by neural activation of lower leg musculature, whereas models of unconstrained jumps are more complex. Optimal control theory predicts that the jump must be partitioned into three epochs regardless of the details of the muscle model. In the first epoch, with the feet flat on the ground any one of many ankle torque trajectories can be used to statically position the center of body mass over the toes and any one of innumerable muscular activation patterns would suffice. In the second epoch, leg muscles must develop a unique, but less than maximal, ankle extensor torque to propel the upper body to an optimal position and velocity. Again, there exist many activation patterns that could be used.

During the third and final epoch of propulsion the heels must be off the ground, ankle flexor muscles must be de-activated and ankle extensor muscles must be fully activated until lift-off. Ground force, body trajectory (using a Selspot system) and medial gastrocnemius and tibialis anterior intramuscular activity were recorded. We found that for the third epoch of propulsion to be consonant with physiological observed forces, trajectories, and calculated ankle torques, both the force-length and lag properties of muscle must be accurately specified. However, these muscle properties severely degrade performance and there is no way by which the nervous system can provide compensation, even with a priori knowledge of these actuator properties, since theory predicts and experiment suggests that ankle extensor muscles are already subjected to maximum activation. For the prior two propulsion epochs, compensation for these muscle properties is possible, and apparently occurs, via either anticipatory or feedback-controlled neural commands.

Supported in part by NIH NS 11971 and Swedish MRC 3026 and 5402. Experimental work was conducted at the Karolinska Institute (Prof. S. Grillner).

155.12 ABSENCE OF NEUROMUSCULAR BLOCK IN FATIGUE OF MAXIMUM VOLUNTARY ISOMETRIC CONTRACTIONS. B. Bigland-Ritchie, C.G. Kukulka and J.J. Woods*. John B. Pierce Foundation and Quinnipiac College, New Haven, CT. 06519.

Merton (1954) found no change in the size of muscle surface mass action potentials (M waves) evoked by single maximal shocks to the motor nerve during fatigue of sustained maximum isometric voluntary contractions (MVC) of the adductor pollicis muscle. From this he concluded that loss of force is not due to progressive neuromuscular block. This observation has been confirmed, also on adductor pollicis, by Bigland-Ritchie, Jones and Woods (1979) and by Bigland-Ritchie and Lippold (1979); but it is at variance with that of Stephens and Taylor (1972). Using similar techniques on the first dorsal interosseous (FDI) muscle they found a decline in the M wave and considered that neuromuscular block was a major factor in this type of fatigue. We have repeated these experiments on both the adductor pollicis and FDI muscles using intramuscular as well as surface recording. When supramaximal stimulation was assured and the whole potential area measured, all changed in a similar manner and no decline in response was seen. A slight slowing of conduction velocity increased the potential duration without loss of amplitude, increasing the total area by some 30% during 60s MVC. This may account for the apparent decline observed by Stephens and Taylor who measured the area only over a fixed time interval. Cross contamination by potentials from other muscles also innervated by the ulnar nerve but not involved in the voluntary fatiguing contraction is an unlikely source of error. With surface recording some cross contamination between either FDI and/or adductor pollicis and the adjacent opponens pollicis was seen when the ulnar and medial nerves were stimulated alternately, but not when the recording was intramuscular. Any cross contamination between FDI and adductor pollicis would not, in any case, influence our results since co-contractions fatigued both muscles simultaneously. For both muscles we confirm a progressive reduction in surface EMG during this type of fatigue but cannot attribute this to neuromuscular block. It must, therefore result from a reduced motor drive from the central nervous system. The effects on force production will be discussed.

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155.13 MOTOR AFTER-EFFECTS OF SHORT-TERM VOLUNTARY MOVEMENT. D.G.D. Watt. Aviation Med. Res. Unit, McGill University, Montreal, Canada, H3G 1Y6.

Following prolonged exposure to moving environments (boats, aircraft), many persons experience sensations of self-motion which may come and go for up to several days. Craske has demonstrated recently that involuntary decaying oscillations of the arm follow a mere 80 sec of alternating isometric flexion and extension of that limb. The present experiments were designed to test the functional significance of motor after-effects during normal locomotor activity.

It is known that subjects asked to hop rhythmically up and down on both feet without external cues will do so at a particular and reproducible preferred frequency, typically about 2.2 times per sec. In these experiments, 4 subjects were asked to hop steadily for 1 minute in synchrony with a periodic auditory cue adjusted to 10% below or above their previously measured preferred hopping frequencies. The effect of this forced hopping on their preferred frequencies was then tested repeatedly for up to 8 minutes.

The preferred hopping frequency of each subject was lowered significantly following forced hopping at a lower rate (from an average 2.20Hz to an average 2.06Hz, $P<.001$), and increased significantly following forced hopping at a faster rate (from an average 2.20Hz to an average 2.25Hz, $P<.001$). In both cases, the effect diminished systematically, but was still present 8 minutes after forcing. The effect of forced hopping at a slower than normal rate was always greater than the effect of forced hopping at a faster than normal rate, but this may simply reflect the fact that all subjects were exposed to slower and than faster hopping, and the former may have influenced the latter.

These findings suggest that certain kinds of voluntary movement are not only the result of what an individual wants to do, but also depend on what he has just done. This is compatible with theories that postulate an internal model for motor control that generates motor commands as required, but which is continuously updated on the basis of recent experience. According to the present results, these changes occur with a time constant of minutes. This "fine-tuning" of motor programs could explain the effectiveness of a short warm-up period just before an atheletic or musical performance.

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155.14 ALPHA-GAMMA BALANCE DURING HUMAN VOLUNTARY ACTIVITY. B. T. Shahani and R. R. Young Clinical Neurophysiology Laboratory, Massachusetts General Hospital, Boston, Ma 02114.

Although it has been reported that there may be a rise in fusimotor activity preceding alpha activity in some instances in experimental animals, there is no evidence that spindle sensitization precedes voluntary extrafusal muscle contractions in man. In order to determine whether or not there is appropriate balance between skeletomotor and fusimotor systems during graded voluntary activity, unloading reflexes were recorded from biceps brachii muscles of 5 healthy volunteers. Subjects, seated in a comfortable chair and resting the arm on the table, were asked to pull, at different times, against 1, 2, 3 and 4 Kg weights attached by an electromagnet which could be electronically de-energized. EMG activity from biceps and triceps muscles, a marker signalling release of the magnet and angle of displacement at the elbow joint were displayed on the oscilloscope and permanent records (4 superimposed traces) were made with Polaroid film. Duration of the silent period (SP) produced by unloading was relatively constant for each individual and ranged in different subjects from 31 ± 3.5 msec to 65 ± 5 msec. when a 1 Kg weight was released. Although all showed a tendency for SP duration to increase ($p < .01$ only in one) when heavier weights were released, these changes were not statistically significant. In one subject (who had great difficulty holding to heavier weights) release of 4 Kg weight produced SPs of shorter duration (71% of SP produced by 1 Kg release). Since this SP is presumed to be due to withdrawal of Ia input and the motor activity following the SP due to renewal of spindle afferent discharge, it is suggested that during moderate voluntary contraction there is appropriate alpha-gamma balance whereas during maximal voluntary effort the increase in fusimotor activity may be out of proportion to the alpha activity resulting in shortening of the SP and early resumption of motor activity. Our preliminary data also suggest that Ia facilitation is more important for maintaining alpha motoneurones at their firing level during moderate voluntary contractions rather than during minimal and maximal effort.

155.15 MECHANISMS OF TRAJECTORY FORMATION IN INTACT AND DEAFFERENTED MONKEYS. E. Bizzi, N. Accornero*, W. D. Chapple and N. Hogan*. Dept. of Psychology, M.I.T., Cambridge, MA 02139.

The formation of forearm trajectories was studied in monkeys performing a simple visuo-motor task. Recently, it has been proposed that the transition from a given position to another may occur whenever the central nervous system generates a signal shifting the equilibrium point between opposing muscle groups by selecting a new set of length-tension curves. In these experiments we have studied the time-course of this transition. The monkeys were seated in a primate chair with the right arm strapped to a splint which allowed rotation of the forearm about the elbow in the horizontal plane. Ten target lights were placed along a perimeter centered on the axis of rotation of the elbow. To obtain a reward the monkey had to point to an electrically defined target (10°). During the experimental sessions an opaque cover was placed over the arm to prevent the animal from seeing the moving arm. We performed a bilateral section of the dorsal roots from C1 to T3. After deafferentation, the forearm visuo-motor responses which had been learned in the preoperative state could be evoked by presenting the targets.

In deafferented animals the position of the arm was displaced and maintained in a new location by the action of a torque motor. After completion of a displacement, a target light was turned on at the location corresponding to the new arm position. After the appearance of the agonist EMGs in the muscles, the arm was released. We observed that the arm moved first towards the position from which it had initially been displaced, then changed direction and returned to the position specified by the target light. While the to-and-fro movement took place, the agonist muscle developed an EMG comparable to that observed during undisturbed movements. This cannot be explained if the muscles are regarded as pure force generators, but is readily explained if the length dependence of muscle force is taken into account.

In additional experiments in both intact and deafferented animals the limb was clamped and released at various times after the onset of EMG activity. We measured the acceleration of the limb immediately after the release and the isometric force during the holding. Both acceleration and isometric tension showed a gradual increase with time.

Taken together, these findings suggest the existence of a gradually changing control signal during movement of the forearm from one position to the next and are not consistent with the view postulating a step-like shift to a final equilibrium point. (Research supported by NIH grants NS09343, NS06318 and EY02621.)

155.16 TRAJECTORIES OF BALLISTIC MOVEMENTS CAN BE ALTERED BY MODIFYING ANTAGONIST MUSCLE ACTIVITY. D. S. Hoffman and P. L. Strick. V. A. Med. Ctr. and Depts. of Neurosurg. and Physiol., SUNY-Upstate, Syracuse, NY 13210.

Ballistic movements are initiated by a burst of agonist muscle activity which precedes movement and are decelerated by a burst of antagonist muscle activity which occurs at or just after movement onset. Prior studies have demonstrated that the amplitudes of both bursts of muscle activity are directly related to movement velocity. These interrelations have suggested that the antagonist burst might be generated by the same central mechanisms which generate the agonist burst and/or by reflex mechanisms related to peripheral feedback. The present experiments were designed to test whether the magnitude of the antagonist burst could be uncoupled from the magnitudes of both the agonist burst and the initial parameters of movement.

Human subjects were asked to perform a visual reaction time task in which ballistic wrist movements (20 degrees of ulnar deviation) were made to a visual target. After a series of 20 degree movements, a "trick" trial was presented to the subject. The visual target for these trials unexpectedly required only a 10 degree movement. Muscle activity was recorded from extensor carpi radialis longus (ECR) and extensor carpi ulnaris (ECU) while subjects repeatedly performed this task.

As expected, the magnitudes of both the agonist burst in ECU and the antagonist burst in ECR were directly related to movement velocity for the 20 degree trials. Agonist bursts and initial movement parameters on some "trick" trials and some 20 degree moves were identical. In contrast, the antagonist burst on the same "trick" trials was larger (in some cases nearly doubled) and earlier (as much as 10 msec.) than that observed with 20 degree moves of comparable initial velocity. The changes in the antagonist burst during the "trick" trials resulted in a greater and earlier movement deceleration than was observed during the 20 degree moves. Thus, for "trick" trials there was a clear dissociation between the magnitude and timing of the antagonist burst and the magnitude of both the agonist burst and the initial movement parameters.

These observations indicate that the antagonist burst is not determined solely by kinesthetic feedback and may be modulated independent of changes in agonist muscle activity. Furthermore, the trajectory of a ballistic movement can be modified shortly after movement onset by altering the magnitude and timing of the "brake" applied by antagonist muscle activity. Supported by funds from the V.A. Medical Research Service.

155.17 RAPID VOLUNTARY ARM MOVEMENTS IN MAN: ELECTROMYOGRAPHY AND PERFORMANCE. P. J. Cordo and L. M. Nashner, Neurol. Sci. Inst., Portland, OR 97209.

Electromyographic (EMG) signals associated with rapid (reaction time) arm flexions in man, monkeys and cats have been previously described by a number of other investigators. The relationships between these muscle activation patterns and peformance are still somewhat controversial. We have studied a variety of visually triggered rapid arm movements, some of which required force tracking and others which were not metrically constrained, to determine the relationships between activity of involved muscles and performance.

Adult subjects stood against a chest-height support and pulled and pushed on an isometric handle by flexing or extending the elbow joint. Multiunit, filtered EMG was recorded from the biceps and triceps brachii muscles during the following visually-triggered tasks: 1) rapid flexion with no end-point specification, 2) rapid flexion followed by rapid extension also without metrical specificity, and 3) step and ramp tracking using a visual compensatory display.

Rapid flexions and extensions with unspecified metrics (the first two paradigms) were executed with approximately synchronous coactivation of biceps and triceps. Single, 100-150 msec phasic bursts of EMG in both muscles were associated with movement in each direction. EMG associated with the two tracking tasks was also highly stereotyped. In these tracking paradigms, biceps activation (EMG) was more sustained in character with phasic bursts occurring only with overshoots. Furthermore, changes in the level of triceps activity were minimal during the movement. In all movement paradigms, modulation of the level of activation in both muscles often appeared to be synchronized, as if both muscles were driven with the same control signal. The accuracy of tracking (performance) was also clearly correlated with the associated patterns of muscle activation. We conclude that, with consideration of the mechanical properties of the involved musculature, movement performance may be predicted from patterns of muscle activation or, conversely, the functional role of segments of EMG activity in the performance of accurate movements may be evaluated. Furthermore, the synchronous patterns of activity in the agonist and antagonist musculature suggest that the movement commands to the muscle pair have common organizational properties.

155.18 ELECTROMYOGRAPHIC ANALYSIS OF THE FINAL POSITION HYPOTHESIS. J. Matheson*, A. Berardelli* and M. Hallett. Lab. of Clinical Neurophysiology, Brigham & Women's Hospital, Boston, MA 02115.

Ghez has proposed that rapid limb movements can be analyzed into successive pulse and step phases, the pulse specifying the dynamic features of the movement and the step specifying final position. Recent investigations by Bizzi and colleagues have suggested that final position of a limb is specified by a relationship in the activity of the antagonist muscles acting about a joint. The following experiments were undertaken to see if the complex EMG activity in biceps and triceps following the initial "triphasic pattern" of a ballistic elbow movement could be explained on the assumption that this activity is specifying the final position of the limb.

EMG activity was recorded with surface electrodes from biceps and triceps, rectified and integrated in 100-500 ms windows. The arm was held in a light splint with the shoulder at 90° and the forearm fully supinated. Elbow position was measured using a potentiometer in the splint. The arm was held at several specified angles and EMG at each angle was recorded during different levels of activity. These different levels were achieved by voluntarily altering the amount of co-contraction. For each angle there was a clear relationship between the amount of biceps and triceps activity and this relationship changed for each angle.

Subjects then made short and long, ballistic elbow flexion and extension movements using as final positions the same angles as used during the holding task. Slow movements were also studied. EMG activity after the limb arrived at the final position was analyzed and compared to the EMG obtained during the holding task. The relationship was sometimes the same in the two circumstances, but often it was different. It appears that features in addition to final-position control play a role in setting the level of EMG activity in the seconds immediately following a dynamic movement.

155.19 MUSCLE MOLECULAR MECHANISMS: IMPLICATIONS FOR MOTOR PROGRAMS. P.Dev. Rehab. Eng. R&D Ctr., V.A. Medical Ctr., Palo Alto, CA.

Crossbridge models of muscle have been shown to possess emergent properties of force generation, viscosity and series elasticity (Huxley, 1957). We show that these models provide insight into muscle and load dynamics during the translation of different motor programs into movement.

A simplified, two-state, crossbridge model of muscle was selected. The intensity of neural input determines the fraction of attachment sites available. To simulate a movement, a limb was modelled as a mass with two opposing muscles, agonist and antagonist.

We postulate two kinds of motor programs -- timing programs and equilibrium setting programs. In a timing program, the temporal pattern of neural input to the muscle is shown to be significant in starting and stopping a movement. To highlight this effect, we hypothesize that isometric muscle tension has no dependence on length. In the equilibrium setting program, we assume that muscle tension does depend on length, i.e. the fraction of available sites depends on muscle length, and that the input intensities to the two muscles specify the equilibrium position. Timing Programs . Three elements of neural input patterns were investigated : 1) a pulse of increased input to the agonist muscle, 2) a pulse of decreased input to the antagonist, 3) a braking pulse of increased input to the antagonist. Increased input to the agonist alters the balance of agonist and antagonist crossbridges and accelerates the movement. Cessation of this increased input is sufficient to halt the movement eventually as the crossbridge balance is restored. The amplitude of the movement depends on the duration of the agonist pulse and on the final input intensities to the two muscles. While a braking pulse is not necessary to halt the movement, it is extremely effective in halting a fast movement or the movement of a large mass. Equilibrium Setting Programs. The input patterns were a maintained increase in agonist input and a maintained decrease in antagonist input. This pattern is effective in initiating movement. The specified equilibrium position is approached asymptotically, and the movement duration is considerably longer than occurs for timing programs.

Therefore, both kinds of programs can generate complete movements. For muscles in which tension depends on length, equilibrium setting is necessary to specify a position to be maintained. However, the addition of timing programs supplies control over the speed with which the equilibrium position is approached.

Huxley, A.F., Prog. Biophys. Biophys. Chem., 7:257, 1957.

155.20 KINEMATICS OF POINTING AND GRASPING MOVEMENTS. F. Lacquaniti* and J. F. Soechting. Lab. Neurophysiol., Univ. Minnesota Med. Sch., Minneapolis, MN 55455.

Arm movements involving forward projection of the hand toward a target were studied in two experimental conditions. In the first task, the subjects performed a pointing movement so as to touch one of four targets presented on a TV screen with their index finger. The movement involved two degrees of freedom (forward flexion at the shoulder and extension at the elbow). The second task was to reach for and grasp a handle at different orientations: the subjects started with the hand supinated and had to pronate it (or vice versa) during the movement in order to grasp the handle. Elbow and wrist angular positions were measured goniometrically and shoulder angular position was estimated indirectly. For each target location the relation between elbow and shoulder instantaneous angular position proved to be consistent and was independent of movement speed. Furthermore, this relation was not appreciably affected by the presence or absence of wrist rotation. During the deceleratory phase of the movement the ratio of elbow angular velocity to shoulder angular velocity was constant and independent of target position. These results indicate that the motions at the elbow joint and the shoulder joint are coupled and one can conclude that such a link comes about because of the inertial coupling between the motion at the two joints and the modality of its central control. On the contrary, no invariant relationship exists between wrist rotation and elbow or shoulder movements. The rotation of the wrist (in both pronatory and supinatory directions) appeared to be a multiphasic movement highly variable in timing and duration. However, it can not be said that wrist rotation is unrelated to the elbow movement; on the basis of our data (including the pattern of EMG activity of some of the muscles involved in wrist rotation) it appears that wrist rotation is constrained by the presence of simultaneous elbow movements, the constraint deriving from the bifunctional nature of most of the muscles involved.

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156.1 ACTIVITY OF VESTIBULAR NUCLEI NEURONS AFTER FLOCCULECTOMY DURING VISUAL-VESTIBULAR STIMULATION IN THE MONKEY. Walter Waespe* and Bernard Cohen, Dept. of Neurology, Mount Sinai School of Med., New York, N.Y. 10029.

Recordings of neuronal activity in the vestibular nuclei and in the flocculus suggest complementary processing of vestibular and visual (optokinetic) information (Waespe & Henn, Exp. Brain Res., 1981, in press). Each structure is tuned to specific stimulus ranges. These results suggest that modulation of vestibular nuclei neurons during visual-vest. interactions would not be dramatically affected by flocculectomy. This postulate was tested in two monkeys in whom the flocculus was removed on both sides, and recordings were done in the vestibular nuclei. Stimuli consisted of rotation in darkness (vestibular stimulation), rotation of the visual surround around the stationary monkey (optokinetic stimulation), rotation in a lighted stationary surround (combined stimulation) and rotation of the monkey and the visual surround together in the same direction (conflict stimulation). Twenty horizontal type I neurons, i.e. neurons that were activated during ipsilateral rotation and inhibited during contralateral rotation, were recorded. Sensitivity of the type I neurons to vestibular stimulation was low. No horizontal type II neurons or omnipause type I cells with a high vestibular sensitivity were found. These omnipause neurons are typical for the medial vestibular nucleus and most likely project to the flocculus. Firing rates of the type I neurons were modulated during optokinetic stimulation in the usual way. They increased their activity during contralateral drum rotation up to velocities of 60^{o}/sec. The time to the build up of steady state frequencies was very long, up to 40-60 sec, paralleling the development of OKN slow phase velocity after flocculectomy. During OKAN, neuronal activity decayed in parallel with slow phase velocity. During conflict stimulation the decay time constant of neuronal activity and of nystagmus was shortened to less than 8-10 sec. The results indicate that flocculectomy does not dramatically alter activity of type I cells in the vestibular nuclei. These cells seem capable of processing visual information in the absence of floccular projections. Steady state values during optokinetic stimulation were similar in flocculectomized as in normal animals, but a longer time was needed to reach these values. The fact that no type II horizontal and no omnipause type I neurons with high sensitivity were found is an interesting but preliminary finding. It can be explained by assuming that the vestibular neurons which project to the flocculus retrogradely degenerate after lesions of this area.

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156.2 LACK OF TOPOGRAPHIC SEPARATION OF SEMICIRCULAR CANAL AND OTHER VESTIBULAR AFFERENTS IN THE PIGEON'S VESTIBULO-CEREBELLUM. I.E. Schwarz* and D.W.F. Schwarz, Lab. of Otoneurology, Dept. of Otolaryngology & Physiology, Univ. of Toronto, Toronto, Ontario.

We have previously shown that directional information about rotational planes of head movements is encoded in firing-patterns of cerebellar neurons more precisely than in vestibular afferents coming from semicircular canals. It was, therefore, interesting to know if rotational planes are topographically represented in the pigeon's vestibulo-cerebellum. Since all mossy fiber afferents originating in one semicircular canal represent the same head rotation plane, we investigated if there is a topographical segregation of mossy fibers originating in different organelles of the labyrinth. A number of tracers were used to achieve the necessary transneural labeling, but only tritiated leucine yielded sufficiently strong clean mossy fiber labeling patterns. 50 uC of ^{3}H leucine in 0.5 ul were injected into one crista of either the lateral, anterior or posterior canal or into the macula-utricui. After one day survival the pigeons were fixed by transfusion and frontal serial sections were processed for radioautography. Labeled mossy fiber rosettes were semiautomatically plotted on section charts in order to permit precise topographic mapping. Controls consisted of injections of the same dosage, a few hundred um away from the crista in either the perilymphatic space or the endolymph. No labeled rosettes were found under these conditions, indicating that label injected into one crista could not have been effectively spread to other cristae. Injections into each crista resulted in similar spatial distributions of labeled rosettes. Only a minority of all rosettes was labeled in all cases, however, the rosettes were distributed throughout the ipsilateral side of the total vestibulo-cerebellum with no spatial concentrations for individual canal afferents. Utricular injections resulted in virtually identical distributions and injections into the cochlea yielded fewer labeled rosettes which appear randomly distributed throughout the ipsilateral vestibulocerebellum. These can be attributed to labeled lagenar afferents. It must be concluded that mossy fibers signaling specific movement directions and planes are not concentrated in specific regions of the vestibulocerebellum. It is unknown if cerebellar neurons carrying sharp plane information are orderly arrayed, however, it can be assumed that each of the highly plane and directionally specific neurons requires information from many if not all vestibular afferent categories.

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156.3 HORSERADISH PEROXIDASE AND GOLGI INVESTIGATIONS OF NUCLEUS Y IN THE MOUSE. C.D. Smith*, A.F. Scoville, M.H. Frederickson, and C.J. Frederickson (Spon: G. Moushegian). Dept. of Psychology, Univ. of Texas at Dallas, Richarson, Tx. 75080.

Brodal and Pomeiano (J. Anat., 1957, 91:438) were first to separately identify (as area Y) the aggregate of vestibular neurons which lie just dorsal to the restiform body, and it was Gacek (Acta Oto-laryngol., 1969, Supp. 254:1) who showed that the primary afferents from the saccular statolith organ project extensively to that vestibular region. As a prelude to study of the effects of altered statolith sensory input upon the structure of second-order neurons of the Y nucleus, we have examined the Y nucleus in normal, adult mice using HRP and Golgi methods.

HRP was used to label the primary vestibular afferents from the saccular macula. To label the afferents, we made stab wounds in the saccular neuroepithelium, perfused the vestibule with HRP solution, and then (after 24 hr survival) reacted the brain-stem tissue with TMB. In the resulting material, the labeled fibers showed the typical, vivid, Golgi-like anterograde staining, and could be readily followed to the Y nucleus. The Y nucleus was distinctly demarcated by the dense plexus of fine, afferent fibers and preterminal fibers which ramified extensively within the region. In coronal sections, the Y nucleus appeared as a small triangle (about 250 μm across) lying on top of the restiform body with the lateral vertex adjacent to the glial cap of cochlear nucleus and the medial vertices oriented along a vertical line just at the lateral margin of the lateral vestibular nucleus.

In the Golgi material, Y neurons proved to be rather homogeneous. The cells were generally small (12 - 20 μm), typically either bipolar, with a fusiform soma, or tripolar, with a more spherical soma. The few, slim primary dendrites branched sparsely, most often into only two daughter branches. Dendrites were generally short (100 - 200 μm) and rarely extended beyond the boundaries of the Y nucleus; dendrites approaching the borders of the Y area were frequently observed to curl away from the border, back into the nucleus. Exceptions to the latter rule were dendrites which descended ventrally and laterally (amidst the ascending primary afferent fibers) along the medial margin of the restiform body.

Fine, hairlike appendages, classical "lollipop" spines, and synaptic crests were all in evidence on the dendrites of Y neurons, although, except at the extreme distal tips, the appendages were not particularly numerous. Both spines and filamentous hairs were identified on the somas of Y nucleus neurons. (Supported by NSF)

156.4 VESTIBULAR CENTERS IN THORNBACK RAY: EVOKED POTENTIAL AND UNIT RECORDING. Wolfgang Plassmann*. (SPON: H.H. Zakon). Neurobiol. Unit, Scripps Instit. Oceanog. and Dept. Neurosci., U.C.S.D., La Jolla, CA 92093.

Little is known about central processing of vestibular input in the elasmobranchs. This study locates vestibular nuclei and pathways at different brain levels and undertakes to distinguish among vestibular modalities represented in these areas in Platyrhinoidis triseriata.

Electrical stimulation of nerve VIII or of individual vestibular nuclei in the medulla and recording of evoked potentials leads to the following findings: (a) the anterior (AN), magnocellular (MN), and descending nuclei (DN) are connected with their opposite medullary counterparts via commissural fibers; (b) a certain vestibular area of the torus semicircularis (TS) receives its main input from AN and MN via the lateral lemniscus; (c) the oculomotor nucleus (OMN) gets vestibular information from AN and MN via the medial longitudinal fasciculus; (d) vestibular input reaches a contralateral diencephalic area as well as the upper and lower leaves of the corpus cerebelli.

Delivery of pulse trains with varying stimulation frequencies reveals at least three response types. The first type is mainly found immediately dorsal to MN, in the dorso-medio-caudal part of AN, and in TS; it responds to stimulation frequencies up to 5/s. The second type occurs predominantly in DN and MN, in TS, and in the cerebellum, while responding to frequencies up to 50/s. The third type responds up to 100/s and preferably occurs in AN and OMN.

Unit recording during presentation of various natural stimuli allows one to establish areas of preference for different vestibular modalities. Pitch and yaw produce phasic responses of neurons located mainly in AN, in a medial portion of DN, and in OMN. Static changes in head position generate a phasic-tonic response in neurons throughout DN. Neurons highly sensitive to vibration stimuli occur predominantly in a region dorsal MN which continues into a caudo-medial portion of AN. They can also be found in some areas of DN and TS.

The findings suggest that TS and OMN receive vestibular input via AN and MN. The information passed on to them, however, appears to be partly different. DN is apparently not involved at this level.

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156.5 A MODEL OF THE VESTIBULO-COLLIC REFLEX. R. J. Peterka* (SPON: D. L. Tomko). Div. Physiological Acoustics, Dept. Otolaryngology, Univ. Pittsburgh Sch. Med., Eye and Ear Hospital, Pittsburgh, PA 15213.

The vestibulo-collic reflex (VCR) tends to stabilize the head in space during whole body movements. The VCR places the semicircular canals (SSCs) in a negative feedback loop in which compensatory head rotations null the stimulus to the canals. To understand the VCR's dynamic properties, it is necessary to determine the consequences of this feedback arrangement. The purpose of this paper is to present control system models of the VCR which can contribute to this understanding and provide specific predictions (hypotheses) which are testable by appropriately designed animal experiments.

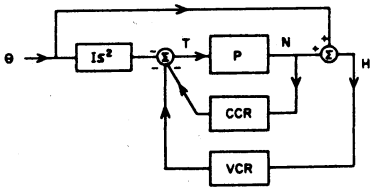
The simplest VCR model predicts that the gain of the VCR will decline at low frequencies since the SSCs cannot accurately encode head velocities below $1/(2\pi\tau_c)$ Hz, where τ_c is the SSC long time constant. However, the VCR corner frequency, above which compensatory head movements are most accurate, is not determined solely by τ_c, but by the sum of τ_c and the open loop gain, K, of the reflex. Above the corner frequency, the model predicts that the gain approaches a value of $K/(K + \tau_c)$ and will therefore always be less than unity. Larger values of K and/or τ_c will increase the VCR bandwidth, and increasing the $K{:}\tau_c$ ratio will enhance VCR gain. The value of these parameters might differ in different species of animals to match their VCR characteristics to specific behavioral needs, neck muscle properties, or various head sizes.

This model can also include an optokinetic system (OKS) component with the same functional form as described by D.A. Robinson (in Control of Gaze by Brain Stem Neurons, Baker and Berthoz eds., Elsevier, 1977) for the vestibulo-ocular reflex (VOR). The OKS provides the VCR with low frequency head rotation information which is combined with higher frequency information from the SSCs to give the overall VCR a response to DC during rotations in the light. The overall VCR has constant gain across all frequencies if the time constant associated with the OKS is equal to the SSC time constant. This choice of an OKS time constant is also consistent with Robinson's VOR model. Since this makes the OKS for the VOR and VCR functionally and parametrically identical, it can be hypothesized that a single OKS can provide the required low frequency head rotation information to both the VOR and VCR.

156.6 A MODEL OF THE NECK MOTOR SYSTEM IN THE ALERT CAT. J. Goldberg* G. Bilotto, B.W. Peterson (Spon: J. Winson). The Rockefeller University, New York. N.Y. 10021

To determine the roles of the vestibulocollic reflex (VCR) and the cervicocollic reflex (CCR) in controlling head position, we have observed head rotation (head free) or head torque (head restrained) about an axis through C_{1-2} during forced horizontal rotation of the body where body angle (θ) was equal to a sum of ten sinusoids ranging from 0.2 to 4.0 Hz. The model below shows how inertial (Is^2) and reflex (VCR, CCR) forces can be expected to sum to produce a torque (T) which acts on the neck motor plant (P) to induce rotations of the head relative to the body (N) and to space (H). Observations in anesthetized cats indicated that P could be modeled as a linear second order system (Eqn. 1, where I is head inertia, B - muscle viscosity, K - muscle spring constant, and s - the Laplace operator). Assuming similar dynamics and linear summation of torques (predicted from our earlier EMG studies), the alert cat's neck motor system will have the transfer function given in Eqn. 2.

In blindfolded, alert cats rotation of the free head was 180° out of phase with θ and gain, $|N/\theta|$ was 0.5-0.7. Our model predicted this behavior from the torque measurements obtained with the head fixed. Increasing I with weights showed that $Is^2 \ll$ VCR below 3 Hz. Torque and EMG measurements indicated that $CCR \cong VCR > BS + K$. Thus the observed head rotation appears to be dominated by antagonistic interaction of VCR and CCR so that: $N/\theta \cong \frac{-VCR}{VCR + CCR}$. (Supported by EY02249, EY00100, NS02619.)



$$(1)\quad P = \frac{1}{Is^2 + Bs + K}$$

$$(2)\quad \frac{N}{\theta} = \frac{-Is^2 - VCR}{Is^2 + Bs + K + CCR + VCR}$$

156.7 EFFECT OF REVERSIBLE LESIONS AND ELECTRICAL STIMULATION OF THE OLIVOCEREBELLAR SYSTEM ON GAIN OF THE VESTIBULO-OCULAR REFLEX (VOR). J. L. Demer* and D. A. Robinson. The Johns Hopkins University School of Medicine, Baltimore, Maryland 21205

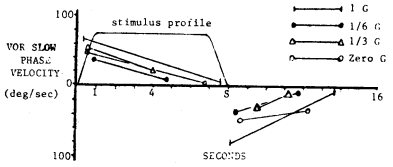
Lesions of the inferior olive (IO) or flocculus abolish plasticity of VOR gain. Modifiable synapses mediating this motor learning might be in cerebellar cortex, or might be elsewhere and the learned component simply carried through the climbing fiber (CF)-Purkinje cell (PC) system. The former theory predicts that momentary CF block would not alter VOR gain, regardless of its adapted value; the latter predicts that gain would immediately revert to some fixed value. Electrical stimulation of the CF's during head rotation might produce a lasting change in VOR gain if modifiable synapses are in the cerebellum, but only a transient change if not. These predictions were tested in 12 cats adapted to low (0.3) and high (1.2-1.5) gains by chronic wearing of optical devices. The field potential created by stimulating the inf. cerebellar peduncle (ICP) was used to locate the IO. An electrode and cannula were placed in the midline at the CF decussation (CFD). 5-10 µL of lidocaine were infused into the CFD of alert cats during VOR gain measurement in darkness. Abolition of CF activity was demonstrated by loss of ICP field potential on CFD stimulation. Regardless of initial state of gain adaptation, there was an immediate increase in VOR gain at 1.2 and 0.05 Hz to some value specific to each cat. Mean gain of 9 cats at 0.05 Hz was 1.50 ± 0.37 (mean ± SD, range 1.02-2.20). Mean gain of 5 cats at 1.2 Hz was 1.23 ± 0.58 (range 0.71-2.18). These gains were obtained in darkness; VOR gain in light was 3-20% lower. Procaine, lidocaine, and tetrodotoxin produced gain increases; glucose and saline did not. As the anesthetic wore off, gain declined to prelesion values as the ICP field potential reappeared. The 26 effective infusion sites were in the rostral half of the CFD; infusions into 20 surrounding sites caused no VOR gain changes.

Bilateral ICP stimulation in darkness (20-100 Hz, 20-100 µA) produced a 15-60% VOR gain decrease in 6 cats, no change in 4, and a 15-75% increase in one cat. Changes, observed at 1.2 and 0.05 Hz, came and went within 5-20 sec of the onset and cessation of stimulation. The effect of ICP stimulation was the same regardless of the initial state of gain adaptation, and was also observed during CFD anesthesia. ICP stimulation produced smaller gain changes in light than darkness. CFD stimulation in darkness produced 10-25% gain decreases in 8 cats. Stimulation never produced lasting changes in VOR gain. These results suggest that learning does not reside in the cerebellum. More must be known, however, about CF action on PC's before this interpretation can be confirmed.

156.8 VESTIBULO-OCULAR REFLEX RESPONSE DYNAMICS DURING PARABOLIC FLIGHT MANEUVERS IN THE SQUIRREL MONKEY. Lionel O. Greene, Jr.* and Nancy G. Daunton. NASA, Ames Res. Ctr., Moffett Field, Ca. 94035

It is implied from manned space flight that the change in certain sensory cues, including gravitational biases from the otolith end organs, may contribute to the onset of disorientation, motion illusions and sickness. Vestibulo-ocular reflex (VOR) nystagmus, an index of vestibular function, has been shown modifiable by otolith stimulation.

An instrumented, restrained squirrel monkey was accelerated about the Earth vertical axis during Learjet meneuvers producing 1, 1/3, 1/6 and zero gravity (G). Horizontal optokinetic (OKN) and vestibular (VOR) nystagmus were evoked by 90°/sec² acceleration to 70°/sec constant angular velocity, and decelerations 70°/sec² to 0°/sec. Baseline (1 G) data were obtained on the ground and in flight. OKN. Optokinetically driven eye movements were not affected by varying G level. VOR. The rate of nystagmus response decay was similar across G levels per acceleration, but the rate and duration of post-rotary nystagmus decay differed significantly at 1 vs. zero G. At 1/6 G, per acceleration VOR gain and latency increased significantly from 1 G, while the per vs. post acceleration average beat frequency (ABF) was significantly from the remaining G forces. There was no difference in the per vs. post accelerations at zero G. These data are presented in figure and table below.



	GAIN per	GAIN post	LATENCY per	LATENCY post	DURATION per	DURATION post	ABF per	ABF post
1 G	.55	.51	0.4	0.3	7.3	5.5	2.6	3.4
1/3 G	.50	.45	0.5	1.5	6.0	3.2	1.5	1.4
1/6 G	.55	.37	1.2	0.7	6.5	1.4	1.2	4.0
Zero G	.47	.43	0.5	0.8	5.2	4.5	1.8	1.4

Orbiting Astronauts have reported motion illusions and episodes of sickness that may be explained by the change in VOR response topography as reported. Adaptation of the vestibular apparatus is speculated the reason for similar experiences being reported upon return to Earth. Long-term habituation to environments of G levels differing from Earth may require an equivalent rehabituation period before normal behavioral activity may ensue.

156.9 RESPONSE OF THE RABBIT VESTIBULO-OCULAR REFLEX TO DIFFERENT MAGNITUDES OF ACCELERATION. R.W. Baloh* and J. Kimm. Animal Vestibular Lab., U.C.L.A. Sch. of Med., Los Angeles, Calif.90024

The vestibulo-ocular reflex of 5 normal adult Dutch pigmented rabbits was tested over a wide velocity range (15-120 deg./s) using step (140 deg./s^2) and sinusoidal (0.0125-0.4 Hz) accelerations. Eye movements were recorded with an implanted induction coil and were analyzed with digital computer techniques. The time constants of decay in slow phase eye velocity after a step change in platform velocity (defined by decay to 37% of initial velocity) were compared with the time constants predicted from the phase lead of slow phase eye velocity during sinusoidal oscillations. There was good agreement between observed and predicted time constants at any given peak velocity but both measurements were markedly velocity dependent. For example, the mean time constants for step changes in platform velocity of 30 and 120 deg./s were 5.8 ±1.5s and 15.8s ±5.2s respectively and the corner frequencies for sinusoidal oscillation at the same peak velocities were 0.038 Hz and 0.009 respectively. These latter values correspond to time constants of 4.2s for a peak velocity of 30 deg./s and 17.5s for a peak velocity of 120 deg./s. By contrast, gain measurements were minimally dependent on velocity (e.g. the average gain values for the 30 and 120 deg./s steps were 0.82 ±0.16 and 0.87 ±0.15 respectively.

The observed increasing time constants and decreasing corner frequencies with increasing platform velocities could be explained on the basis of recruitment of afferent neurons with progressively longer time constants as stimulus magnitude increases. Recent studies documenting a broad range of behavior of primary afferent vestibular neurons in the ray, frog, and cat are consistent with such speculation.

156.10 EFFECTS OF CEREBELLAR STIMULATION AND ABLATION ON THE GOLDFISH VESTIBULO-OCULAR REFLEX (VOR). J.J. Michnovicz* and M.V.L. Bennett, Div. of Cellular Neurobiology, Dept. of Neuroscience, Albert Einstein College of Medicine, Bronx, N.Y. 10461.

The goldfish cerebellum is essential for adaptive gain changes in the VOR (Schairer and Bennett, Soc. Neurosci. Abstr. 3:485, 1977). Cerebellar units were recorded while training fish to lower VOR gain by rotating the fish inside a brightly lit striped drum rotating in phase (Michnovicz et al., Soc. Neurosci. Abstr. 6:693,1980). Only units showing an immediate change in firing rate when the training stimulus was presented were followed for longer periods. These units(n=31) had a resting discharge of 10-30 impulses/sec (ips), and were modulated less than ±5 ips when rotated in the dark(1/8 Hz, ±20 deg.). The training stimulus immediately increased modulation as much as ±20 ips. 19 units responded maximally to contralateral table velocity, and 12 to ipsilateral. 5 units were held 30-90 minutes, while VOR gain dropped up to 70%. Depth of modulation increased in the dark, as well as during the training. Increases in depth of modulation were correlated with the change in gain.

Cerebellectomy in alert fish is performed in about one minute by aspiration, with no apparent damage to the underlying brain. Three types of fish were operated on: A)untrained(n=5); B)trained to gain of 0(n=5); C)trained to gain of 2(n=3). The average gain for all fish was initially 0.82±0.12. Training lasted 5 hrs in B and C, with resulting gains of 0.13±0.10 and 1.82±0.17. The immediate post-aspiration gains of A, B and C were 1.29±0.13, 1.18 ±0.08 and 1.21±0.18. This hypermetric gain was no longer altered by subsequent training for periods up to 2 hrs.

The cerebellum was stimulated through 3 M NaCl-filled microelectrodes (DC resistance 0.5-1 MΩ) with pulse trains (33/sec, 20 msec duration) of 1-10 μamps. Most vigorous nystagmus was evoked when the electrode was at the depth where most vestibular-sensitive units are recorded, which is in a well-defined layer of Purkinje cells. Sensitivity 0.5 mm deeper was greatly reduced, indicating that there was negligible current spread to the underlying brainstem.

Cathodal current in the dark evoked nystagmus with ipsilateral slow movements. The velocity of the slow phases was approximately linearly related to current strength, and was reversed by anodal current. Strong nystagmus elicited in the dark was suppressed when the fish was surrounded by a brightly lit stationary striped drum. Ipsilateral optokinetic nystagmus produced by moving the drum was increased by electrical stimulation.

These results demonstrate a powerful cerebellar influence on teleost eye movements, which could be mediated by monosynaptic inhibition of neurons in the ipsilateral vestibular nucleus.

156.11 A QUANTITATIVE EVALUATION OF VESTIBULAR COMPENSATION IN FISH. J. F. Ott. Dept. of Biology, Univ. So. Cal., Los Angeles, CA 90007.

Vestibular compensation is the term applied to gradual restoration of normal function after loss of gravistatic input from the inner ear. The functional loss and subsequent recovery have been studied in many species. An especially useful group is the fishes which have no tactile cues or head-neck-trunk interactions. I have studied goldfish after unilateral removal of the utricle, their major gravistatic organ, to find reliable behavioral correlates of the plastic changes involved in vestibular compensation. Although other species of fish have been studied, changes in post-operative lateral tilt were measured strictly by observation (Schoen, L., Z. fur vergl. Physiol. 32: 121, 1950; von Holst, E., Z. fur vergl. Physiol. 32: 60, 1950). I reevaluated this behavior using an automated photographic method in an effort to decrease observer bias. Goldfish were used because their anatomy is well described and they are hardy animals on which it is easy to do further physiological and behavioral experiments.

I have found that vestibular compensation in goldfish takes place in two distinct phases. The acute phase begins immediately after recovery from anesthesia and normally lasts 15-30 minutes. It is characterized by several features. The muscles of the operated side (ipsilateral) are contracted so the body is flexed. Swimming is sporadic and uncontrolled, with fin movements rolling the animal to the ipsilateral side. There is no nystagmus as seen in vestibular compensation in other vertebrates; instead, both eyes roll strongly toward the operated side. The acute phase ends abruptly when the eyes "unlock" from their ipsilateral position. Seconds later, the muscles of the ipsilateral side relax and swimming is normal. In this acute phase, there is very little variation in the duration or intensity.

The chronic phase extends for many days beyond the end of the acute phase. It is characterized by a lateral tilt to the operated side, with an amplitude that decreases to approach zero asymptotically over a period of 5-20 days. In this phase there is a very large variation in both duration and intensity. Some animals appear to compensate immediately, with no residual tilt at all, while others take up to three weeks to return to control values. A single fish may have consecutive daily means that differ by more than 20°, and standard deviations as great as 25°. Pooled data, however, have standard errors of the mean in the range of 1-7°. These results are in contrast to the previous work on fish in which such detailed quantification is not offered. (This work was supported by a Grants-in-Aid for Research from Sigma Xi.)

156.12 OPTOKINETIC AND VESTIBULOOCULAR REFLEXES IN RABBITS WITH BILATERAL PLUGS OF THE HORIZONTAL OR ANTERIOR SEMICIRCULAR CANALS N. H. Barmack and R. G. Erickson*. Neurological Sciences Institute, Good Samaritan Hospital & Medical Center, Portland, Oregon 97210.

Bilateral labyrinthectomies not only abolish vestibulooocular reflexes, but also reduce the gain of the horizontal optokinetic reflex (HOKR) in rabbits. One possible explanation for this reduction in gain of the HOKR is that the spontaneous activity of secondary vestibular neurons is reduced by the loss of spontaneous primary afferent input. The consequent reduction in secondary vestibular neuron activity would restrict the range of discharge frequencies over which this activity could be modulated by other sensory inputs; specifically vision. The present experiment was undertaken with the purpose of testing whether an equivalent reduction in the gain of vestibulooocular reflexes (eye velocity/head velocity) produced by bilateral plugs of the horizontal or anterior semicircular canals would reduce the gain of the HOKR (eye velocity/stimulus velocity).

Fifteen rabbits were subjected to either bilateral plugs of the horizontal (HSC) or anterior semicircular canals (ASC). The horizontal optokinetic reflex (HOKR), horizontal vestibulooocular reflex (HVOR), and vertical vestibulooocular reflex (VVOR) were tested before and after the plugging operation, and at various times following removal of the plugs. Plugs were constructed from silver wire formed into a spindle under heat. After a small opening was made in the bony portion of the semicircular canals at least 1 mm from the ampullae in anesthetized rabbits, plugs were inserted into the semicircular canals where they compressed the membranous labyrinth. The plugs were left in place for 24-48 hr, after which the rabbits were re-anesthetized and the plugs removed. Bilateral plugs of the HSC or ASC caused no significant decrease in the gain of the monocularly-evoked HOKR tested with a stimulus field of 72 x 72 deg at velocity of 00.4-50.0 deg/sec. Bilateral plugs of the HSC abolished the HVOR over the frequency range of 0.02-0.80 Hz. The gain of the VVOR in rabbits with bilateral HSC plugs was not reduced. Conversely, bilateral plugs of the ASC caused a reduction in the gains of both the VVOR and HVOR. Upon removal of the plugs of the HSC or ASC, the gains of the HVOR and VVOR returned to within 75% of normal within 10 days. We conclude that bilateral plugs of the HSC and ASC cause a reduction in the gain of the appropriate vestibulooocular reflex by restricting the flow of endolymph rather than by directly damaging the ampullae. Unlike labyrinthectomies, bilateral plugs of the semicircular canals do not reduce the gain of the HOKR. (Supported by NIH grant EY00848 and the Oregon Lions Sight and Hearing Foundation.)

156.13 ETIOLOGY OF SPACE MOTION SICKNESS: ROLE OF OTOLITH- SEMICIRCULAR CANAL INTERACTIONS. J.R. Lackner and Ashton Graybiel* Psychology Department, Brandeis University, Waltham, MA 02254.

Astronauts often experience symptoms of motion sickness (MS) during their first days in orbital flight, especially when making head movements (Graybiel, 1980, Aviat. Space Environ. Med. 51, 814-822). These symptoms may be related in part to alteration of the vestibulo-ocular reflex (VOR) in free fall: relative to earth-gravity values, VOR gain diminishes in the free fall phases of parabolic flight and increases in the high force (≥1.8g) phases (Lackner and Graybiel, 1981, Aviat. Space Environ. Med.52, 154-158).

To test this hypothesis, 20 subjects (Ss) were exposed on the ground and in parabolic flight to provocative vestibular stimulation until a MS endpoint of nausea was reached. Ss were accelerated at $15^{o}/s^{2}$ to a clockwise angular velocity of $300^{o}/s$, maintained at this velocity for 30s, and decelerated to rest within 1.5s. For the first 20 stops, each S was blindfolded, for the next 20 his eyes were open and he viewed passively the vertically-striped cylindrical experimental enclosure, for the final 20 stops, his eyes were open and the direction of rotation was reversed. The number of stops tolerated before the MS endpoint was reached served as an index of susceptibility. Each S was first tested in the laboratory and then in parabolic flight maneuvers in a Boeing KC-135 aircraft. In one flight test, Ss were accelerated to constant velocity in free fall and decelerated to rest in the high force (≥1.8g) phase of flight. In another test, accleration was in the high force phase and deceleration in free fall. Test order was balanced across Ss.

MS susceptibility was significantly greater ($p<.001$) both in free fall and in the high force phases of parabolic flight than in 1g test conditions. Ss developed symptoms more rapidly and the symptoms also persisted longer. Susceptibility did not differ, however, for the free fall and high force test conditions ($p>.05$).

These findings demonstrate that departures in either direction from earth-gravity force levels increase MS susceptibility. This suggests that the otolith organs, which are influenced by gravitoinertial force level,affect canalicular responsiveness. This interpretation accords with changes in the VOR in supra- and infra-g test conditions and the report by Igarashi et al., 1977 (Arch. Oto-Rhino-Laryng. 217, 183-188) that pendular rotation nystagmus decreases after ablation of the otolith organs.

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156.14 TORSIONAL EYE MOVEMENTS IN MAN DURING LINEAR ACCELERATIONS UPON EMERGING FROM WEIGHTLESSNESS. A. P. Arrott*and L. R. Young.
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Experiments were conducted to investigate the relation between gravitoinertial force (GIF is gravity minus linear acceleration) and the resulting torsional eye movements (ocular torsion, OT) in man. The OT of human subjects was measured using a binocular photographic technique. OT is known to occur in response to lateral head tilt and in response to lateral acceleration with the head upright. Both of these stimuli consist of a rotation of the GIF vector relative to the head. The question arises whether the rotation of the vector or merely the change in its lateral component is the adequate stimulus for OT.

A linear acceleration cart was used to accelerate subjects sinusoidally at a frequency of 0.4 Hz at amplitudes between 0.2g and 0.8g. Over this range the resulting OT was sinusoidal, with sensitivity of about 3 deg/g in the upright position and about 1 deg/g in the supine position (deg/g: deg. of OT per g of head lateral GIF). Head tilt at this frequency results in OT of about 3 deg/g.

The conditions of parabolic flight were used to achieve lateral acceleration with no pre-existing GIF and consequently no rotation of the GIF vector. OT was measured during the transition from the free fall phase (0g) to the pull-up phase (about 2g) of the flight trajectory. Subjects were positioned in the aircraft such that this change in GIF occurred laterally with respect to the head (lying on the side). OT was observed in the same direction as if the subject had been tilted to this position from the upright. The sensitivity of OT was about 4 deg/g. Actual tilting in gravity using a similar time course would produce OT of about 6 deg/g.

The results of these experiments support the notion that a change in the lateral component of GIF rather than a rotation of the gravitoinertial vector, is the adequate stimulus for ocular torsion.

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156.15 VESTIBULAR RESPONSE DECLINE, SKEWNESS, & INPUT-DEPENDENT GAIN & THRESHOLD ARE EXPLAINED BY A SINGLE MECHANISM. Bernard N. Segal and John S. Outerbridge*. Dept. Physiol., Biomed. Eng. Unit, Dept. Otolaryngol., McGill Univ., Montréal, Québec, H3G 1Y6.

It was previously shown that during prolonged 0.3 Hz sinusoidal rotation most bullfrog semicircular canal primary neurons exhibit input-dependent gain and threshold, as well as response decline. This could be accounted for by assuming that significant hair cell non-linearities precede adaptation and high frequency lead phenomena associated with the hair cell-primary neuron synapse (Neurosci. Abst. 5:692). Neural response skewness has now been characterized by the skewness coefficient (g_1) in order to further test these assumptions. It was found that (1) most (73%) cells tended to exhibit positively skewed responses (had longer right-hand tails), (2) skewness was significantly correlated with stimulus amplitude, and with 'high-frequency phase discrepancy', attributable to a high-frequency lead element and (3) skewness was uncorrelated with either viscoelastic (semicircular canal) or adaptation time constants, estimated from responses to lower-frequency stimuli.

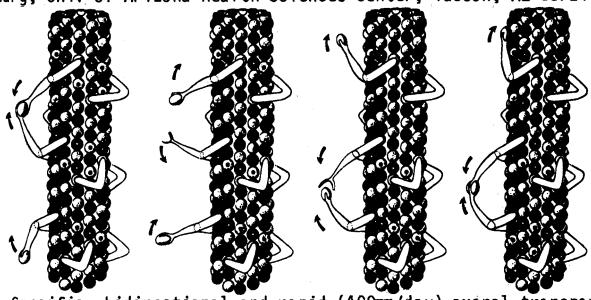
Simulation studies have shown that a single mechanism - approximate differentation of the output of a thresholding and saturating non-linearity - can account for these observations, as well as response decline and input-dependent gain and threshold. If a saturating rectifier is followed by approximate differentiation due to an adaptation element, response decline occurs during prolonged high-frequency sinusoidal stimulation and input-dependent gain and threshold characterizes the late non-declining response; at low-frequencies, the positive peaks of the response are positively skewed. If a saturating rectifier is followed by approximate differentiation due to a lead-lag element, response decline occurs during higher-frequency stimulation; at mid-frequencies, the peaks of the phase leading response exhibit positive skewness which increases with stimulus amplitude. If both adaptation and lead-lag elements follow a saturating rectifier, as was assumed for the bullfrog semicircular canal primary afferent pathway, the positive peaks of the response are positively skewed, skewness increases with stimulus magnitude, and skewness increases with the phase lead introduced by the lead-lag element. The lack of correlation, in the neural data, between skewness and adaptation, or viscoelastic,time constants was expected because adaptation had negligible effects at 0.3 Hz, and because canal mechanics were assumed to precede the dominant (hair cell) non-linearity of this pathway. Thus the proposed mechanism accounts for a wide variety of phenomena which otherwise appear unrelated.
(Supported by Canadian MRC.)

156.16 VESTIBULAR INVOLVEMENT IN SPATIAL ORIENTATION. S.L. Miller*, M. Potegal and L. Abraham (SPON: A.M. Gentile). Teachers College, Columbia Univ., New York, NY 10027; N.Y. State Psychiatric Inst., New York, NY 10032 and Dept. of Physical & Health Ed., Univ. of Texas, Austin, TX 78712.

The importance of vestibular contributions to spatial orientation has long been debated. Studies have yielded conflicting results; while some investigators found long distance bird navigation unimpaired by vestibular dysfunction, others continued to report indirect evidence for vestibular involvement in shorter distance tasks (Barlow, J. Theoretical Biol., 6, 1964). Beritov demonstrated that labyrinthine damage impaired performance of a vestibular/spatial orientation task requiring animals to return to a target point from which they had been passively transported (Beritov, Neural Mechanisms of Higher Vertebrate Behavior, 1965). We have developed a similar "passive transport and return" task (PTR) for rats. In PTR, water deprived rats are passively transported through right angle trajectories of varying distances in an opaque vehicle from one of eight identical spouts bordering a visually homogenous room. To obtain water upon release, the rat must return to the target spout from which he was transported on that trial. We have demonstrated that blinded rats can perform PTR, and that vestibular nuclei, but not cerebellar cortex lesions severely impair PTR performance in blinded rats (Miller et al., E.P.A., 1979), not effecting their performance in a control, olfactory/spatial task.

We report here an extension of these findings. Seven visually intact rats received vestibular nuclei lesions; six rats, dorsal column nuclei (DCN) lesions; and eight rats, sham operations. In PTR acquistion, vestibularly lesioned rats were significantly impaired compared to sham operated rats; DCN lesioned rats' performance was intermediate. In a control task, eight vestibular nuclei, nine DCN and eight sham rats were required to choose a scented string from one of seven visually identical strings and follow it to its end for a water reward. There were no significant differences among groups on this olfactory/spatial task which was at least as difficult (judging by the sham rats' performance) as the vestibular/spatial task. The efficacy of vestibular lesions in producing vestibular dysfunction is indicated by air righting responses for rats in both tasks, which revealed that the vestibular group was significantly different from sham and DCN groups in angle of head inclination at landing. These findings support the hypothesis that vestibular information is necessary for successful performance of specific spatial tasks.

157.1 "COMPUTER-LIKE" MICROTUBULE MODEL OF AXONAL TRANSPORT. S.R. Hameroff, R.C. Watt*, J.C. Oakley*,Dept Anesthesiology,Div Neurol Surg, Univ of Arizona Health Sciences Center, Tucson, AZ 58724



Specific, bidirectional and rapid (400mm/day) axonal transport depends on microtubules (MT) and associated ATPase proteins. Mechanisms of spatial arrangement and temporal control of attached contractile proteins remains unknown in axonal transport and similar MT functions. Atema (J Theor Biol 38:181, 1973) proposed that sensory cilia MT transferred information by propagated conformational changes among MT subunits (alternating α and β tubulin). Tubulin conformational states may be coupled to transferable Ca^{2+} binding (via calmodulin), energy state, electron occupancy, or electron excitation in aromatic hydrophobic regions (Frolich H: Proc Natl Acad Sci 72:4211, 1975). The cylindrical grid-like MT structure, connecting proteins, and intracellular trabecular networks (Porter KR, Tucker JB: Sci Am 244:56, 1981) could provide programmable switching matrices for information transfer resulting in temporal and spatial control of axonal transport. Media of information transfer among the 4 nm, 55,000 dalton subunits could include conformational states coupled to any of the previously mentioned modes. Tubulin switching programming regulating directional transfer could ensue from genetically-controlled primary protein structure, or post translational effects including binding of proteins, ions, or other substances. Pulsed reading, switching, and transduction could be driven by nerve membrane depolarization or calcium ion fluxes. Key intraprotein hydrophobic environments could thus integrate and transduce several input modes to determine conformational state and mechanical activities. Aspects of this model are analogous to information and computer technologies including programmable Boolean switching matrices, transistor circuits, bubble memory, charge transfer devices, and/or surface acoustic wave resonators.

157.2 COBALT INDUCED DISRUPTION OF THE GOLGI APPARATUS BLOCKS ASSEMBLY OF FORMING FACE ELEMENT. James D. Lindsey and Mark H. Ellisman, Department of Neurosciences, University of California, San Diego, School of Medicine, La Jolla, CA 92093.

Low levels of cobalt have been shown to selectively block the initiation of fast axonal transport. Ultrastructurally, such cobalt treatment produces a specific depletion of the Golgi apparatus (GA). Concomitantly, there is a dramatic accumulation of smooth membranous cisternae which do not stain for thiamine pyrophosphatase (TPP). To examine the interaction of cobalt with the GA cis (forming) element, bullfrog spinal ganglia were incubated for 12 hrs. in a modified Ringer's solution both with and without 0.18 mM cobalt and then subjected to an osmium impregnation protocol. Thin and thick sections were studied by conventional as well as high voltage electron microscopy.

This impregnation technique results in deposition of osmium in the cis element of the GA. Also stained were a few isolated small rounded profiles. In thick section, the cis element is seen to be large, regularly perforated, sheet-like cisterns. Anastomotic with these cisterns were two distinct types of "accessory tubules". The first, 50-80 nm in width, was smooth surfaced and often ran in pairs or small bundles. The second, 20-30 nm in width, was characterized by regularly spaced swellings and usually coursed as isolated individuals through the cytoplasm. These tubules probably correspond to the small isolated profiles seen in thin section.

Examination of the cobalt treated ganglia revealed that many of the accumulated smooth membranous cisternae were stained. In thick section, the osmiphillic elements appeared as small, flat, perforated cisterns and as clustered vesicles varying from 50-120 nm in diameter. The accessory tubule systems appeared largely intact except that they now ended blindly or in small end bulbs.

These findings suggest two major conclusions. First, the "accessory tubule system" is seperate from and probably antecedent to the cis element. Second, low levels of cobalt specifically block GA cis element assembly which consequentially results in falure of the trans element's maturation.

157.3 EFFECTS OF ANTIMITOTIC DRUGS ON AXOPLASMIC TRANSPORT IN TISSUE CULTURED NERVE CELLS. H. HORIE*, T. TAKENAKA* and K. INOMATA* (SPON : M. Sato) Dept. of Physiology, Sch. of Med., Yokohama City University, Minamiku, Yokohama, Japan 232

The role of microtubules in the axoplasmic transport was studied by using the antimitotic drugs in tissue cultured nerve cells. Dorsal root ganglion cells dissected from 6-day-old chick embryos were cultured in Eagle's minimal essential medium supplemented with 10% horse serum and 5% chick embryo extract. A cultured dish was made of a silicon plate fixed a cover glass on the bottom. After 3 days, the transported particles in the neurites (dia : 1.5-2.5μm) near the growth cones were observed with Nomarski differential interference contrast microscopy and cultured medium was perfused with the solution containing the antimitotic drugs. The movements of the particles were recorded with a video tape recorder.

Colchicine at $1x10^{-6}$M caused the decrease of the number of the transported particles in the neurites. At 30 min after the application of colchicine the number of the transported particles decreased to about 50%. Two types of the particle movement were observed. Small particles (<0.5μm) were transported smoothly and those velocities were 1.0-1.8μm/sec, which values were almost same as those in control. On the other hand the movements of the large size particles were to-and-fro. At 60 min the transported particles could not be observed. Morphologically the neurites did not retract and the position of the neurites did not change. But some resions in the neurites swelled and the particles transported into those resions stopped. The same effects on the axoplasmic transport were observed in vinblastine at $1x10^{-6}$M. The concentration required to supress the axoplasmic transport of 50% at 60 min after the drug application was approximately $1x10^{-7}$M, which value coincides with the binding constant of colchicine to chick embryo brain tubulin, $5x10^{-7}$M. Cytochalasin D at $2x10^{-5}$M had no effect on the number of transported particles and their velocities at 60 min after application. These results suggest that colchicine or vinblastin disrupted the microtubules in the neurites and as a result of it the movements of the transported particles were supressed. So microtubules play an important role in axoplasmic transport in tissue cultured nerve cells.

157.4 TUBULIN ADP-RIBOSYLATION IS CATALYZED BY CHOLERA TOXIN. Dan J. Hawkins* and Edward T. Browning (SPON: Roger C. Duvoisin). CMDNJ-Rutgers Medical School, Dept. of Pharmacology, Piscataway, NJ 08854.

The covalent modification of proteins by an ADP-ribosyl moiety derived from NAD^+ is catalyzed by cholera toxin. Cholera toxin catalyzes the ADP-ribosylation of several proteins including the regulatory component (G/F) of hormone-sensitive adenylate cyclase. The ramifications of cholera toxin-catalyzed ADP-ribosylations are still poorly understood.

Rat C6 glioma cell membranes and cytosol were prepared by the method of Jett et al. (JBC 252: 2134). Membranes and/or cytosol were incubated with ^{32}P-NAD^+ and cholera toxin and the proteins resolved on the basis of isoelectric point and mol. wt. using 2-dimensional gel electrophoresis. Gels were stained with Coomassie blue, destained, and the dried gels subjected to autoradiography. Cholera toxin catalyzed the ^{32}P-ADP-ribosylation of putative subunits of the membrane G/F protein. Cholera toxin also catalyzed the ADP-ribosylation of several cytosolic proteins. One of these cholera toxin-dependent ^{32}P-ADP-ribosylations in cytosol was located by autoradiography as an acid satellite relative to stained tubulin. This ^{32}P-ADP-ribosylation was preferentially associated with beta tubulin. Tubulin modified by ADP-ribosylation would undergo an acid shift in 2-D gels due to the charge of the ADP-ribosyl moiety. No toxin catalyzed acidic shift was discernible for the stained tubulin. The data suggested that a small amount of tubulin may have undergone a cholera toxin-catalyzed ADP-ribosylation. The presence of GTP promoted the cholera toxin-catalyzed ^{32}P-ADP-ribosylation of the ^{32}P-ADP-ribosylated protein associated with tubulin and the ^{32}P-ADP-ribosylated membrane G/F peptide subunits.

Bovine brain tubulin was prepared by three cycles of warm/cold polymerization/depolymerization according to Shelanski et al. (PNAS 70: 765). Bovine brain tubulin incubated with ^{32}P-NAD^+, GTP, and cholera toxin was observed in 2-D gels to possess a ^{32}P-ADP-ribosylated acidic satellite in autoradiograms relative to stained tubulin just as was found with rat C6 glioma cell tubulin. Peptide mapping of bovine brain tubulin and the associated ^{32}P-ADP-ribosylated protein was performed using the method of Cleveland et al. (JBC 252: 1102). Correspondence was observed between the autoradiographic images of the peptide map and the stained peptide fragments. This served to confirm that cholera toxin catalyzes the ADP-ribosylation of tubulin. ADP-ribosylation of even a small fraction of tubulin might effect alterations in certain microtubule-associated processes (e.g., treadmilling). (Supported by NIH grant NS 08436 and CMDNJ Foundation grant 29-81.)

157.5 SODIUM REQUIREMENT FOR FAST AXONAL TRANSPORT IN THE FROG DORSAL ROOT GANGLION-SPINAL NERVE PREPARATION. P.-A. Lavoie. Département de pharmacologie, Université de Montréal, Montréal, Canada.

A previous study had showed that the substitution of sucrose for the NaCl of the incubation medium decreased the quantity of [^{3}H]leucine-labeled proteins carried by the fast axonal transport system (Lavoie, Can. J. Physiol. Pharmacol. 59: 31-36, 1981); the effect was demonstrable when either the dorsal root ganglion or the desheathed spinal nerve was exposed to the modified medium. Since sucrose is not only a Na^+ substitution but also a Cl^- substitution, it was appropriate to study the influence of a selective substitution of Na^+ ions: to that effect, the 114 mM NaCl of the normal incubation medium was replaced by 114 mM LiCl in the modified medium used for the present experiments.

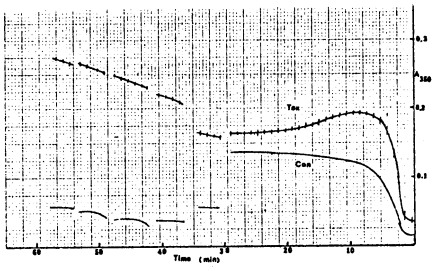
Frog dorsal root ganglia were exposed to [^{3}H]leucine for 1 h *in vitro*, and the ganglia and spinal nerves were incubated for a further 16-17 h at 18°C in medium free of added radioactivity; the ganglion was isolated from most of the nerve trunk by a silicone grease barrier during this incubation. For experiments on the dorsal root ganglion, control ganglia and spinal nerves and experimental nerves were in normal medium whereas experimental ganglia were in modified medium; with this set-up, a 60% reduction in the quantity of fast-transported [^{3}H]proteins was observed. For the set of experiments on spinal nerves, all spinal nerves were desheathed along 4-5 mm of their length (approx. midway between the ganglion and the junction of the spinal nerve to the sciatic nerve) prior to labeling of ganglia with [^{3}H]leucine. The desheathed nerves of the control preparations were incubated in normal medium as were the control and experimental ganglia, but the desheathed nerves of the experimental preparations were incubated in modified medium. Axonal transport through the desheathed region of nerve was quantitated by the level of trichloroacetic acid-insoluble radioactivity present at a ligature distal to the desheathed area of the nerve, and it amounted to approx. 50% of control in nerves exposed to the LiCl medium.

Thus the presence of Na^+ ions in the extracellular fluid seems required if normal fast axonal transport is to be maintained in this biological preparation from frog. It has been speculated that sodium deficiency may inhibit transport in the cat hypogastric nerve by increasing intracellular Ca^{2+} (Esquerro et al., Brit. J. Pharmacol. 70: 375-381, 1980), but this is probably not the mechanism of inhibition in the frog preparation since Li^+ is a form of Na^+ substitution which does not modify Ca^{2+} fluxes across the plasma membrane in neurons of the frog spinal nerve (Stout & Diecke, Can. J. Physiol. Pharmacol. 58: 1366-1372,1980).
Supported by grants from MRC of Canada, MDA of Canada, and the "Conseil de la recherche en santé du Québec".

157.6 EFFECTS OF L. QUINQUESTRIATUS SCORPION VENOM ON THE IN VITRO POLYMERIZAITON OF TUBULIN. Lelland C. Tolbert and George B. Brown. Neurosciences Program, University of Alabama in Birmingham, Birmingham, AL 35294.

We have studied the effects of crude and fractionated scorpion venom from Leiurus quinquestriatus on the polymerization of cycle-purified tubulin from bovine brain. Tubulin at a concentration of 1-2 mg/ml was incubated under the assembly conditions of Weingarten et al (Biochem. 13:5529, 1974) with or without addition of scorpion venom and polymerization was followed by the increase in light scattering at 350 nm. Crude scorpion venom (separated from insoluble mucopolysacccharides), at final concentrations of 10-15 µg protein/ml, had dramatic effects on both the rate of increase in light scattering and the lability of tubules to Ca^{++}-induced depolymerization. Incubations containing scorpion venom actually gave an increase in light scattering in response to addition of Ca^{++}. These effects are indicated in the figure below. Each break in the curves corresponds to the addition of CaCl such that the net concentration of calcium is increased by 0.75 mM. Similar effects were obtained from one and only fraction resulting from ion exchange chromatography of crude scorpion venom on SP-Sephadex. These effects are distinguished from those of other agents affecting the polymerization of tubulin, e.g., nerve growth factor, taxol and natural and synthetic polycations.

The active fraction is also the most lethal fraction (determined by bioassay in mice) resulting from the chromatography and contains the component which binds to voltage sensitive sodium channels and enhances the binding of batrachotoxin to its sodium channel site. This fraction contains less than 10% of the protein in the crude venom. Thus, the effective concentraiton for the effects on tubulin is $\leq$ 1 µg protein/ml.



157.7 REDISTRIBUTION OF FAST AXONAL TRANSPORT OF PROTEINS IN β,β'-IMINODIPROPIONITRILE (IDPN) INTOXICATION. AN ULTRASTRUCTURAL AUTORADIOGRAPHIC STUDY. S. Ch. Papasozomenos* L. Autilio-Gambetti*, P. Gambetti. Division of Neuropathology, CWRU, Cleveland, Ohio 44106.

IDPN intoxication selectively impairs the transport of neurofilament proteins, while the rate of the fast anterograde and retrograde transports are not affected (Griffin et al., Science, 202:633, 1978). We have recently shown that IDPN intoxication produces a rearrangement of axonal organelles, with displacement of neurofilaments towards the periphery and of microtubules, smooth endoplasmic reticulum (SER) and mitochondria towards the center of the axon (Papasozomenos et al., J. Neuropathol. Exp. Neurol., 39:380, 1980). Quantitative electron microscopic autoradiography (EMAR) was undertaken to determine the distribution of proteins migrating with the fast component of axonal transport in IDPN-treated and control rats. A mixture of ^{3}H-proline and ^{3}H-lysine was injected into the spinal cord, and six hours after, the sciatic nerve was dissected out and sectioned into 2 mm pieces. Radioactivity was determined by liquid scintillation counting in alternate segments and the region of the nerve containing the front of the transported proteins was processed for EMAR. The results show that proteins migrating with the fast component of axonal transport are displaced towards the center of the axon, as compared to the control. This redistribution parallels that of microtubules and SER previously found by us in this experimental model.
(Supported by NIH Grants NS-14509 and AG_00795).

157.8 COMPARISON OF RAPIDLY TRANSPORTED PROTEINS IN FROG AND RAT SENSORY NEURONES. G.W. Perry* and David L. Wilson. Dept. of Physiol. and Biophys., Univ. of Miami, Sch. of Med., Miami, Fl 33101.

Some studies have suggested that the electrophoretic profiles of rapidly transported proteins might be similar in the axons of different species (Barker et al 1975; Bisby, 1977), but Neale et al (1980) report very little correspondence in the profiles of these proteins from the sensory neurones of rat and frog. We have resolved this issue by studying rapidly transport proteins on two dimensional polyacrylamide gels. Dorsal root ganglion (DRG) neurones from rat and frog were labeled in vitro with ^{35}S-methionine, and the newly synthesized, rapidly transported proteins were collected at ligatures on the sciatic nerves approximately 30 mm from DRG as described in Stone & Wilson (1979).

The rat preparation was incubated for 10 hrs at 30°C and the frog preparation for 24 hrs at 18°C. A co-migration analysis (triplets of gels compared: one gel from frog, one gel from rat, one gel from frog + rat) revealed three classes of proteins. (1) Some proteins spots, including A2, A17-18, $B3_{a-g}$, $B14_{a-i}$, and C1 co-migrated on the gels, suggesting that these abundantly transported proteins have been well conserved during evolution (gel spot notation as in Stone & Wilson, 1979; Perry & Wilson, 1981). (2) Other protein spots had similar, but not identical positions on the gels. The gels separate proteins according to isoelectric point in one dimension and molecular weight in the second dimension. Thus slight differences in position reflect differences in size and charge which could result from (minor) evolutionary modifications of proteins with similar functions. (3) A number of the rat and frog proteins appeared to be species specific. However, these could have resulted from very radical abundance changes (protein abundance or methionine-residue abundance) or very radical position changes on the gels. The species apparantly unique to frog include $A1_{a-i}$, $A4_{a-d}$, A10, C2-6, C8-11, and C18.

The gel staining patterns of unlabeled proteins in the sciatic nerves of rat and frog show some similarities: for example, actin, serum albumin, and the α and β subunits of tubulin are in identical positions on the gels. Each pattern also has major spots that are unique.

It appears then, that there are not only a number of highly (evolutionarily) conserved proteins in rapid transport and in the sciatic nerve, but also a number of species-specific proteins.

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Supported by NIH grant NS14328.

157.9 HIGH AND LOW AFFINITY MUSCARINIC CHOLINERGIC RECEPTORS UNDERGO AXONAL TRANSPORT. M.A. Zarbin*, J.K. Wamsley and M.J. Kuhar. Dept. of Neuroscience, Johns Hopkins Univ., Sch. of Med., Balto., MD 21205.

Axonal flow of opiate and muscarinic receptors has recently been found in the rat vagus and dog splenic nerve, respectively (Young et al., Science 210:76, 1980; Laduron, Nature 286:287, 1980). Here, we report that muscarinic receptors are also undergoing transport in the rat vagus nerve. Transported muscarinic binding sites were assayed as described previously (Wamsley et al., Brain Res., in press).

Muscarinic binding sites accumulate in a time dependent manner both proximal and distal to ligatures placed around the vagus nerve trunk. The rate of accumulation appears to be constant during the first 24 hrs. of ligation. The accumulation proximal to the ligature is greater than the accumulation distally. The calculated average rate of receptor transport is roughly 15 mm/day for receptor accumulation proximal to the ligature and roughly 10 mm/day for receptor accumulation distal to the ligature. These values are an underestimate of the true rate of receptor transport because only a small fraction of the axonal receptors seems to be moving.

The transported muscarinic binding sites have pharmacologic characteristics appropriate for a muscarinic receptor.

Muscarinic receptor transport is inhibited by the injection of low doses of colchicine into the vagal nerve trunk. Double ligature experiments reveal a build up of binding sites proximal to both ligatues and distal to the ligature furthest from the nodose ganglion. These data are consistent with the notion that peripherally transported receptors move by fast transport while centrally tranported receptors move by retrograde transport.

Preliminary experiments indicate that the bulk of peripherally transported binding sites are high affinity muscarinic receptors, while most of the receptors accumulating distal to the ligature are low affinity muscarinic receptors. Similar results have been obtained for tranported muscarinic receptors in the rat sciatic nerve (Wamsley, et al., ibid.).

The presence and transport of cholecystokinin and insulin receptors has also been detected in the rat vagus nerve. Neither the presence nor the transport of glycine, H-1 histamine, beta-adrenergic, and benzodiazepine receptors has been detected in the rat vagus. In the rat sciatic nerve, however, the presence and transport of beta-receptors and H-1 histamine receptors has been detected. Thus, receptor transport may be a property of most neurotransmitter receptors.

Supported by grants DA00266, MH00053 (MJK), NS15080 (JKW) and a MSTP award GM07309 (MAZ).

157.10 AXONAL TRANSPORT OF GANGLIOSIDES IN MAMMALIAN PERIPHERAL NERVE. U.R. Tipnis*, J.H. Hofteig, H.W. Palay*, K.S. Leon*, and A.J. Yates. Department of Pathology, Ohio State University College of Medicine, Columbus, Ohio 43210

The following studies were performed to determine the time course of incorporation of radioactivity from (^{3}H)-glucosamine injected into dorsal root ganglia (DRG) into gangliosides of 3 segments of this nerve unit: DRG, lumbosacral trunk (LST) and sciatic nerve (SN). Normal adult female New Zealand white rabbits were anaesthetized with nitrous oxide and halothane. Both L-7 DRG were aseptically exposed and injected with 50µci (^{3}H)-glucosamine (S.A=39.6Ci/mmole). Animals were allowed to survive 1, 2, 7, 15, and 21 days at which times they were killed and DRG, LST, and SN removed and frozen. Specimens were homogenized and extracted in chloroform-methanol (C-M) 2:1 and C-M-water 1:2:5%. Gangliosides were purified by a Folch partition, treatment of upper phase with alkaline phosphatase and phosphodiesterase, alkaline methanolysis, dialysis and silicic acid column chromatography. Radioactivity was determined on aliquots of initial homogenate, acid soluble fraction, and purified gangliosides. Expressed as a percent of the total radioactivity present in the homogenate of all 3 segments (DRG + LST + SN) radioactivity in the DRG decreased from 95% at day 1 to 80% at day 21. Radioactivity in homogenates of LST and SN increased from 3 to 14% and 1 to 5% respectively, and acid soluble radioactivity progressively decreased over the same time in all 3 segments. Radioactivity in the gangliosides of DRG at day 21 was only 46% that of day 1, but radioactivity in LST and SN increased 5-and 6-fold respectively (Table 1). Colchicine injected into DRG prior to (^{3}H)-glucosamine completely blocked the transport of all lipids into LST and SN. These results are compatible with the synthesis of gangliosides in DRG and their subsequent transport into LST and SN. However, the possibility of local synthesis of gangliosides within LST and SN cannot be ruled out.

TABLE 1. RADIOACTIVITY (DPM) IN GANGLIOSIDES

Anatomic Segment	Days Following Injection 1	2	7	15	21
D.R. Ganglion	26,800	18,500	14,200	13,200	12,600
Lumbosacral Trunk	1,070	1,700	1,300	3,600	5,400
Sciatic Nerve	590	1,140	1,600	1,800	3,500

Supported by a grant from USPHS-NS10165.

157.11 ^{3}H-PUTRESCINE IS INCORPORATED INTO PROTEINS AFTER INJECTION INTO THE GIANT NEURON R2: RAPID AXONAL TRANSPORT OF THE LABELED PROTEIN. Richard Ambron and Leon Kremzner*, Depts. Anatomy, Neurology, and Rehab. Med., P&S, Columbia Univ., N.Y., N.Y. 10032.

A relationship between cell surface macromolecules and the intracellular cytoskeleton has been suggested by a number of studies but the nature of the interaction remains unknown. Transglutaminase, an enzyme that cross-links proteins, is a good candidate as an effector of such surface-cytoskeletal interactions and recently was shown to participate in carrier-mediated endocytosis (Davies et al., Nature, 1980). The function of this enzyme appears to be regulated by polyamines, which act as substrates, and are incorporated into protein (Folk et al., J. Biol. Chem., 1980). We have found transglutaminase activity in R2, the giant cholinergic neuron of Aplysia (Kremzner and Ambron, in prep.) and have shown that ^{3}H-putrescine (^{3}H-Put) injected directly into R2, becomes covalently linked to protein. This was accomplished by isolating and characterizing γ-glutamylputrescine (Kremzner and Ambron, These Proceedings). Among the soluble proteins labeled by putrescine are several (m.w. 56Kd, 48Kd, and 42Kd) that are precipitated by vinblastine: the 56 Kd protein migrates on gradient gels with tubulin isolated from rat brain. Several high m.w. membrane proteins also become labeled. We have not established whether the putrescine is covalently linked to each of these specific proteins, however.

^{3}H-Put injected into R2 is rapidly metabolized to spermidine, spermine, GABA, and and monoacetylputrescine, as determined by HPLC. Analysis of Aplysia nervous tissue detected the polyamines in approximately the same proportion as found for the labeled compounds in R2, indicating that injected putrescine is metabolized like the endogenous compound. The soluble polyamines diffuse along R2's axon where additional metabolism can occur: assays of extruded axoplasm indicate the presence of S-adenosylmethionine decarboxylase activity. In contrast, examination of radioactivity in sequential segments of R2's axon shows that ^{3}H-polyamine(s) associated with protein is rapidly transported along the axon toward R2's synapses. (Supported by Grants from NIH, Muscular Dystrophy Assoc. and by a Career Development Award to R.A.)

157.12 AXONAL TRANSPORT OF POLYAMINES IN INTACT AND REGENERATING MOTOR AXONS OF THE RAT SCIATIC NERVE. T.D. Lindquist*, N.A. Ingoglia and J.A. Sturman, Depts. of Physiol. and Neurosci., CMDNJ-New Jersey Med. Sch., Newark, NJ 07103 and Dept. of Pathol. Neurobiol., Inst. for Basic Research in Mental Retardation, Staten Island, NY 10314

The axonal transport of polyamines has been demonstrated in regenerating optic axons of goldfish (Ingoglia et al., 1977, Brain Res., 130:433-445) but has been reported not to occur in motor axons of the sciatic nerve of rats (Harik et al., 1979, Exper. Neurol., 63:311-321). The present experiments are similar in design to those of Harik et al. but in contrast to their findings, we report evidence that following injection of ^{3}H-putrescine into the spinal cord, radioactive polyamines are transported axonally along both intact and regenerating motor axons of the rat sciatic nerve.

The left sciatic nerve was crushed 75 mm from the spinal cord in 3 rats, and 10 ds later 66 µCi of ^{3}H-putrescine was injected bilaterally into the lumbar region of the spinal cord. At the same time, ligatures were applied to the right nerve 60 and 75 mm from the cord and to the left (regenerating) nerve 60 mm from the cord. Rats were sacrificed 3 ds later; both sciatic nerves were cut into 5 mm segments and assayed for radioactivity by tissue digestion and liquid scintillation counting. A small but significant peak of radioactivity was found at the distal ligature of the normal nerve and at the site of the crush in regenerating nerves. Since the proximal ligature prevents radioactivity from arriving by axonal transport, we conclude that this represents uptake of radioactive material from the blood at the ligation. In intact nerves, approx. 2xs the radioactivity at the distal ligature accumulated in the nerve segment central to the proximal ligature, indicating axonal transport. In regenerating nerves the amount of radioactivity accumulating central to the proximal ligature was 4.5xs the peak at the more distal crush, and 2.5xs the amount transported in normal nerves. The nature of the transported radioactivity was determined in separate experiments. The majority of the radioactivity was present as spermidine and as a more acidic compound. Further analysis of this compound suggests that it is likely to be a metabolite of spermidine, perhaps an acetylated derivative.

These experiments indicate that polyamines are axonally transported in motor axons of the rat sciatic nerve, that the amount transported is increased several fold in regenerating nerves, and that the polyamines are likely to be metabolized following transport into the nerve.

(Supported by grant EI 02887 from NIH.)

157.13 CHANGES IN FAST AXOPLASMIC TRANSPORT DURING HYPERTHERMIA, J.-H. Kim* and J.L. Johnson, Division of Biochemistry, Physiology, and Pharmacology, The University of South Dakota School of Medicine, Vermillion, South Dakota, 57069.

While the effects of hypothermia on mammalian fast axoplasmic transport have been well documented, there is a singular lack of detailed studies concerning any actions that hyperthermic conditions may have on this process. The purpose of this study was to discern the effects of hyperthermia on fast axoplasmic transport via analysis of the advancing crest of axonal labelled material. This has permitted the detection of any component blocking effects separately from any transport rate effects. ^{3}H-phenylalanine was injected into the L5 sensory ganglia of large Sprague-Dawley rats anesthetized with sodium pentobarbital and 1.5 hrs of in vivo transport was allowed at 37°C for all experiments. In vitro incubation for 1.5 hrs at temperatures ranging from 30-45°C resulted in an exponential increase in fast transport rate from 30-44°C. 1.5 hrs at 45°C, however, completely and irreversibly blocked all transport. Incubation at 45°C for times ranging from 10-60 min enabled estimation of the transport rate at 45°C prior to block (696 mm/day). The Q_{10} for the transport process was 1.9 between the entire range of 30-45°C, and activation energies averaged 11,300 cal/mole. Marked changes during hyperthermia were also occurring in the advancing crest behind the leading edge used to calculate the above rates of transport. The more severe the hyperthermia, the more was the advancing crest amplitude depressed. Simultaneously, there was an increasing amount of stationary material (blocked) left behind this advancing wave. In addition, the slope of the leading edge ahead of the crest peak declined sharply during hyperthermia. Unlike previous data (J. Neurobiol., 7, 1976, 339) there was no decline in the fast axoplasmic transport rate associated with hyperthermic block, with the transport rate increasing continually right up to the point of complete and irreversible block. From 37-45°C there was a progressive block of transport in nerve fibers resulting in a declining edge. These blocked fibers did not contribute, then, to the obtained fast transport rate estimates. Transport rate estimates alone would not detect this fiber loss, which could be occurring even in relatively mild hyperthermic conditions if long enough in duration. Further studies are needed to assess the irreversibility of the transport blocking effects at temperature below 45°C. Supported by USD General Research Support Funds to J.L. Johnson.

158.1 COMPARISON OF TWO TECHNIQUES FOR SEQUENTIAL LIGHT (LM) AND ELECTRON MICROSCOPIC (EM) ANALYSIS OF NEURAL ELEMENTS LABELED WITH HORSERADISH PEROXIDASE (HRP). G.M. Mawe*, M.S. Beattie, and J.C. Bresnahan (SPON: J.S. King). Dept. of Anat. and Div. of Neurosurg., Ohio State Univ. Sch. Med., Columbus, OH 43210.

We have examined two modifications of the HRP technique which have been reported to be more sensitive than the standard diaminobenzidine (DAB) procedure and which yield electron-dense reaction products. The goal has been to develop reliable methods for the sequential LM and EM analysis of retrogradely and transganglionically transported HRP from peripheral nerves.

The sciatic nerves of 6 rats were transected and HRP was applied via a polyethylene tubing cuff. Survival times were 2 or 3 days. Sections of the spinal cord and dorsal root ganglia (DRG) were cut on a vibratome and alternate sections were processed for the visualization of HRP by the glucose oxidase method for enhancement of DAB reaction product (Itoh et. al. Brain Res., 175: 341, 1979) and the benzidine dihydrochloride (BDHC) technique described by Mesulam (J. Histochem. Cytochem., 24:1273,1976). Sections were either mounted on glass slides and counterstained with neutral red or flat embedded in Maraglas between two sheets of clear plastic for sequential LM and EM observations.

Labeling in cells in the DRG and in motoneurons as well as in axons in the dorsal horn was granular in appearance after processing with the GOD protocol. Label was visible mainly in the primary dendrites and somata of motoneurons. In adjacent sections processed using BDHC the label was more diffuse, with occassional dense granules, and typically showed many more dendritic branches, often giving a "Golgi-like" appearance to the cells reminiscent of intracellular labelling, or of the tetramethyl benzidine (TMB) technique. Additionally, many more axons were visible in the DRG.

To date we have only examined labeled motoneurons with the electron microscope. Cells identified as labeled were trimmed from the plastic sections and thin-sectioned for EM. Cells processed by the GOD technique contained numerous lysosome-like inclusions which stained positively for HRP. The BDHC technique, however, produced labeling which was not only confined to lysosomes, but was also dispersed in restricted regions. In these regions the label was aggregated on the surfaces of cytoplasmic organelles. This dispersal of reaction product may account for the increased axonal and dendritic labelling afforded by this technique at the LM level. Additionally, the dispersed label provides for easier identification of HRP-positive elements at the EM level. (Supported by Grants NS-]4457 and -10165.)

158.2 ANTERO- AND RETROGRADE TRANSPORT OF WHEATGERM AGGLUTIN FOLLOWING EXTRA- AND INTRACELLULAR INJECTION IN THE CAT VESTIBULO-OCULOMOTOR SYSTEM. R. Baker, H. Baker, J. Blanchard*, M. Shaw* and D. Soriano*. Dept. Physiol. & Biophys., New York Univ. Med. Ctr. and Dept. Neurol., Cornell Med. Ctr., New York, NY 10016.

Representative of a class of proteins with selective binding affinity for carbohydrate moities, wheatgerm agglutin (WGA) shows considerable promise as a sensitive marker for study of neuronal connectivity, especially due to its transynaptic transport (Coulter, et al, Neurosci. Abst. 6: 389, 1980). In order to test anterograde, transneuronal labeling following intracellular injection of WGA, we employed as a model pathway the extensive synaptic contact between second order vestibular neurons and extraocular motoneurons. To confirm the antero- and retrograde labeling reported above, WGA (0.2-2.0%) was injected intraocularly and into slips of extraocular muscles. Using an immunocytohistochemical technique with anti-WGA antibody provided by E-Y Laboratories (1:5000 dilution) both brown (DAB) and black (TMB) reaction products were localized in appropriate layers of the superior colliculus and motoneuronal subdivisions of the oculomotor complex with particularly striking sensitivity. For intracellular injection, WGA and HRP (the latter for comparison) were dissolved in 0.5M KCl (2.0-4.0%) and electrophoretically injected utilizing 1/2 duty cycle stimulation and a range of 60-200 nA minutes of positive current. Both WGA and HRP were recognized as diffuse brown reaction products (DAB) when injected either in identified second order vestibular neurons 3 mm from their termination sites in the oculomotor nucleus or in motoneuronal axons or somata. Unfortunately, we must report that WGA appears to bind immediately to the intraaxonal membrane at the injection site and it does not diffuse more than about 1 mm from that point, even up to 72 hrs after injection. In contrast, a similar survival time with HRP results in intense labeling of the entire vestibular neuron extending from the vestibular to oculomotor nucleus (1.5 cm). Injection of WGA directly into motoneuronal somata and dendrites produces only localized reaction product, and within 2-4 hrs it is incorporated into lysosomes. We conclude that WGA is indeed an extremely sensitive marker for both antero- and retrograde study of neuronal connectivity including transneuronal targets; however, it cannot function in this capacity when introduced directly into the cytoplasmic compartment. In fact, this lectin as well as others like it, may be more useful for marking injection sites. Other lectins including various conjugated forms of WGA are also being examined in the above model system. Supported by USPHS grants NS13742, EY02007 and NHLB18974.

158.3

WITHDRAWN

158.4 PEROXIDASE ANTI-PEROXIDASE AND OTHER LABELED IMMUNOGLOBULINS ARE SENSITIVE RETROGRADE TRACERS. Joseph N. Riley, Department of Neurology, SUNY Stony Brook, Stony Brook, NY 11794

The horseradish peroxidase (HRP)retrograde tracing method has become a widely used neuroanatomical technique. One of the major problems associated with the method is the extensive diffusion of the enzyme. Diffusion severely limits the HRP method in the analysis of local circuit connections. Recently we reported (Brain Research, 205, 1981) that there is significantly less diffusion from microelectrophoretic deposits of fluorescein isothiocyanate-conjugated HRP compared to unconjugated HRP. Speculating that the increased size of the conjugate may slow diffusion, a number of HRP-immunoglobulin conjugates were tested for retrograde transport. These conjugates were found to be transported more effectively than unconjugated HRP.

A number of conjugates have been tested, including peroxidase anti-peroxidase (PAP) produced in goat (Miles) and rabbit (Miles and Polysciences), HRP-labeled goat anti-rabbit (Miles), and HRP-labeled rabbit anti-rat (Polysciences).

For parametric studies, adult Sprague-Dawley rats were injected in the tongue bilaterally with 10 ul of conjugate at various concentrations or a control solution of HRP (Miles or Sigma, Type VI). Following 18 hrs survival, animals were sacrificed and alternate sections through the hypoglossal nucleus were reacted according to Mesulam's TMB procedure. The number of clearly identifiable labeled neurons was used to compare the effectiveness of conjugates to unconjugated HRP. With the exception of one brand of PAP (Polysciences), all conjugates labeled more cells than unconjugated HRP at equimolar concentrations. The concentration necessary to demonstrate retrograde transport was 10-30 times lower for the conjugates. Goat anti-rabbit and rabbit anti-rat labeled up to 200 cells at concentrations that transport of unconjugated HRP could not be demonstrated. Preincubation of conjugates with Protein A or appropriate control serums had no effect on transport.

In additional experiments, conjugates were deposited microelectrophoretically into various brain regions. Using appropriate parameters, conjugates are effective in tracing local circuitry. For example, using PAP, it has been possible to examine intranuclear connections in the suprachiasmatic nucleus of the hypothalamus.

Conjugates were injected into the eye to determine the degree of anterograde transport. At stock concentration, little or no anterograde transport could be demonstrated. A ten-fold concentration of PAP produced only weak labeling as well as a moderate to severe allergic responses.

(Supported by NIH Grant NS 16814)

158.5 POLYETHYLENE GLYCOL: A SUPERIOR EMBEDDING COMPOUND COMPATIBLE WITH IMMUNOHISTOCHEMISTRY AND INTRACELLULAR STAINING.
Kenneth G. Smithson*, Brian A. MacVicar and Glenn I. Hatton. Neuroscience Program and Psychology Department, Michigan State University, East Lansing, MI, 48824.

A technique is described which permits rapid processing of neural tissue for light microscopic analysis of sections from 1-40 μm thickness. This technique was developed as an alternative to paraffin embedding which has several serious disadvantages, such as causing profound tissue shrinkage, producing relatively great reduction in tissue antigenicity, destroying enzymatic activity and often resulting in tissue distortion due to the high temperatures and/or harsh organic solvents used. We needed a technique that: would allow ∿5 μm sections of flat-embedded tissue slices, was rapid, was compatible with locating intracellularly injected fluorescent dyes and would permit differential immunocytochemical analyses. Polyethylene glycol (PEG) is a water soluble polymer which has been used in the past as an embedding medium with a wide variety of stains and satisfies the above criteria. When compared to paraffin, PEG offers the following advantages: 10-15°C lower embedding temperature, 20% tissue shrinkage in PEG which diminishes to 5% upon rehydration vs. 50% in paraffin, approximately one-half the embedding time, and, because PEG is water soluble, ethanol dehydration is not required. Furthermore, tissue orientation during embedding and sectioning is particularly easy to control because of the working properties of PEG, i.e., brain slices can be routinely flat-embedded and sectioned to form excellent ribbons. Also, PEG can be easily removed by washing the tissue with a variety of aqueous solutions such as buffered saline. However, because PEG is hygroscopic, tissue blocks become soft and difficult to section in high ambient humidity. Finally, we have found PEG to be compatible with intracellular staining with Lucifer Yellow or horseradish peroxidase as well as immunohistochemical demonstration of neuronal peptides. Therefore, we conclude PEG is a whole "new" ball of wax.

This research was supported by NIH grants NS 09140 and NS 16942.

158.6 EXTRACELLULAR IONTOPHORETIC INJECTION OF LUCIFER YELLOW STAINS LOCALIZED ARRAYS OF NEURONS WITHIN ORGANIZED CNS EXPLANTS.
D.R. Friedlander and S.M. Crain. Dept. of Neuroscience, Albert Einstein College of Medicine, Bronx, New York 10461.

Although extracellular injection of the fluorescent dye, lucifer yellow (LY), does not stain neurons when applied in amounts that completely fill cells intracellularly injected (e.g.Stewart, Cell 14:741,'78), LY can penetrate into neurons by submerging a cut nerve in a solution of the dye (S. Sharma, pers. commun.).

We have applied LY by iontophoretic injection, via extracellular glass microelectrodes (10 μm tips) filled with 3% LY, placed in selected regions of fetal mouse neural explants, and observed staining of cell bodies and neurites with epi-fluorescence microscopy. Most of these neurons could not be visualized in our relatively thick organotypic CNS explants, even with Nomarski interference optics. Successful results were obtained with negative currents of the order of 1 μA for 10 sec. This localized dye injection generally produced high-contrast Golgi-like labeling of dozens of nearby neurons, within a few min, distributed in variable arrays over areas of the order of 100 μm diam. Large diameter axons (> 1 μm) stained for distances > 1 mm within about 10 min and did not fade for > 1 hr. Extrusion of dye via a pipette almost touching the explant did not stain neurons, even when nearby tissue was damaged with a saline-filled pipette.

In retinal explants (Smalheiser et al, Br. Res. 204:159,'81) ganglion cell bodies and their processes were labeled, and also the growth cones of unidentified neurons. In explants of spinal cord with attached dorsal root ganglia (DRG), where DRG neurites develop specific connections with dorsal horn neurons (Crain and Peterson, Br.Res. 79:145,'74), LY injection into the DRGs labeled a number of cell bodies and also axons which entered the dorsal cord. Application of the dye into dorsal regions of the cord explants stained local cord neurons and neurites and, more faintly, some distant cells including DRG perikarya. LY injections into ventral cord labeled large neurons with stout dendrites, presumably motoneurons, but did not stain DRG neurons.

These results are consonant with horseradish peroxidase injections of similar cultures (Smalheiser et al, Dev.Br.Res. '81, in press) and are thus indicative of the potential value of this new LY method for more rapidly mapping axonal projections to target tissues within CNS explants. Furthermore, since neurons can be made visible in selected regions of living CNS explants, it is now possible to guide microelectrode placements to particular cells within organized multi-layered neural networks for electrophysiologic analyses under direct visual control.
(Supported by grants NS-14990 and DA-02031 to S.M.C.; and NIH fellowship award EY0 5471 to D.R.F. from National Eye Institute.)

158.7 SUICIDE TRANSPORT: DESTRUCTION OF NEURONS BY RETROGRADE AXONAL TRANSPORT OF THE TOXIC LECTIN, RICIN.
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Ricin, the toxic lectin from castor beans (Ricinis communis), can be taken up and retrogradely transported by peripheral sympathetic nerves. (Lab Invest. 42; 396, 1980). We sought to determine if this lectin, which kills cells by inactivating ribosomes, can be taken up and retrogradely transported by peripheral somatic sensory and motor nerves; if such transport will kill parent neurons; and if so whether the perikaryal response differs from the chromatolytic reaction. Rats or rabbits were anaesthetized and either ricin or ricin conjugated to horseradish peroxidase (HRP) was applied to the cervical vagus (X) or to the hypoglossal nerve (XII). Toxin was applied either by soaking the proximal end of the cut nerve or injecting (0.25-2.5ug of ricin in 0.5ul of saline) into the intact nerve. In control experiments, the nerves were either transected or injected with saline. After 1-16 days, animals were anaesthetized and perfused with aldehyde fixative. The medulla and nodose ganglia (NG) were sectioned and stained with cresyl violet or, for HRP conjugate, with tetramethylbenzidine (ricin-HRP experiments.) One day after injection of ricin-HRP into the cervical vagus, HRP staining was intense & Nissl staining was reduced in neurons in both the ipsilateral NG and the dorsal motor nucleus of the vagus (DMNX.) By 2 days these neurons had lost their Nissl substance without change in nuclear position or morphology. At 3 days, neurons were beginning to disintegrate and HRP staining became diffuse in DMNX. After 4 days there was extensive neuronal loss and some gliosis was evident in DMNX. At 16 days there was almost complete neuronal loss in both ipsilateral DMNX and NG. Similar changes were observed in the XII nucleus after injection of ricin into the XIIth nerve. These cellular events initiated by ricin contrasted to the classic chromatolytic responses observed in NG and DMNX after cervical vagotomy which only resulted in a modest loss of neurons.
We conclude: (a) sensory and motor axons will transport ricin to the perikaryon; (b) such transport results in rapid death of these neurons; (c) the toxic response differs from the retrograde reaction elicited by nerve transection.
We have called this transfer of toxic agents to the nerve cell body "suicide transport". It may prove a powerful tool for correlated anatomical, neurochemical and physiological experiments.
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158.8 TRACING NEURONAL CONNECTIONS BY ELECTRON MICROSCOPY USING HORSERADISH PEROXIDASE: A COMPARISON OF EIGHT METHODS.
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Electron microscopy is being used frequently in studies of neuronal connectivity to visualize the synapic relationships of neurons labeled with tracers such as horseradish peroxidase (HRP). Recently the most commonly used HRP histochemical methods were compared (Mesulam and Rosene,1979, J. Histochem. Cytochem., 27: 763). This quantitative light microscopic comparison showed that methods using tetramethyl benzidine (TMB) and benzidine dihydrochloride (BDHC) were among the most sensitive. Our experiments were undertaken to evaluate and improve the TMB and BDHC ultracytochemical methods for HRP and then compare them to several other commonly used methods for the electron microscopic demonstration of HRP activity. A spinal cord-peripheral nerve system was chosen so that HRP which entered the spinal cord by retrograde or transganglionic axonal transport could be visualized in the same section. Horseradish peroxidase-wheat germ agglutinin conjugate was injected in the gastrocnemius muscle complex in mice. After 48 hours the mice were anesthetized and perfused through the heart with fixative. The lumbar spinal cord was cut coronally on a vibratome at 75 microns into 8 series of about 20 sections each. HRP activity was demonstrated by the following methods: TMB, BDHC, o-tolidine, paraphenylenediamine-pyrocatechol, diaminobenzidine (DAB)-Streit and Reubi, DAB-Malmgren and Olsson, DAB-glucose oxidase and DAB-LaVail and LaVail.

The results showed that TMB and BDHC could be readily used for the ultracytochemical demonstration of HRP activity. In the case of TMB, the temperature and pH of osmication were crucial variables in obtaining an electron dense and insoluble reaction product. A pH of 6.0 and temperature of 45°C gave the best results in this system. The TMB, BDHC and o-tolidine procedures demonstrated the largest distribution of HRP activity in the dorsal and ventral horns. The TMB reaction product was electron dense and had a unique, angular, crystalloid appearance that aided identification of labeled neurons. This useful feature combined with the apparent sensitivity of the TMB ultracytochemical method indicated its potential value in demonstrating processes of labeled neurons containing low amounts of HRP, such as distal dendrites and synaptic boutons. This investigation was supported by NIH Grants 14625, NS 090211, NSF Grant BNS 7823610 and an NIH Post-doctoral Fellowship NS 06550 to K.A.C.

158.9 PHYSIOLOGICALLY ENHANCED HRP LABELING OF SALIVARY NEURONS IN RATS

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Neurons in the superior salivary nucleus of rats were labeled with HRP by injecting 2μl of a 50% HRP solution (Sigma,type VI) into the lingual nerve.14 1/2 to 24 hrs after injection,the rats were sacrificed by perfusion with physiological saline followed by 2.5% glutaraldehyde.50μm sections were cut and reacted using Mesulam's TMB method (J.Histochem.Cytochem.26;106,1978).Marked cells form a diagonal band in the ipsilateral brainstem,just caudal to the exiting root of the facial nerve and extending about 950μm in a rostrocaudal direction (Hiura,Br.Res.137;145, 1977). A ventral and dorsal cluster of cells are evident at the largest expanse of the nucleus.The neurons are multipolar,with the cell body measuring 30x20μm across.The TMB reaction product is seen as fine granules distributed throughout the cytoplasm and proximal dendrites,leaving the large,centrally placed nucleus (10μm in diameter) unlabeled.Increasing the transport time from 14 1/2 to 24 hrs,led to marking of an exponentially increasing number of cells,from 2 labeled cells at 14 1/2 hrs to 272 cells at 24 hrs.The regression of cells marked on transport time could be fitted with the equation $N=0.001\exp(0.51T)$,$r=0.995$.

In five pairs of rats,the effect of stimulating activity of salivary neurons on HRP labeling was studied.In one rat of each pair,salivation was stimulated by swabbing its mouth with 1% acetic acid for one hr,immediately following HRP injection into the lingual nerve.At each transport time,more HRP labeled cells were counted in stimulated than in unstimulated salivary nuclei. The largest ratio of stimulated:unstimulated cells,13.5:1,was seen following 18 hrs transport time.In stimulated animals,the relationship between number of cells marked and transport time followed a normal cumulative frequency distribution (ogive) curve,with a mean at 18.2 hrs,S.D.=1.6 hrs.Using several dissected specimens,axonal length from injection site to cell body was estimated to be 23-25 mm.Taking visible cell marking as an endpoint,the range of retrograde transport rates was 23-38mm/day.

In these studies,labeling occurred by diffusion of HRP into injured axons,followed by retrograde transport.It seems likely, therefore,that enhanced labeling of stimulated cells was not due to increased pinocytic activity, but may reflect either increased intravesicular packaging and/or increased retrograde transport rate in the stimulated cells.

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159.1 THE DISTRIBUTION OF CHOLINERGIC COMPONENTS IN LIMULUS. R. Sukumar*, M. Ivy* and J.G. Townsel. Department of Physiology and Biophysics, University of Illinois Medical Center, Chicago, IL 60612.

The distribution of several biochemical parameters generally accepted as being associated with cholinergic transmission was studied in Limulus muscle and nerve tissue. These studies included a determination of tissue levels of acetylcholine (ACh), choline (Ch), acetylcholinesterase (AChE:E.C.3.1.1.7) and choline acetyltransferase (ChAC, E.C.2.3.1.6). Choline uptake was also studied in these tissues. A high affinity component of the measured uptake was assessed by the determination of tissue/medium distribution ratios (T/M).

In general, ACh and Ch were distributed among all the tissues assayed. The ACh values ranged from a high of 3.00± 0.49 nmole/mg protein in abdominal ganglia to a low of 0.54± 0.04 nmole/mg protein in cardiac muscle. Similarly, AChE was detectable in all tissues assayed. However, the range of activity was an order of magnitude greater with brain roots having the highest level of activity at 2.81±0.73 μmoles of ACh hydrolysed/liter/mg protein/min and skeletal muscle having the lowest level of activity at approximately 1.8% of brain root activity. ChAC was not so widespread in distribution. ChAC activity was not detected in any of the muscle tissues assayed nor was it detected in the cardiac ganglion. The highest level of ChAC activity was measured in the corpora pedunculata (43.15±0.07 nmoles of ACh synthesized/mg protein/min). No direct correlation was found between the level of ChAC activity and the amount of measurable ACh in a tissue. Similar levels of ACh (c.a.2.4 nmoles/mg protein) were found in circumoesophageal ring (CR) and the dorsal roots of the abdominal ganglia (DR-AG); however, the CR had a ten fold greater level of ChAC activity than that measured in the DR-AG (33.2±1.6 nmoles of ACh/mg protein/min vs 3.4±0.9 nmoles of ACh/mg protein/min).

TM ratios indicative of high affinity uptake were selectively distributed ranging from 13.22 to 4.62. In three of the four tissues where ratios in this range were found we also observed an elevated potassium triggered, Ca^{2+}-requiring, specific release of [H^3]-ACh. The significance of these results will be discussed.

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159.2 EVIDENCE FOR TWO FUNCTIONALLY DISTINCT POOLS OF BOUND (VESICULAR) ACH IN RAT BRAIN SYNAPTOSOMES. M. E. O'Leary* and J. B. Suszkiw, Dept. of Physiol., Univ. of Cincinnati Med. Ctr., Cincinnati, OH 45267.

Compartmentation of ACh was studied in resting and in K+-depolarized rat brain synaptosomes. In standard Krebs-Ringer solution, [^{3}H]ACh synthesized from [^{3}H]Ch in resting synaptosomes was distributed 40% in bound and 60% in free form. In the presence of 20 μM HC-3, sequential 3-minute exposures of synaptosomes to 50mM K+ resulted in the release of decrementing amounts of [^{3}H]ACh until maximal cumulative release reached 40% of the total initial [^{3}H]ACh in synaptosomes. Bound [^{3}H]ACh decreased in proportion to [^{3}H] ACh released. Resting synaptosomes first labelled with [^{14}C]ACh and then sequentially depolarized by 3-minute exposures to high K+, each followed by a 10-minute recovery period in normal saline containing 1μM [^{3}H]Ch, released [^{14}C]ACh decrementally but the release of the newly forming [^{3}H]ACh continued unabated or increased with each depolarization. The [^{3}H]ACh/[^{14}C]ACh ratio in the perfusate nearly doubled for each successive depolarization and did not correspond to [^{3}H]ACh/[^{14}C]ACh in synaptosomes, vesicles or cytoplasm. These results suggest that:

1) K+ depolarization results in Ca^{++}-dependent-enhanced release of bound vesicular but not of cytoplasmic ACh.

2) After displacement of total reserve ACh, the emptied vesicles cannot be readily replenished from the cytoplasm.

3) A fraction of vesicles can be replenished readily with newly synthesized ACh before this ACh equilibrates with the cytoplasmic pool. This implies tight coupling between choline transport, synthesis of ACh and reloading of the active pool. Functioning of this system appears to be essential for the unabated, evoked release of ACh.

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159.3 HETEROGENEITY OF CENTRAL NERVOUS SYSTEM α-BUNGAROTOXIN BINDING SITES. Ronald J. Lukas. Division of Neurobiology, Barrow Neurological Institute, Phoenix, AZ 85013.

Ligand binding characteristics of putative mammalian central nervous system nicotinic-type acetylcholine receptor sites have been reinvestigated. Purffied α-bungarotoxin has been radioiodinated and resolved into unlabeled, monoiodinated (I-Bgt) and diiodinated species according to established procedures (Biochem. 17, 3208, 1978). Using fresh preparations of I-Bgt, subcellular membrane fractions rich in synaptic junctional complexes and plasma membranes from brains of male Sprague-Dawley rats; and a modification of published binding assay protocols (Biochem. 18, 2384, 1979), toxin binding saturation profiles have been obtained to ~300nM with non-specific binding levels limited to ~25% of total binding. This increased sensitivity permits resolution of two classes of I-Bgt binding sites in 1:1 stoichiometry, characterized by pre-equilibrium K_d values of ~2nM and ~100nM.

Cholinergic ligand binding competition profiles yield the following rank order of potencies at the high-affinity (at 10nM) I-Bgt site: Nicotine (1uM) > acetylcholine = bromoacetylcholine > carbamylcholine = d-tubocurarine (10uM) > alcuronium > tetraethyl-ammonium > lobeline (100uM) > gallamine = decamethonium = pancuronium > atropine > muscarine (1mM) > mecamylamine = hexamethonium (~10mM).

Delineation of the pharmacological profile for the low affinity I-Bgt site may provide insight into correlates of central nicotinic receptor function.

159.4 PURIFICATION AND IMMUNOCHEMICAL PROPERTIES OF CHICKEN BRAIN CHOLINE ACETYLTRANSFERASE. J. H. Peng*, K. Ma*, P. L. McGeer and E. G. McGeer. Kinsmen Laboratory of Neurological Research, University of British Columbia, Vancouver, B. C., Canada, V6T 1W5.

Chicken brain choline acetyltransferase (CAT) was purified to homogeneity using ammonium sulfate fractionation, followed by chromatography on DEAE-Sephadex (A-25), hydroxyapatite, Sephadex G-150, immunoabsorption and Sephadex-CoA columns. A purification of 3500-fold was achieved and the final preparation had a specific activity of 2.32 μmol ACh formed per min per mg protein. The purified chicken CAT migrated as a single band on polyacrylamide gel electrophoresis in the presence and absence of sodium dodecyl sulfate. The native enzyme, with a molecular weight of 67,000 daltons, consists of two subunits of identical molecular weight. Chicken CAT has a sharp pH optimum of 7.4. It is activated by sodium chloride and potassium chloride but inhibited by cupric ion and N-ethylmaleimide.

An antiserum was obtained from immunized rabbit after three subcutaneous injections of 100 μg each of the pure chicken CAT preparations at various sites near scapular and neck regions. The antibodies appeared to be monospecific for CAT, since they gave only a single precipitin line against pure or crude enzyme preparations from chicken and human brain, and crude enzyme from rat, rabbit, guinea pig and bovine brains in double immunodiffusion and immunoelectrophoresis plates. The antiserum or immunoglobulins also inhibited CAT activities from chicken and mammalian brains in immunoprecipitation studies. As with the inhibition of human enzyme by anti-human CAT, inhibition of chicken CAT was seen after 30 min of pre-incubation with anti-chicken CAT serum or immunoglobulins and the extent of inhibition increased with time of incubation up to 3 h at 37°C and 20 h at 4°C. The maximum levels of inhibition measured was 85% in the reaction mixtures and 96% in the supernatants after centrifugation when compared with similarly incubated samples using pre-immune serum. This and previous studies clearly demonstrated that high titer anti-chicken CAT antibodies do cross-react with CAT from several mammalian species, and vice versa.

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159.5 EXCITATORY EFFECT OF ACETYLCHOLINE ON DIFFERENT TYPES OF ANTIDROMICALLY IDENTIFIED CEREBRAL CORTICAL NEURONS. P. Dutar*, Y. Lamour and A. Jobert*. Unité de Recherches de Neurophysiologie Pharmacologique de l'INSERM, U. 161, 2 rue d'Alésia 75014 Paris, France.

Acetylcholine (ACh) has been shown to excite a large percentage of pyramidal tract neurons in the cerebral cortex. The aim of this experiment was to compare in rat somatic sensory cortex (SI) the sensitivity to ACh of pyramidal tract neurons (PTNs), cortico-thalamic neurons (CTNs) and commissural neurons (CNs), identified by antidromic activation. Stimulating electrodes were respectively located in the pyramidal tract, the ipsilateral ventro-basal nucleus of the thalamus and the corpus callosum (in the opposite hemisphere). Rats were anaesthetized with urethane (1.5 g/kg, i.p.). ACh (0.5 M, pH 4) was iontophoretically applied from one channel of a seven barrel electrode. Single unit activity was recorded using a second pipette rigidly fixed to the side of the multibarrelled electrode. Each microelectrode penetration was reconstructed on a camera lucida drawing of 100 µm frozen sections stained with cresyl violet using a dye deposit made at the last recording site. The location of each stimulating electrode was also verified on the sections. A given penetration was taken into account only if at least one excitatory response to ACh was recorded. ACh responses were tested with currents of up to 120 nA for up to 1 minute. Criteria for antidromic activation were : fixed latency, ability to follow high stimulation rates (above 200 Hz) and collision with orthodromic action potentials.

ACh excited 59 % of the PTNs, 52.6 % of the CTNs and only 4.3 % of the CNs. The percentage of unidentified cortical neurons excited by ACh was 27.5 % of the total population (n = 461). PTNs were located in layer Vb and the CTNs in layers V and VI, whereas CNs were scattered in the cortical layers, in agreement with previous anatomical observations. These results suggest that ACh-sensitivity is specific for certain neuronal types. Most of the other unidentified neurons excited by ACh were located in layer Vb and upper part of layer VI, where cortical neurons projecting on subcortical structures are to be found. There might therefore be a relationship between the cells of origin of cortico-subcortical systems and ACh sensitivity. ACh might increase the excitability of cortical output neurons involved in the control of sensori-motor activities.

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159.6 AN INHIBITORY ACTION OF ACETYLCHOLINE ON THE DENDRITIC EPSPs IN THE AMMON'S HORN. Y. Ben-Ari, E. Cherubini and C. Rovira, Laboratoire de Physiologie Nerveuse, CNRS, 91190 Gif-sur-Yvette, France.

When microiontophoretically applied on pyramidal cells of the ammon's horn, Acetylcholine (Ach) has a double action : a postsynaptic depression of K conductance and a suppression of inhibitory control apparently mediated by a presynaptic action on GABAergic terminals (Ben-Ari, Krnjevic, Reinhardt and Ropert, Neurosci. In Press). We now report an additionnal effect, namely an inhibitory, primarily muscarinic, action on the fields EPSPs recorded in the dendritic layer. Our experiments were done on intact, urethane anaesthetized rats, using multibarreled micropipettes to record field responses evoked in CA1 of the dorsal hippocampus by stimulation of the ventral hippocampal commissure. On several occasions an assembly of glued micropipettes enabled to simultaneously record (and eject various drugs) in the somatic and dendritic layers. At low frequencies of stimulation (usually 1/s a large positive wave, which reflects the powerful IPSPs generated by recurrent collaterals of pyramidal axons (Andersen et al. J. Neurophysiol. 27, 592, 1964) and a negative population spike are recorded in the pyramidal layer and a negative field (with a small positive spike) in the dendritic layer. Application of Ach on the soma produces a reduction of the positivity and the appearance of multiple (2-3) population spikes (see, Reiffenstein, Krnjevic and Ropert Neurosci. abst, 1980). At the dendritic layer, Ach produced a 10-25 % reduction of the field EPSP. This action has typically a slow onset (often more than 20 sec) and recovery. It is also produced by muscarine, and antagonized by atropine or scopolamine and not by benzyl-penicilline. The Ach effect - in contrast to the more powerful inhibitory effect of GABA - is quite localized (approximately 200-250 um of the soma). It is not due to diffusion of the substance to the soma, since simultaneously recorded somatic IPSP and population spikes are not altered. These resultats are in keeping with previous observations of Hounsgaard (Exptl. Neurol. 62, 787, 1978) who reported in the slice preparation a presynaptic inhibitory action of Ach on dendritic EPSP. Since eserine also reduces the fields EPSPs and potentiates the effects of Ach it is possible that cholinergic inputs modulate the excitatory inputs to the dendrites. Interestingly, recent observation suggest that following ipsilateral destruction of the septo-hippocampal cholinergic projection (as controlled by means of acetylcholinesterase staining) there is a denervation suppersensitivity of Ach effects in the dendrites (in preparation).

159.7 CHOLINERGIC GENERATION OF DESYNCHRONIZED SLEEP SIGNS:LOCALIZATION TO THE PONTINE RETICULAR FORMATION. H.A. Baghdoyan*, R.W. McCarley, J.A. Hobson. Laboratory of Neurophysiology, Department of Psychiatry, Harvard Medical School, 74 Fenwood Road, Boston, MA 02115.

Carbachol injection into the pontine reticular formation (RF) elicits D carb, a state resembling physiological desynchronized sleep (D). The anatomical specificity of this response is being examined by comparing the effects on sleep-wake states of carbachol injections into the pontine (FTG), midbrain (FTC) and medullary (FTL) RF. Our results show: 1) D carb can be induced only from the pontine RF, and 2) synchronized sleep (S) is markedly suppressed following carbachol injection into all brainstem sites.

Cats are implanted stereotaxically with guide tubes. 0.5-1.0 ul of carbachol (8ug/ul) or saline is pumped through a stainless-steel cannula. Every site receives at least one administration of carbachol and saline. Polygraphic recordings and behavioral observations are conducted for 4 hrs post injection.

Injection of carbachol into the pons produced a 4-fold increase in the percent of total recording time spent in D carb as compared with baseline D values (31 vs 8%, N=7). In contrast cholinergic stimulation of the medullary RF totally suppressed D (N=7). The midbrain effect of carbachol on D is a slight reduction (6 vs 8%, N=4). Carbachol dramatically reduced S at all brainstem sites: S was abolished after all 7 pontine, 5 of 7 medullary and 2 of 4 midbrain injections. Following the other medullary and midbrain injections S was reduced. Waking was increased by carbachol from 39% of total time during baseline recordings (N=36) to 61% for pontine, 98% for medullary and 79% for midbrain sites. In 5 of 7 pontine injections a unique pattern of REMs and PGO waves was induced during D carb: a cluster of large amplitude PGO waves alternates with a cluster of small amplitude PGO waves in one LGB while the reverse occurs in the other. The larger amplitude waves occur in conjunction with ipsilateral REMs, while the small amplitude waves are accompanied by REMs in the opposite direction.

At the medullary site carbachol produced a state of hyperarousal characterized by ataxia and increased motor activity. Midbrain injections caused an arousal lasting at least 2 hrs but with an overall decrease in motor activity; hissing, growling and contralateral turning were elicited at this site. Pontine injections also were characterized by little movement during waking; upon entering D carb the cats would assume a sleep posture and the nictitating membranes would partially close. Pending histological confirmation of injection sites we tentatively conclude that cholinergic generation of D is not medullary and not mesencephalic; it is pontine.

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159.8 PRODUCTION AND CHARACTERIZATION OF ANTIBODIES TO CHOLINE ACETYLTRANSFERASE OF RAT STRIATA FOR IMMUNOCHEMISTRY AND IMMUNOCYTOCHEMISTRY. D.H. Park, M.E. Ross, H. Baker, D.J. Reis, and T.H. Joh. Laboratory of Neurobiology, Cornell University Medical College, New York, N.Y., 10021.

We sought to develop antibodies to choline acetyltransferase (CAT) suitable for immunochemistry and immunocytochemistry. CAT was purified from striata of 500 rat brains by the following steps: Homogenization in 10 mM K-phosphate buffer pH 7.5, centrifugation at 100,000 g, lyophilization of the supernatant, followed by five sequential column chromatographic procedures-Sepharose 4B, hydroxylapatite (fast flow), hydroxylapatite (high resolution), Sephadex G150 and CM-Sephadex. The purified enzyme was then subjected to polyacrylamide gel electrophoresis and the active protein band was identified by assaying CAT activity of the gel slices. The active protein in the gel slices was then electrophoretically eluted by ISCO model 1750. The eluted protein showed a single stained band on the gel and was enzymatically active. Antibodies to CAT, either monoclonal (Ross, Neurosci Abs. 1981) or raised in rabbits, were highly specific. They exhibited a single immunoprecipitin arc when run on immunoelectrophoretic plates against a crude enzyme preparation or purified CAT, and strong inhibition of CAT activity when incubated with CAT preparations. However attempts to localize CAT by immunohistochemistry using the PAP technique failed. No cells were stained in tissue preparations of CNS or PNS, even those of cholinergic structures including the neuromuscular junction of diaphragm, hypoglossal nucleus and anterior horn cells of spinal cord.

In contrast, these structures were all autoradiographically labelled by a radiolabelled monoclonal antibody (Ross et al, Neurosci. Abstr 1981). The failure of the PAP to localize CAT may indicate that the CAT-CAT Ab complex formation on the surface of tissues after fixation is weak, although some primary antigen-antibody binding does occur, as demonstrated by autoradiographic localization of the radio-labelled monoclonal Abs. Conceivably, the primary binding of CAT-CAT Ab may be broken off by the addition of secondary antibody, i.e. goat antirabbit IgG or goat antimouse IgG, or may reflect stronger binding of secondary antibody to primary antibody. The results suggest that although antibodies to CAT are specific, the PAP method does not always reveal specific binding of antibodies to antigen. In this case, a direct staining procedure, such as autoradiographic localization of radiolabelled Ab is preferable.

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159.9 LOW ACTIVITY OF THE PYRUVATE DEHYDROGENASE COMPLEX AS WELL AS OF CHOLINE ACETYLTRANSFERASE IN HUNTINGTON CAUDATE AND PUTAMEN.
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Previous reports have described a correlation between the activities of choline acetyltransferase (CAT) and of the pyruvate dehydrogenase complex (PDHC) in cat and rat brain. We examined the distribution of these two enzymes and of the mitochondrial marker fumarase in regions of human brain from controls without neurological disease and from patients with Huntington's disease, who have decreased CAT in affected areas. Samples from a brain bank were studied double blind. Homogenates were assayed for CAT (by Fonnum's radiochemical method), for PDHC (by a spectrophotometric coupled enzyme assay using pigeon-liver arylamine acetyltransferase), and for fumarase (by a standard spectrophotometric method). The correlation between CAT and PDHC activity for all 84 samples studied was $r=0.93$ ($P<0.001$). Fumarase activity did not correlate well with either CAT or PDHC. Activity of PDHC, but not fumarase, was decreased in Huntington caudate (to 50% of controls; $P<0.005$) and putamen (to 40% of controls; $P<0.001$). As expected, CAT activity was lower in Huntington caudate and putamen. These observations are consistent with other data indicating a close relationship between cholinergic function and the capacity for pyruvate oxidation in the mammalian CNS. They suggest that decrease of PDHC is a consequence of Huntington's disease in affected areas. This decrease may prove to play some role in the pathophysiology of the disease, particularly in view of the suggestions that PDHC participates in synaptic plasticity.

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159.10 3,4-DIAMINOPYRIDINE AMELIORATES THE EFFECTS OF HYPOXIA ON BEHAVIOR AND ACETYLCHOLINE METABOLISM
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Hypoxia (reduced O_2 availability) decreases mental function in man, impairs performance on standardized behavioral tests in animals and inhibits brain acetylcholine (ACh) metabolism. Although the molecular basis of these changes is unknown, they may be explained in part by a decreased release of ACh. Thus, we studied the Ca^{2+}-dependent-K^+-stimulated release of ACh in hypoxia. The release of ACh from brain slices declined from 100±2% to 46±4% or 35±3% when the percent oxygen was lowered from 100% to 2.5% or 0%, respectively. Since 3,4-diaminopyridine (3,4-DAP), a K^+ channel blocker, enhances the influx of Ca^{2+} into nerve terminals, we examined its pharmacological action on the Ca^{2+}-dependent release of ACh during hypoxia. After treatment with 3,4-DAP (10 nM), the release of ACh only declined from 100±2% to 80±3% (2.5% O_2) or 71±2% (0% O_2).

In vivo, anemic hypoxia (injection of $NaNO_2$, 150 mg/kg) inhibited the synthesis of ACh (nmol/100 mg protein/min) in the hippocampus from 7.6±0.7 to 3.4±0.2 (45% of non-hypoxic control), striatum from 80±7 to 14.8±1.4 (18%) and cortex from 12.2±1.0 to 3.9±0.3 (32%). 3,4-DAP (10 nM) partially ameliorated the effects of hypoxia on ACh synthesis in the hippocampus from 3.4±0.2 to 5.5±0.5 (72% of non-hypoxic control) and the striatum from 14.8±1.4 to 20.1±1.7 (25%) but not in the cortex 3.9±0.3 to 3.9±0.3 (0%). 3,4-DAP did not stimulate ACh synthesis in non-hypoxic control mice. Brain lactate concentrations increased with hypoxia (>300%) but did not show regional specificity and were unaltered by 3,4-DAP treatment. Behavior, as measured by tight rope performance, declined with anemic hypoxia from 13.2±0.1 to 1.4±0.5 (11% of control). Treatment of these hypoxic mice with 3,4-DAP (10 nM) improved their scores to 6.9±1.0 (53% of control), while it did not alter non-hypoxic control scores.

In conclusion, 3,4-DAP, an agent which has been reported to increase influx of Ca^{2+} into nerve terminals, ameliorates some of the effects of low O_2. During hypoxia, 3,4-DAP increased the in vitro release of ACh, stimulated the in vivo synthesis of ACh in the hippocampus and striatum and improved performance on a standardized behavioral test.

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159.11 EFFECTS OF HYPNOTIC AND CONVULSANT BARBITURATES ON HIGH AFFINITY CHOLINE UPTAKE IN THE HIPPOCAMPUS.
J.A. Richter, J.M. Gormley*, J.R. Holtman, Jr.* and J.R. Simon. Dept. of Pharmacol. & Inst. Psych. Res., Indiana Univ. Sch. Med., Indianapolis, IN 46223.

Pentobarbital (PB) administration has been shown to inhibit high affinity choline uptake (HACU) in the hippocampus (Simon, Atweh and Kuhar, J. Neurochem. 26: 909, 1976). We have initiated a series of experiments to determine the locus and the mechanism of this effect and the relationship of the physiological effects of barbiturates to their effects on choline uptake.

Experiments were done on male Wistar rats, 200-300g and male Swiss mice, 25-30g. Drugs were injected i.p. and the animals killed by decapitation at selected times after injection. Synaptosomes (P_2 fraction) were prepared from the hippocampus and incubated with 0.5 µM ^{3}H-choline at 30°C for 4'. Blanks were incubated in medium without sodium. Rats were injected with 50 mg/kg ^{14}C PB and decapitated at loss of righting reflex (about 3') or 33', 63' or 123' after injection. The hippocampal choline uptake was inhibited only at 33' and 63' and not at the loss of righting reflex. The concentration of PB in the hippocampus was the same at loss of righting reflex as it was at 63' (with a peak at 33'), indicating no correlation between hippocampal PB levels and the inhibition of choline uptake. These data suggested that either the drug does not act in the hippocampus, or if it does, either the drug levels in the whole region do not reflect the levels at the exact site of action or there is a time lag before the drug effect is manifest. To test the possibility of a long time lag, we injected doses of ^{14}C PB from 35-150 mg/kg and decapitated the rats 7' after injection. At this time the standard 50 mg/kg dose caused an inhibition of uptake, so if there is a time lag, it is very short. However, the degree of uptake inhibition was still not well correlated with the levels of PB in the hippocampus.

Tested in mice, which were decapitated at convulsion, the convulsant barbiturate CHEB (5-(2-cyclohexylidine-ethyl)-5-ethyl B.A.) did not cause a change in HACU in the hippocampus. Another convulsant barbiturate (+) MPPB (1-methyl-5-phenyl-5-propyl B.A.) did cause an increase in hippocampal HACU similar to that originally reported for the convulsant petnylenetetrazol. These data coupled with the lack of inhibition of HACU by PB at the point of loss of righting reflex, suggest that the actions of these drugs are not directly correlated with the behavioral state and therefore are probably not caused by convulsion or hypnosis per se. Further studies are underway to determine the site of action of these drugs on hippocampal HACU by testing them in rats with fimbria/fornix lesions and also by local administration of the drugs into selected CNS sites. Grant DA00796.

159.12 EFFECTS OF ACETYLCHOLINESTERASE INHIBITORS ON 2-DEOXY-D-GLUCOSE UPTAKE IN THE RAT BRAIN.
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The ^{14}C-2-deoxy-D-glucose (2-DDG) technique was employed to determine the effects of two acetylcholinesterase inhibitors, physostigmine and neostigmine, on glucose metabolism in the central nervous system of the rat. Animals were injected with either physostigmine (0.1 or 0.4 mg/kg) or neostigmine (0.03 mg/kg), followed by an injection of 50 µCi 2-DDG. After 30 minutes the animals were killed by quillotine, the brains quickly removed, frozen in liquid freon and cut at 32 µm on a Damon/IEC cryostat. Radioautograms prepared from these animals were compared to saline injected control rats. Following either dose of physostigmine the most striking increase in 2-DDG uptake was found in the superficial layer of the superior colliculus (37%). Smaller increases were seen in caudal retrosplenial cortex, substantia nigra pars compacta, and in the motor nucleus of the Trigeminal nerve. Decreased uptake was observed in lateral thalamic nuclei. There were no significant effects in any other cortical region. After neostigmine administration the autoradiograms were indistinguishable from those of control animals. The results of these experiments are consistent with our knowledge of the distribution of both muscarinic and nicotinic cholinergic receptors. However, most cholinergic areas of the brain did not show demonstrable metabolic effects.

159.13 MODIFICATION OF CHOLINERGIC DYNAMICS IN RAT BRAIN BY INTRAVENTRICULAR ADMINISTRATION OF ADENOSINE ANALOGS. T. F. Murray,* D. L. Cheney and E. Costa. Lab. Preclin. Pharmacol., NIMH, St. Elizabeths Hosp., Washington, D.C. 20032.

Endogenously generated adenosine has been shown to behave as a neuromodulator that inhibits the firing of most central neurons. The inhibitory effects of adenosine on both spontaneous neuronal activity and on the generation of postsynaptic potentials in central pathways are mediated by an interaction with specific adenosine receptors which can be blocked by theophylline or caffeine. Considerable evidence indicates that adenosine and its analogs affect adenylate cyclase via two types of extracellular receptors, the A_1 which is a high affinity inhibitory receptor and the A_2 which is a lower affinity stimulatory receptor (Daly, J.W. et al. Life Sci., 28:2093, 1981). We have examined the effects of intraventricular (i.vt.) administration of adenosine receptor agonists and antagonists on the turnover rate of acetylcholine (TR_{ACh}) in various areas of the rat brain in an effort to better understand the role of adenosines as a neuromodulator or cotransmitter. TR_{ACh} was determined by gas chromatographic-mass fragmentographic analysis of the incorporation of deuterium into ACh and choline following an infusion of deuterated phosphorylcholine. The i.vt. administration of the adenosine receptor agonist, 2-chloroadenosine (2-ClAdo), in a dose of 83 nmoles did not alter the ACh or choline content of any of the brain areas examined. This dose of 2-ClAdo elicited significant reductions in the TR_{ACh} in both the hippocampus and frontal cortex, but not in the striatum. The extent of this reduction in TR_{ACh} was greatest in the hippocampus with a 67% decrease, while the TR_{ACh} in the frontal cortex was decreased by 36%. This effect of 2-ClAdo was antagonized by the prior i.vt. injection of theophylline (278 nmoles) suggesting that an activation of adenosine receptors is operative in the action of 2-ClAdo. Further support for this notion was obtained from experiments in which we compared the effects of the L and D isomers of phenylisopropyladenosine (PIA) on TR_{ACh}. The i.vt. administration of L-PIA (65 nmoles) elicited a 79% reduction in the TR_{ACh} in the hippocampus, while D-PIA (65 nmoles i.vt.) had no significant effect on hippocampal TR_{ACh}. This finding that L-PIA is more potent than D-PIA suggests that these effects on TR_{ACh} may be mediated by adenosine A_1-receptors which have been shown to display marked stereospecificity for the isomers of PIA rather than A_2-receptors at which L- and D-PIA have similar potencies.

159.14 EFFECTS OF A SINGLE DOSE OF SOMAN ON LEVELS OF ACETYLCHOLINE AND CHOLINE IN RAT BRAIN AREAS: A TIME COURSE STUDY. Tsung-Ming Shih. U.S. Army Biomedical Laboratory, Aberdeen Proving Ground, MD 21010.

The aim of these studies has been to investigate the time course of the levels of acetylcholine (ACh) and choline (Ch) in discrete brain areas of the rat following a single subcutaneous administration of Soman (120 µg/kg; 9/10 LD50), a completely irreversible anticholinesterase, in order to understand the relationship between neurotransmitter elevation and Soman toxicity. Rats were killed by microwave irradiation focused to the head 5, 10, 15, 20, and 40 min, and 1, 2, 3, 4, 8, 12, 16, 20 and 24 hrs after injection of Soman. One additional group of animals was killed in parallel with experimental groups at different times of day to ascertain possible circadian variations. The levels of ACh and Ch were determined in brain stem (B), cerebral cortex (C), hippocampus (H), midbrain (M), cerebellum (R) and striatum (S) by gas chromatography/mass spectrometry (Anal. Biochem. *55*, 438, 1973). A single dose of Soman caused a different degree and duration of increase of ACh and Ch levels in each brain area. Selective data (mean ± SE) for ACh are summarized in the following table:

Area	Time After Injection (Hours)			
	0	1	4	20
B	13.1±0.37(38)	14.7±1.82(10)	12.6±2.05(11)	11.9±0.42(10)
C	13.7±0.36(36)	32.6±4.80(15)	36.1±7.24(14)	20.2±1.46(10)
H	17.1±0.49(42)	27.7±4.66(15)	30.4±4.06(14)	21.4±1.36(10)
M	22.1±0.52(38)	29.1±2.86(15)	28.4±2.27(15)	23.9±1.69(10)
R	3.7±0.31(37)	4.9±0.42(11)	4.4±0.57(12)	2.3±0.46(10)
S	64.9±2.22(43)	82.4±8.27(11)	79.3±6.50(8)	59.1±3.82(10)

Maximal ACh elevation was reached in B, H, and S at 20 min (↑34.4, 68.3, and 33.3%, respectively); in R at 40 min (↑51.9%); in M at 1 hr (↑31.8%); and in C at 2 hrs (↑320.3%) after Soman. Duration of elevation of ACh was approximately 1/2 hr in B; 2 hrs in R; 4 hrs in S; 8 hrs in M; and 16 hrs in C and H. Thus injection of Soman caused the greatest change in ACh content in areas C and H, both in degree and duration. However, in the case of Ch, time for maximal effect was varied with, and duration of its elevation was shorter than that of ACh in each area. These data indicate that the perturbation of ACh and Ch levels caused by Soman was not uniform throughout the brain regions and suggest that the toxicity produced by Soman may be related to changes in neurotransmitter levels in specific brain areas.

159.15 ELEVATION OF PLASMA ACETYLCHOLINE DURING DEVELOPMENT OF THE ECC SYNDROME. A.C. Sconzert, G. Karp* and B. Haber. Marine Biomedical Institute, UTMB, Galveston, Texas 77550.

The ECC syndrome is a complex of stereotyped behavior characterized by general excitation, circling, and choreiform head and neck movements. This syndrome is induced by the daily intraperitoneal administration of 2,2'-iminodipropionitrile (IDPN; 300 mg/kg) to rats for one week. The expression of the symptoms usually follows a course such that choreiform head movements are first evident at about day 3 and rapid circling activity is present at day 6. During the week following the last injection the syndrome gradually stabilizes until the 15th day after the initial IDPN injection when the behavioral abnormalities can be said to be stabilized. Once induced, the ECC syndrome is permanent and irreversible. Thus the ECC rat may represent a useful animal model in which to explore some of the neurochemical parameters associated with movement disorders. We have previously reported data that regional markers of CNS cholinergic function (levels of acetylcholine, activity of choline acetyltransferase and acetylcholinesterase, and the binding of *QNB) are altered in ECC rats. We have also observed that the plasma GABA levels change in parallel with those seen in the CNS. Therefore it was of interest to measure ACH in the plasma, which is present at the level of 31 nmoles/ml. In the present study, female rats were injected with IDPN and blood obtained by cardiac puncture under pentobarbital anaesthesia at select times during the development of the ECC syndrome and analyzed for acetylcholine (ACH) content by the radioenzymatic method of Goldberg and McCaman (1976). Plasma levels of ACH rose to 171% of control values after a single injection of IDPN, 143% after 3 injections, were the same as control on day 6, and were 236% of control on day 14. This temporal pattern of ACH alterations parallels that seen in plasma GABA, and is the reverse of that seen in the CNS levels of ACH. Therefore, we feel that the measurement of plasma ACH may be useful in the assessment of the cholinergic changes in the development of the permanent ECC syndrome by IDPN.

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160.1 PRESENCE OF AN ENDOGENOUS FACTOR IN RAT CNS WHICH INHIBITS BINDING OF α-BUNGAROTOXIN 2.2 TO ITS RECEPTOR. M. Quik. Dept. Pharmacol., McGill Univ., 3655 Drummond St., Montreal, Que. H3G 1Y6.

Although the α-bungarotoxin 2.2 (α-BGT) binding site in nervous tissue has the characteristics of a nicotinic cholinergic receptor, the identity of the site remains unclear as the toxin fails to inhibit cholinergic receptor function at neuronal sites. The possibility of an endogenous ligand for the α-BGT binding site was thus investigated. Cerebral cortical membranes and supernatant were prepared by centrifugation of tissue homogenates at 45,000 xg for 10 min. The supernatant fraction obtained was found to significantly inhibit α-BGT binding to the membrane preparation. After a 3 min incubation period, the supernatant inhibited toxin binding by approximately 65%, while the inhibition declined to about 40% after 30 min of incubation, presumably due to the slow reversibility of α-BGT binding. The choice of buffer was found to be an important determinant of the degree of inhibition observed, with 10 mM Tris pH 7.3 providing the most effective condition. This inhibition of toxin binding to cortical membranes by the supernatant was shown not to be due to adsorption of the radiolabelled compound to soluble or residual particulate material in the supernatant fraction. Specificity of the supernatant for the α-BGT site was demonstrated; a supernatant fraction could be prepared which inhibited toxin binding by 50%, but had no effect on ^{3}H-spiroperidol (DA_2 and 5-HT_2), ^{3}H-prazosin (α_1-adrenergic), ^{3}H-5-hydroxytryptamine (5-HT_1) and ^{3}H-quinuclidinylbenzilate (muscarinic cholinergic) binding. Saturation kinetics indicated that the inhibiting substance affected the maximal number of binding sites (B_{max}), while the apparent affinity (K_d) of α-BGT for its receptor was not altered. The inhibition of toxin binding also occurred in several other CNS regions including hippocampus, brainstem, spinal cord and cerebellum, with an 80 to 90% inhibition of binding occurring in the latter two regions. In addition, the 45,000 xg cortical supernatant completely prevented the binding of α-BGT to extrajunctional neuromuscular receptors and inhibited the binding to junctional receptors by 50%. Supernatants prepared from heart, liver and kidney or bovine serum albumin, at a concentration similar to the cortical supernatant fraction, did not alter radiolabelled toxin binding to cortical membranes. A preliminary characterization of the inhibitory substance indicated it was of molecular weight greater than 1000 and unaffected by heat and trypsin treatments. Supported by the MRC of Canada.

160.2 INTERACTION OF RADIOLABELED ACETYLCHOLINE WITH MUSCLE PARTICULATE FRACTION. V.A. Eterović, P.A. Ferchmin, R. Hann and G. Escalona de Motta¶. Dept. of Biochem. School of Medicine, Univ. Central del Caribe; ¶College of Pharmacy and Lab. of Neurobiology, Medical Science Campus, Univ. Puerto Rico. Cayey, P.R. 00633.

The particulate fraction of toad rectus abdominis muscle binds tritiated acetylcholine (^{3}H)ACh) with high affinity. Two sites are detected by displacement with excess decamethonium. (^{3}H)ACh associates with each binding site through a fast process, which is completed in less than 15 sec under our assay conditions, and dissociates with similar celerity. The higher-affinity site is seen at (^{3}H)ACh concentrations of 10^{-9} to 3 x 10^{-9}M; thre are 1-2pmoles of this site per mg of protein. In addition to (^{3}H)ACh and decamethonium, this site interacts with carbamylcholine and nicotine, but not with d-tubocurarine. Preliminary experiments indicate that about half of the higher-affinity sites are inhibited by α-bungarotoxin. (^{3}H)ACh binding to this site is stimulated by succinic anhydride, which has been previously found to potentiate ACh potentials in the same muscle (Escalona de Motta and del Castillo, Nature 270: 178-180, 1977). We believe that the higher-affinity site is the nicotinic receptor.

Binding of (^{3}H)ACh to the lower-affinity site is seen at free (^{3}H)ACh concentration between 3 x 10^{-9}M and 5 x 10^{-7}M. This binding is partially inhibited by excess carbamylcholine, succinic anhydride, and d-tubocurarine. Decamethonium at low concentrations (10^{-8}M) stimulates (^{3}H)ACh binding to the lower-affinity site, while higher concentrations (10^{-4}M) are inhibitory.

This work was partially supported by the NSF Grant RIM 78-16333.

160.3 IN VIVO DEVELOPMENT AND REGULATION OF THE MUSCARINIC ACETYLCHOLINE RECEPTOR FROM EMBRYONIC CHICK HEART. S.W. Halvorsen* and N.M. Nathanson. Department of Pharmacology, School of Medicine, Univ. of Washington, Seattle, WA 98195.

Sustained exposure of 8-day embryos to cholinergic agonists decreases cardiac muscarinic acetylcholine receptor (mAChR) number as much as 87% as measured by the specific binding of the potent muscarinic antagonist [^{3}H]quinuclidinyl benzilate. The decrease in receptor number is both dose and time dependent. Muscarinic receptor number recovers to control levels when further agonist-receptor interaction is blocked by the mAChR antagonist atropine. When receptor number is decreased 50% by in vivo agonist treatment, isolated atria require a 12-fold greater concentration of the cholinergic agonist carbachol than controls to arrest spontaneous beating. Analysis of the binding of carbachol to the mAChR indicates this shift in response can be accounted for by a decrease in the absolute number of receptors and a change in the relative fraction of the high and low affinity forms of the mAChR with no change in their respective affinities for carbachol.

Other investigators have demonstrated that atria isolated from 3-day embryos are much less responsive to the negative chronotropic effect of muscarinic agonists than are atria from 8-day embryos (Pappano, A.J. and Skowronek, C.A., J. Pharmcol. Exp. Ther. 191: 109-118, 1974). We have found that 3 and 4-day embryos require at least a 10 fold greater dose of agonist to achieve equivalent in vivo loss of cardiac mAChR number as observed in 8-day embryos. Analysis of agonist binding to the mAChR in 4-day embryonic atrial membranes indicates the receptor is significantly less responsive to regulation by guanyl nucleotides than in atrial membranes from 8-day embryos. This suggests that 4-day atria which have not developed a full negative chronotropic response to muscarinic agonists either do not have or are not functionally coupled to a guanyl nucleotide binding protein. In addition, the percentage of high affinity agonist binding sites is greatly enhanced in 4-day atria. Comparison of binding and physiological data from 4-day atria with 8-day atria suggests that the high affinity form of the receptor is not uniquely required for the negative chronotropic response to muscarinic agonists and that the low affinity form is the active one.

160.4 N-ETHYLMALEIMIDE (NEM)-INDUCED ALTERATION IN THE INTERACTION OF AGONISTS WITH MUSCARINIC CHOLINERGIC RECEPTORS (MR). T.K. Harden and M.M. Smith*. Dept. of Pharmacology, Univ. of North Carolina School of Medicine, Chapel Hill, NC 27514.

Pretreatment of washed rat heart membranes with 100 μM NEM resulted in a 10-20 fold decrease in the apparent affinity of the agonist oxotremorine (OXO) for MR assessed in competition binding experiments with ^{3}H-quinuclidinyl benzilate (^{3}H-QNB). No effect of NEM-pretreatment on antagonist interaction with MR was observed. The apparent affinity of OXO for MR in NEM-pretreated membranes was similar to the apparent affinity of OXO in the presence of GTP in control membranes. GTP had no effect on the competition binding curve for OXO in NEM-pretreated membranes. NEM-pretreatment of membranes or the addition of GTP to control membranes increased the steepness of competition binding curves for OXO. Calculated Hill coefficients were 0.66±.01, 0.86±.03, 0.89±.05, and 0.92±.04 for control membranes, control membranes + 100 μM GTP, NEM-pretreated membranes, and NEM-pretreated membranes + 100 μM GTP, respectively. Maximal effects on agonist affinity of a 60 μM NEM pretreatment on ice were observed after 25 min of incubation. The $K_{0.5}$ value for the effect of NEM on OXO affinity was 40 μM for a 25 min incubation on ice and 5 μM for a 20 min incubation at 37°. Pretreatment of membranes with NEM at concentrations as high as 1 mM had no effect on agonist interaction at β-adrenergic receptors. The effects of NEM-pretreatment on the interaction of MR with adenylate cyclase (AC) were also studied. Both basal and isoproterenol-stimulated AC activities in heart membranes were inhibited (25 to 45%) in a concentration-dependent manner by OXO. Pretreatment of membranes with NEM under conditions which reduced the apparent binding affinity of OXO and blocked the effects of GTP on agonist binding affinity did not cause an equivalent reduction of the efficacy of OXO for inhibition of AC. The MR system has also been investigated in WI-38 fibroblasts. PGE_1 and isoproterenol stimulate AC activity 4-6 fold in membranes from these cells. OXO inhibits basal, as well as PGE_1- and isoproterenol-stimulated activities by 40%. Pretreatment of WI-38 membranes with NEM decreases the apparent affinity of OXO measured in ^{3}H-QNB competition binding assays and prevents the effect of GTP on agonist affinity. The concentration-effect curve for NEM is similar to that observed with heart membranes. Preliminary experiments with WI-38 membranes suggest that NEM-induced blockade of the inhibitory effects of OXO on AC occur at concentrations of NEM higher than that necessary to produce large effects on apparent OXO binding affinity. Supported by NIH grant 29536.

160.5 FACTORS INFLUENCING THE CONCENTRATION AND CHOLINERGIC REGULATION OF MUSCARINIC RECEPTORS IN NEURON-LIKE HYBRID CELLS. Hemin R. Chin, Neri M. Cohen*, and W.L.Klein. Dept.of Neurobiology and Physiology, Northwestern University, Evanston, IL 60201.

Sustained cholinergic stimulation of cultured cells causes a loss of muscarinic receptor binding sites measured in cell homogenates(Klein,W.L.,Proc.West.Pharmacol.Soc.23:449,1980). We have investigated this process further using cultured NG108-15 neuron-like hybrid cells and also have assessed the ability of other factors to influence muscarinic receptor levels. Incubation of cells with 10^{-3} M carbachol for 4 hours caused a 45-55% loss of (^{3}H)-QNB and (^{3}H)-N-methylscopolamine binding sites whether assayed in homogenates or in intact cells. This loss occurred in defined physiological buffer as well as in culture medium supplemented with serum. Treatment of homogenates with up to 1% digitonin or 10^{-3} M GppNp did not result in the appearance of any new binding sites. All available evidence is consistent with the hypothesis that the cholinergic-induced decrease in binding is due to a loss of receptor molecules on the cell surface. We have further found that, in addition to cholinomimetic drugs, several other physiological and biochemical factors influence the concentration of muscarinic receptors in NG108-15 cells. Removal of calcium ions from culture medium with EGTA resulted in a 40% decrease in binding sites within 4 hours. Binding was measured in the presence of calcium. Cells maintained with calcium concentrations either higher or lower than 1.8 mM(concentration in DMEM) had fewer binding sites than cells maintained in normal DMEM. Incubation of cells for 4 hours with either 10^{-3} M adenosine or 10^{-3} M GABA resulted in 30% and 45% increases in binding,respectively. Other transmitters and modulators tested showed no effect. This effect was not seen when carbachol was added, and neither adenosine nor GABA inhibited the loss of the sites caused by carbachol. Further exposure of cells to adenosine for 24 hours caused a 35% decrease in maximum binding and this response was not blocked by atropine. These results show that a variety of modulators, in addition to cholinergic activity, may regulate muscarinic receptor concentrations. (Supported by NIH grant 5 R01 NS 15299-02 to WLK.)

160.6 SEPARATION AND CHARACTERIZATION OF NEURONAL NICOTINIC RECEPTORS USING BROMOACETYLCHOLINE. T.H. Large, H. Chin, D.J. Raithel*and W.L. Klein. (Department of Neurobiology and Physiology, Northwestern University, Evanston, Illinois 60201.)

Nicotinic receptors from avian retina and muscle have been investigated using 3H-bromoacetylcholine (BAC) as a covalent ligand for receptor binding sites. In addition, an affinity column, prepared by linking BAC to agarose beads, has been used to separate receptor-rich membranes from crude homogenates.

As in electroplax (Damle, V.N., et al., Biochem. Biophys. Res. Comm. 84: 845, 1978), 3H-BAC labels covalently the reduced receptor from chick skeletal muscle and retina homogenates. Solubilization of labeled proteins followed by precipitation and filtration of the proteins does not abolish specific binding. Likewise, solubilized proteins from labeled membranes applied to DEAE paper can be washed free of unbound 3H-BAC with no loss of labeled receptors. Although 3H-choline uptake in retina is inhibited by acetylcholine (ACh) and nicotine, no 3H-choline labeled molecules adhere to DEAE paper following solubilization. Alkylation of reduced membranes with NEM blocks 80% of covalent 3H-BAC binding. Receptors are protected from reduction and alkylation by nicotinic agonists. The covalent reaction of 3H-BAC with non-receptor proteins is diminished by alkylation of free sulfhydryls with NEM.

To separate receptor-rich membranes, an affinity gel was prepared by linking BAC to 4% agarose beads (Damle, V.N. and Karlin, A., Biochemistry 17: 2039, 1978). The derivatized gel contained 1.6 umoles of choline carboxy methyl group per gram packed gel. Incubation of chick muscle or retina homogenate with the gel beads gave a dose-dependent loss of 125I-Bungarotoxin (125I-BTX) binding. Separation of gel from crude homogenate by slow-speed centrifugation maximally removed from the suspension 95% of the 125I-BTX binding sites. Nearly all of the homogenate protein remained in the supernatant fraction. This procedure should allow for the isolation of nicotinic receptor-rich membranes and may be extended to permit isolation of single cells containing the nicotinic receptor. This will make possible biochemical comparisons of muscle and CNS receptors and associated synaptic elements.

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160.7 NEUROMUSCULAR BLOCKING AGENTS COMPETE FOR AND MAY DISCRIMINATE BETWEEN ^{3}H-QNB BINDING SITES IN RAT ATRIUM. Janet Dunlap* and Joan Heller Brown, Division of Pharmacology, Dept. of Medicine, University of California, San Diego, La Jolla, CA 92093

Muscarinic receptors in the cardiac atrium mediate chronotropic and inotropic responses to acetylcholine, inhibit cyclic AMP formation and modulate norepinephrine release. Physiological studies suggest that muscarinic receptors in the atrium differ from classical muscarinic receptors characterized in other peripheral organs. Among the antagonists that appear to preferentially block atrial muscarinic receptors are gallamine and pancuronium, neuromuscular blocking drugs. We have used radioligand binding studies to compare the affinities of classical muscarinic antagonists and neuromuscular blocking agents for QNB binding sites in rat atrium, and to examine the possibility that subtypes of muscarinic receptors may be selectively antagonized by these agents. Radioligand binding studies were carried out using rat atrial membranes, in a Krebs salt solution buffered with 25 mM Na-Hepes pH 7.4 at 35°C. Binding of ^{3}H-QNB was linear with protein, saturable, and stereospecific. Nonspecific binding determined with 1 μM atropine was always less than 10% of total binding. The K_D for QNB determined by Scatchard analysis was approximately 0.06 nM and $B_{max} \sim 700$ fmol/mg protein. Competition experiments were performed using 0.1-0.3 nM ^{3}H-QNB, and 40-100 μg membrane protein. $K_i$app was calculated from IC_{50} values using the Cheng-Prussof equation.

Antagonist	IC_{50}	$K_i$app	N_H
QNB	0.7 nM	0.11 nM	.95
Dexetimide	16.0 nM	3.6 nM	.94
Atropine	30.0 nM	7.3 nM	.99
Ambenonium	2.0 μM	.44 μM	.70
Pancuronium	2.8 μM	.72 μM	.56
Gallamine	10.0 μM	2.7 μM	.58
d-Tubocurarine	140.0 μM	33.2 μM	.75

Gallamine and pancuronium, as well as ambenonium, a cholinesterase inhibitor with neuromuscular blocking properties, were less potent than classical muscarinic antagonists, but had apparent affinities in the micromolar range. Inhibition by 10 μM gallamine appeared competitive (10-fold increase in K_D with no decrease in B_{max} for QNB). Additionally, Hill slopes for the neuromuscular blocking agents differed from unity. The shallow Hill slopes seen most strikingly with pancuronium and gallamine suggest that the atrium may contain multiple QNB binding sites with equal affinities for classical muscarinic antagonists but differing affinities for neuromuscular blocking drugs. (Supported by NIH 24441.)

160.8 PROTEOLYSIS LEADS TO SELECTIVE LOSS OF ANTIGENIC DETERMINANTS ON ACETYLCHOLINE RECEPTOR. W. Gullick*, B. Einarson*, S. Tzartos* and J. Lindstrom. Receptor Biology Laboratory, The Salk Institute for Biological Studies, San Diego, California 92138.

Proteolytic digestion of Torpedo californica acetylcholine receptor in the membrane or in solution causes loss of antigenic determinants for some monoclonal antibodies. However, binding of monoclonal antibodies to the main immunogenic region on α subunits is not affected by treatment with either trypsin or V8 protease. Protease-labile determinants have been detected on α, β and δ subunits of the receptor. The localization of the proteolytic fragments containing these labile determinants and their role in agonist-induced sodium flux is being studied. Monoclonal antibodies to protease-labile determinants provide a sensitive assay for monitoring the integrity of electric organ receptor during storage and of mammalian muscle receptor during purification.

160.9 MUSCARINIC RECEPTOR REGULATORY MECHANISMS IN MEMBRANE-BOUND AND DETERGENT-SOLUBILIZED PREPARATIONS. Gerald O. Carrier and Robert S. Aronstam. Department of Pharmacology, Medical College of Georgia, Augusta, GA 30912.

Muscarinic acetylcholine receptors can exist in several reactive states which are most clearly defined on the basis of their agonist binding properties. Several mechanisms have been identified which induce interconversions betweeen these states, the functional significance of which is not always obvious. Some researchers have suggested that only the low affinity receptors are active insofar as they are effectively coupled in the membrane to structures involved in the elicitation of cellular responses to receptor stimulation.

Alkylation of neural membranes with N-ethylmaleimide (NEM) increases the affinity of agonists for muscarinic receptors in rat brain. After solubilization of cerebral cortex membranes with 1% digitonin/1 mM Na_2EDTA, NEM treatment still produced a selective increase in agonist binding affinity. The IC50 value for carbamylcholine inhibition of 0.1 nM [^{3}H]QNB binding was reduced from 9 to 1 μM after treatment with 1 mM NEM for 15 minutes.

Certain guanine nucleotides decrease high affinity agonist binding to muscarinic receptors in membranes isolated from rat cerebral cortex. After solubilization, however, 100 μM 5'-guanylylimidodiphosphate did not affect the ability of 10 μM carbamylcholine to inhibit 0.1 nM [^{3}H]methylscopolamine binding, as measured using equilibrium dialysis techniques. In parallel experiments, the nucleotide decreased carbamylcholine binding to membrane-bound receptors. It is possible that receptor and nucleotide subunits were separated during solubilization, although a number of alternate explanations for the failure of nucleotides to affect receptor binding after solubilzation have not been ruled out.

Longitudinal muscles were isolated and mounted in tissue baths for the measurement of isometric contractions. Exposure to 0.3 mM NEM for 30 seconds reduced contractions induced by 1 mM Ba^{++} and 60 mM KCl to a significantly lesser extent than contractions induced by 1 μM ACh. The longitudinal muscles were removed from the bath, homogenized and muscarinic binding properties studied. Specific binding of 0.18 nM [^{3}H]QNB was reduced 30±4% in control and 62±8% in NEM-treated tissues by 1 μMcarbamylcholine.

160.10 PURIFICATION AND SUBUNIT COMPOSITION OF THE ACETYLCHOLINE RECEPTOR FROM DENERVATED CHICKEN SKELETAL MUSCLE. James McManaman,* James Blosser, Stanley H. Appel (Spon: R. Grossman) Department of Neurology, Baylor College of Medicine, Houston, Texas 77030.

Although acetylcholine receptors from the electric organ of the ray have been extensively studied, apart from rat skeletal muscle (Froehner S.C., Reiness C.G. and Hall Z.W. (1977) JBC 252 8589-8596), little is known about the structure of skeletal muscle receptors. In order to provide a basis for further investigation of the cellular regulation of acetylcholine receptors in chick skeletal muscle, we have purified acetylcholine receptors from the leg and breast muscle of denervated chickens.

Acetylcholine receptors were purified from skeletal muscle that had been denervated for seven days. The acetylcholine receptor levels of the denervated muscle were elevated 20 fold over non-denervated muscle. Muscle was homogenized in a 50 mM phosphate buffer pH 7.3 and centrifuged at 38,000 xg for 90 minutes. The 38,000 xg pellet was then extracted with a 50 mM phosphate buffer containing 1% Triton X-100. Acetylcholine receptors were purified 18,000 fold, with a 61% recovery from the Triton X-100 extract by affinity chromatography on an acetylcholine affinity column. Pepstatin A, EGTA and PMSF were included in all purification steps to prevent proteolysis. The binding of ^{125}I-α-bungarotoxin was assayed by sucrose gradient sedimentation, and the purification was monitored by SDS-gel electrophoresis.

For comparison, acetylcholine receptors were also purified from the electric organ of Torpedo californica by the same procedure. The purified acetylcholine receptors from chicken skeletal muscle and Torpedo californica had similar specific activities for α-bungarotoxin binding, and cosedimented on sucrose gradients. Iodination and SDS-gel electrophoresis of purified chicken acetylcholine receptors demonstrated the presence of electrophoretic bands comigrating with the α, γ, and δ subunits of purified Torpedo acetylcholine receptors, and the absence of major contaminants. Supported by NSF grant BNS 7914115 and the Muscular Dystrophy Association.

160.11 THREE AGONIST- AND TWO ANTAGONIST-STATES OF MUSCARINE RECEPTORS. J. Luber-Narod* and L.T. Potter* (Spon. J.N. Barrett) Dept. of Pharmacology, University of Miami School of Medicine, P.O. Box 016189, Miami, Florida 33101.

We have studied the binding of (-) ^{3}H-quinuclidinyl benzilate at saturating concentrations (0.32 nM) to muscarine receptors in unselected membranes from the brainstem and forebrain of rats, in the presence and absence of carbachol, Mn^{++} (1 mM), EDTA (1 mM) and/or GppNHp (10 μM). In EDTA QNB binds to and dissociates from a single class of sites in the brainstem with a kinetic Kd ≃ 15 pM; Mn slows both rates 6-fold. In EDTA QNB binds to one class of sites in the forebrain, but dissociates from two classes, with 3/4ths of the receptors showing a kinetic Kd ≃ 7 pM; Mn slows rates 2-fold. Carbachol competes with QNB in a complex fashion as described by Birdsall et al (Proc. Roy. Soc. 207 1, 1980: evidence for 3 states), with much higher affinity in the brainstem than forebrain. GppNHp irreversibly diminishes carbachol potency in the brainstem, but has little effect in the forebrain. Mn accentuates, and EDTA diminishes, carbachol potency in both regions; these effects are reversible. GppNHp and EDTA reduce carbachol competition with QNB almost to a Langmuir isotherm in the brainstem, but the IC50 (30 μM) is still much lower than that seen in the forebrain.

We conclude that most receptors in the brainstem behave differently than most receptors in the forebrain. While one explanation is that there are two different types of receptors, our working hypothesis is that there is a single receptor type (M) in both regions, with different coupling to a guanine nucleotide binding protein (G). We propose three agonist states: "super-high" affinity = carbachol-MG (a ternary complex with altered conformation), "high" affinity may = carbachol-M...G ± GppNHp (a complex with weaker M...G interaction), and "low" affinity = carbachol-M. We suggest two antagonist states: QNB-M and QNB-M...G ± GppNHp, where QNB-M shows slightly higher affinity. In each case Mn promotes the binding of carbachol or QNB to receptors.

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160.12 UNCHANGED BINDING PROPERTIES OF MUSCARINE RECEPTORS IN SOLUTION. Lynn Kalinoski* and L.T. Potter* (Spon: J.C. Hackman), Dept. of Pharmacology, University of Miami School of Medicine, P.O. Box 016189, Miami, Florida 33101.

Muscarine receptors in the rat forebrain are intrinsic membrane proteins which are not dissolved in the cold by sonication, alkaline extraction, 2 M NaCl or 0.5 M Na perchlorate. However receptors saturated in membranes with (-) ^{3}H-quinuclidinyl benzilate (QNB) are fully soluble in most detergents. In Lubrol-PX, as in membranes, QNB dissociates from two classes of sites, and 3/4ths of the sites show slightly higher affinity for QNB than the rest. When high-affinity sites for carbachol are protected during the labeling of receptors in membranes, only one class of sites is seen in Lubrol. Unfortunately, QNB does not bind to receptors in Lubrol.

Digitonin (3 mg/mg protein; in EDTA) appears to solubilize only 3/4ths of the total receptors in membranes, and QNB dissociates from only one class of sites in solution. In digitonin QNB does bind to receptors at 37°C, and bound QNB can be quantitatively recovered after precipitation on ice with 5% protamine sulfate and 20% polyethylene glycol, by collection on glass-fiber filters. Non-specific binding is negligible. Binding is proportional to the concentration of receptors, and is saturable. QNB associates and dissociates in solution from a single population of sites: the association rate is 0.7×10^9 M^{-1} min^{-1}; the dissociation rate is 7×10^{-3} min^{-1}; and the calculated kinetic Kd is 10 pM. (-) Scopolamine shows simple competition with 0.1 nM QNB for receptors; the IC50 value is 10 nM, and the calculated Kd for scopolamine is 0.9 nM. The inhibition curve for carbachol vs. QNB is close to a Langmuir isotherm, with an IC50 value of 0.3 mM; the apparent calculated Kd for carbachol is 30 μM. These values for QNB, scopolamine and carbachol, determined in solution, are nearly identical with those we have found in membranes.

We conclude that 3/4ths of the receptors in the rat forebrain (those with lowest agonist- and highest antagonist-affinities) can be dissolved in digitonin without alteration of their binding properties. This fact facilitates purification of these receptors by affinity chromatography.

(Supported by grants from the National Parkinson Foundation and the American Parkinson Disease Association.)

160.13 FUNCTIONAL CHARACTERIZATION OF PURIFIED ACETYLCHOLINE RECEPTORS RECONSTITUTED IN LIPID VESICLES. R. Anholt[†Π*], V. Hudson[†*], D. R. Fredkin[#*], M. Montal[Π#*], and J. Lindstrom[†]. (SPON: A. E. Traynor [†]). Depts. of Biology[Π] and Physics[#], Univ. of California, San Diego, and The Salk Institute[†], San Diego, CA 92138.

Acetylcholine receptors (AChRs) from Torpedo californica, purified under conditions which preserve the integrity of the agonist-regulated cation channels, and reconstituted in lipid vesicles, were functionally characterized. The vesicles displayed a carbamylcholine-induced uptake of $^{22}Na^+$ which was dose dependent with a midpoint at $\sim 1.4 \times 10^{-6}$ M. Dose dependent desensitization of the AChR was assayed by preincubation with different concentrations of carbamylcholine in the absence of radioisotope, followed by $^{22}Na^+$ - influx assay at 10^{-4} M carbamylcholine. The equilibrium desensitization curve had a midpoint at $\sim 3.2 \times 10^{-7}$ M. The integrated flux response measured after 10 s incubation is limited by desensitization and is proportional to the amount of active AChR present. Both curves were characterized by a Hill coefficient of 2.0 ± 0.4. AChR function was non-competitively blocked by cobratoxin and competitively inhibited by curare. Computer techniques are currently being used in order to explain the observed $^{22}Na^+$ - flux data measured under various conditions in terms of models for AChR function, based on known ligand-binding properties of the AChR.

160.14 ANALYSIS OF PERIPHERAL PROTEINS ASSOCIATED WITH NICOTINIC ACETYL CHOLINE RECEPTORS. R. Gysin*, M. Wirth* and S.D. Flanagan (SPON: A.R. Dravid). Division of Neurosciences, City of Hope Research Institute, Duarte, CA 91010.

Membranes from Torpedo californica electroplax, highly enriched in acetylcholine receptors contain ∿43,000 dalton proteins that are not involved in the ion translocation and ligand binding functions of the receptor. We are interested in these proteins because of their potential role in the supramolecular organization of cholinergic synapses. We report here the subcellular distribution and partial molecular characterization of these proteins.

SDS-gel electrophoresis suggested the existence of at least two subunits. Further analysis on two-dimensional gels revealed extensive heterogeneity in the isoelectric focusing dimension. Two protein families, designated ν_1 and ν_2, were of slightly different molecular weight and each consisted of three major and some minor isoelectric variants focusing between pH 7 and pH 8, more alkaline than most of the other membrane proteins. A third protein, ν_3, was more acidic and focused around pH 6 as a single spot.

Tryptic peptide mapping of the spots cut out from two-dimensional gels gave different patterns for the three proteins, indicating no similarity in their primary sequence. The major isoelectric variants of both ν_1 and ν_2, however, gave internally consistent peptide fingerprints, suggesting that posttranslational modifications may be responsible for the observed charge differences. Using the same techniques it was found that ν_2 and ν_3, but not ν_1, are prominent proteins in the cytoplasm and are therefore present in a soluble form, besides being membrane bound.

All the 43,000 molecular weight subunits are considered to be peripheral proteins because they are removed from the membranes during a brief incubation at pH 11. Work from other laboratories with such alkali-treated membranes has suggested that the 43,000 dalton proteins are involved in controlling the arrangement, stability, and mobility of the receptor complex in the membrane. Our results raise questions concerning the relative contribution of the different protein subunits to these parameters, and the relationship between membrane bound and soluble forms of ν_2 and ν_3. (Supported by postdoctoral fellowship and grant-in-aid of the Muscular Dystrophy Association. S.D.F. is a recipient of an Alfred P. Sloan Foundation Research Fellowship).

160.15 EFFECTS OF CATIONS ON CLOZAPINE AND THIORIDAZINE BINDING TO THE MUSCARINIC CHOLINERGIC RECEPTOR. J.M. HAPPY* (SPON: R.G. Babington). Sandoz, Inc. E. Hanover, N.J. 07936

Clozapine (CLZ) and to a lesser degree, thioridazine (THZ) are distinquished from the classical neuroleptics by their lack of extrapyramidal side effects which has been speculated to be a result of their anticholinergic properties. Therefore, the binding characteristics of CLZ and THZ to the muscarinic receptor were studied through competition studies with the specific muscarinic antagonist, ^{3}H-QNB. CLZ and THZ were found to differ in their binding properties to this receptor. In Tris buffer (pH 7.4 at 24°C), CLZ is approximately 3 times as potent as THZ in displacing ^{3}H-QNB from calf caudate membranes. This is consistent with their in vivo potencies in antagonizing oxotremorine-induced tremors in rats. However, in Tris buffer containing 125 mM NaCl, 5 mM KCl, 2mM $CaCl_2$ and 1 mM $MgCl_2$ the THZ inhibition curve becomes steeper and the difference in potencies between CLZ and THZ diminishes. If in addition, increasing concentrations of ethanol are added to the medium, CLZ and THZ become equipotent in 1% ethanol. In 10% ethanol THZ is 2X as potent as CLZ.

In the presence of $2X10^{-7}$M THZ and increasing conc. of NaCl or KCl, ^{3}H-QNB binding is progressively decreased, while under the same conditions in the presence of $1X10^{-7}$M CLZ, ^{3}H-QNB binding is increased. In both of these cases the changes are small, but significant. Increasing $CaCl_2$ or $MgCl_2$ conc. in the presence of THZ or CLZ progressively decreases ^{3}H-QNB binding. However, the decrease is significantly more pronounced for THZ.

The effects of monovalent and divalent cations on other muscarinic antagonists and agonists were examined. In general, monovalent cations caused a small significant increase in ^{3}H-QNB binding in the presence of agonists and antagonists, except for QNB which showed no change. THZ was the only compound examined which showed a decrease. Divalent cations caused a decrease in ^{3}H-QNB binding in the presence of either agonist or antagonist. THZ was the most sensitive to divalent cations.

160.16 MUSCARINIC AND NICOTINIC EFFECTS OF ACETYLCHOLINE IN THE HIPPOCAMPUS OF THE RAT. N. Ropert and K. Krnjević, Anaesthesia Research Dept, McGill University, Montréal, PQ H3G 1Y6, Canada.

Previous experiments have indicated that the modulatory effect of acetylcholine (ACh) in the hippocampus is probably mediated through muscarinic receptors. The present experiments show that, even though the ACh effect is mainly muscarinic, a component of it may be mediated by nicotinic receptors.

Experiments were performed on urethane-anaesthetized rats (2.5 Kg^{-1};I.P.) whose head was maintained in a stereotaxic apparatus. Extracellular recordings were done in the CA1 and CA3 regions of the dorsal hippocampus using the central barrel (filled with K acetate and Pontamine sky blue) of multibarrelled electrodes whose peripheral barrels were used for iontophoretic application of various drugs: ACh; some muscarinic agonists (muscarine, bethanecol, acetyl-β-methylcholine, pilocarpine, oxotremorine, arecoline) and some nicotinic agonists (tetramethylammonium (TMA), butyrylcholine, nicotine and dimethylphenyl-piperazinium). The muscarinic antagonists, atropine and scopolamine, were injected into the femoral vein.

Low frequency stimulation (≤1 Hz) of the hippocampal commissure induces in the pyramidal cells IPSPs which can be recorded as positive extracellular waves in the stratum pyramidale of CA1 or CA3. We have already shown that ACh can induce the appearance of population spikes, probably by removing GABA-mediated inhibition (Ben-Ari, Y. et al., Neuroscience, In Press, 1981; Krnjević, K. et al., J. Physiol., 308:73P, 1980). Very low doses of muscarine and other muscarinic agonists had a similar effect, which was dose dependent, typically slower in onset and offset than that of ACh, and was seen only when muscarine was applied in the stratum pyramidale. It was completely blocked by scopolamine (i.v.). In contrast, the effect of ACh was not completely blocked by atropine or scopolamine. After scopolamine, the relative sensitivity of the hippocampus to ACh and muscarine was reversed: before scopolamine, it was more sensitive to muscarine; after scopolamine, it became relatively more sensitive to ACh. Moreover, all the nicotinic agents tested (except TMA) were also able to induce population spikes; this effect was typically fast and was not suppressed by scopolamine. These data indicate the possibility that ACh may also act through nicotine receptors.

(Supported by the Canadian Medical Research Council).

161.1 *INHIBITION OF ^{3}H-FLUNITRAZEPAM BINDING BY THE NOVEL TRIAZOLOPYRIDAZINE, CL 218,872 IN VARIOUS REGIONS OF THE HUMAN BRAIN.* *Micaela Morelli*, Susan Yamamura*, Pushpa Deskmukh and Henry I. Yamamura. University of Ariz., Health Sci. Ctr. Tucson, AZ 85724.*

The inhibition of ^{3}H-flunitrazepam binding by the novel triazolopyridazine, CL 218,872 was examined in 8 regions from membrane preparations of post mortem human brain. Brain regions examined for binding inhibition were the cerebellum, thalamus, frontal cortex, globus pallidus, substantia nigra, putamen, hippocampus and hypothalamus. The potency for CL 218,872 inhibition of ^{3}H-flunitrazepam binding was greatest in the cerebellum with a Ki value of about 60nM. Intermediate potencies were seen in the thalamus, frontal cortex, globus pallidus and the substantia nigra with Ki values of 85, 110, 120 and 125nM, respectively. CL 218,872 inhibited ^{3}H-flunitrazepam binding with least potency in the putamen, hippocampus and the hypothalamus with Ki values of 180, 230 and 240nM, respectively. The Hill coefficient was near unity for the cerebellum (0.95) and approximately 0.8 for the rest of the brain regions examined except for the putamen where the Hill coefficient obtained was about 0.68. The inhibition curve obtained in the human putamen was analyzed using nonlinear least squares regression analyses to determine whether CL 218,872 was binding to one or two classes of binding sites. A two site regression model resulted in a highly significant improvement in the fit of the data. A high affinity binding site resulted with a Ki value of about 60nM with 60% of the sites being associated with the high affinity site. A low affinity binding site with a Ki value of about 1300nM with 40% of the sites being associated with the low affinity site was observed. Autoradiographic studies were performed to determine the discrete localization of these binding sites in human brain. Radiolabelled CL 218,872 (10nM) was used and incubated according to the method of Young and Kuhar. The initial results of our studies show that ^{3}H-CL 218,872 specifically labels the benzodiazepine receptor and that there is evidence consistent with receptor heterogeneity.

Portions of these studies were supported by USPHS grants and a Research Scientist Development Award to H.I.Y.

161.2 SUPERSENSITIVITY OF BENZODIAZEPINE RECEPTORS FOLLOWING DENERVATION OF SUBSTANTIA NIGRA. G. Biggio, M.G. Corda, A. Concas and G.L. Gessa (SPON: Walter J. Wojcik)
Institute of Pharmacology, School of Biology and School of Medicine - University of Cagliari (Italy).

The intranigral injection of kainic acid (0.5 µg) produced a 50% loss in the number of ^{3}H-diazepam binding sites (Bmax) in the rat substantia nigra, suggesting that benzodiazepine receptors are localized, in part, on intrinsic neurons (kainic acid-sensitive receptors) and in part, on glia cells and/or terminals of neurons with cell body outside the substantia nigra (kainic acid-resistant receptors). The number (Bmax) of the kainic acid resistant benzodiazepine receptors increased by 100% following destruction of striato-nigral connections produced by the intrastriatal injection of kainic acid (1.5 µg). The observed denervation induced "supersensitivity" suggests that kainic acid resistant benzodiazepine receptors are innervated by neurons originating in the striatum and that the endogenous ligand for benzodiazepine receptors, might be the transmitter of specific neurons or the co-transmitter in GABA neurons. The latter possibility is suggested by the fact that the markers (GABA content and GAD activity) of GABAergic innnervation are almost completely lost in the supersensitive substantia nigra.

161.3 MORPHOLOGICAL STUDIES ON NEUROTRANSMITTER RECEPTORS IN THE BRAIN S. Kito, E. Itoga*, N. Mori*, M. Togo*, T. Kishida* and Y. Nakamura*. Third Dept. of Int. Med., Hiroshima Univ. Sch. of Med.,Hiroshima 734, Japan.

We performed light and electron microscopic autoradiography of receptors of acetylcholine (Ach), gamma aminobutyric acid (GABA) and opiates using ^{3}H-quinuclidinyl benzilate (QNB), ^{3}H-muscimol and ^{3}H-met-enkephalin as ligands.

As for autoradiographic demonstration of Ach-muscarinic receptor sites with QNB, the ligand was appled in vivo. In the cases of muscimol and met-enkephalin, the ligands were applied to the tissues which were lightly fixed, sectioned by cryostat and then mounted on slide glasses. The slides were coated with carbon and emulsion (Ilford L-4)-coated by dipping method. Binding affinities were measured biochemically and compared between materials fixed with 0.1% paraformaldehyde for light microscopic autoradiography and unfixed ones, and also between materials fixed with 2 fold diluted Zamboni solution for electron microscopic autoradiography and unfixed ones. As displacers, atropine, GABA and met-enkephalin were used.

Electron microscopically muscimol binding sites in the cerebellar molecular layer and met-enkephalin binding sites in the amygdaloid nucleus were observed.

For electron microscopic autoradiography, selection of developing method was critical to get high resolution of the pictures. We used elon ascorbic acid (EAA) development in which developed silver grains were much smaller than grains of silver bromide of undeveloped emulsion. Furthermore, in EAA development, a growing speed of silver grains during the development process is so slow that we can easily select the development time adequate for our purpose. Thus we obtained silver grains in a pattern highly localized to the cytoplasmic membrane, i.e. the receptor site by means of EAA development.

Autoradiographic observation of neurotransmitter receptors has still problems to be solved. Nevertheless, this technique is very important to observe morphological relationship between transmitters and their receptor sites.

161.4 CHANGES IN [^{3}H]-MUSCIMOL BINDING IN SUBSTANTIA NIGRA, ENTOPEDUNCULAR NUCLEUS, AND THALAMUS AFTER STRIATAL LESIONS AS DEMONSTRATED BY QUANTITATIVE RECEPTOR AUTORADIOGRAPHY. Helen S. Pan, Anne B. Young, John B. Penney*, Jr. Department of Neurology, University of Michigan, Ann Arbor, Michigan 48109.

We have developed a quantitative autoradiographic technique for measuring the binding of tritiated ligands to neurotransmitter receptors. This technique allows us to measure receptor numbers and affinities for volumes of brain as small as 0.01 cu. mm. We have also quantitated ligand binding kinetics and drug displacements autoradiographically. We have applied the method to the investigation of GABA receptors in rat brain after kainic acid lesions of striatum.

20 µ frozen sections of brain are mounted on subbed slides. To measure GABA receptors the slides are washed 3 times in 50 mM tris-citrate buffer (pH 7.0) for 5 min., dipped in buffer plus varying concentrations (5-90 nM) of [^{3}H]muscimol for 30 min. at 0°C, rinsed 3 times in 0° C buffer for 5 seconds and blown dry. Tritium sensitive film (Ultrofilm ^{3}H LKB) is apposed to the slides and standards for 12 days then developed. Optical densities of areas of interest are compared to the standards and radioactive ligand concentration is calculated.

The effects of lesioning rat striatum at the level of the anterior commissure (A-7000) with 1 nM of microelectrophoretically applied kainate on distant GABA receptors were measured 7 days after the lesion. There is local loss of receptors in striatum. Receptors increase in numbers but not affinity in the ipsilateral entopeduncular nucleus (EPN) and substantia nigra pars reticulata (SNR). There is a decrease in number but not affinity in the ipsilateral anteroventrolateral (VL), ventromedial (VM) and parafasicular (PF) thalamic nuclei. These findings suggest that a loss of GABAergic striatal output results in GABA receptor supersensitivity in EPN and SNR. These areas in turn contain GABA neurons which project to VL, VM and PF. It is suggested that with loss of inhibitory input the GABAergic neurons in EPN and SNR may become more active resulting in GABA receptor subsensitivity in VL, VM and PF. The data supports the hypothesis that striatal GABAergic output cells directly influence GABAergic output from EPN and SNR to thalamus.

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161.5 OPIATE/ALPHA-2 INTERACTIONS: CO-LOCALIZATION OF BOTH RECEPTORS BY RADIOHISTOCHEMISTRY. J.R. Unnerstall, J.M. Palacios and M.J. Kuhar. Dept. of Neuroscience, Johns Hopkins Univ. Sch. of Med., Balto., MD 21205.

A relationship has been shown to exist between central adrenergic and opioid systems in the control of cardiovascular function and pain sensitivity. The antihypertensive effects of clonidine, a potent alpha-2 adrenergic agonist, can be reversed by naloxone in experimental models of hypertension. Clonidine is also a potent analgesic, and can alleviate some adverse effects of opiate withdrawal. In a previous autoradiographic study (Young and Kuhar, PNAS 77:1696, 1980) it was noted that the distribution of alpha-2 receptors paralleled that of opiate receptors in regions involved in cardiovascular regulation and nociception. We have extended these findings in the rat CNS using the in vitro autoradiographic technique for localization of neurotransmitter receptors by labeling adjacent sections with selective alpha-2 or opiate agonists.

For the localization of alpha-2 receptors, sections were incubated for one hour at room temperature in 1 nM ^{3}H-para-aminoclonidine in the presence of 1 mM $MnCl_2$. Sections were preincubated for 30 minutes in 5 mM EDTA and washed for 10 min. in cold buffer plus Mn^{2+}. Opiate receptors were identified by incubating adjacent sections in 4 nM ^{3}H-dihydromorphine as has been previously described (Young and Kuhar, Brain Res. 179:255, 1979; Goodman, Snyder, Kuhar and Young, PNAS 77:6239, 1980).

In the brainstem, high levels of alpha-2 and opiate receptors were identified in areas involved in cardiovascular regulation; i.e. the nucleus commissuralis, nucleus tractus solatarius, dorsal motor nucleus of the vagus. Elevated levels of both receptors were also observed in areas involved in nociception and attention including the substantia gelatinosa of the spinal cord and trigeminal nucleus, raphe magnus, lateral reticularis gigantocellularis and periaqueductal gray. Particularly high levels of both receptors were observed in the locus coeruleus. In the diencephalon, high densities of both receptors were seen in dorsal and ventral regions of the medial thalamus and in several hypothalamic nuclei. Co-localization of these receptors was also observed in specific amygdaloid nuclei (e.g. central, basal, lateral and posterior cortical), the bed nucleus of the stria terminalis and pyriform and cingulate cortex.

These studies suggest that alpha-2 and opiate receptors are localized to the same areas and possibly the same cells, and may provide an anatomical basis for explaining at least some of the interactions between opiates and clonidine.

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161.6 A LOW COST MICROCOMPUTER BASED DENSITOMETER SYSTEM FOR QUANTITATIVE AUTORADIOGRAPHY. G. Dauth, K. Frey* and S. Gilman, Department of Neurology, The University of Michigan, Ann Arbor, MI 48109

Quantitative autoradiographic methods developed in the past five years provide powerful tools for the investigation of regional neurochemical processes in mammalian brain, such as cerebral blood flow, glucose utilization, protein synthesis, and neurotransmitter receptor pharmacology. These techniques are based upon contact autoradiography of [^{3}H] or [^{14}C] labeled brain tissue using x-ray film or LKB Ultrofilm and subsequent densitometric analysis of the film images.

The available methods for reading film density are either slow and cumbersome or expensive. We have developed a computer assisted densitometry system which provides rapid readings of film density, conversion to radionuclide concentration, as well as subsequent conversions, e.g. local cerebral glucose utilization rates or receptor concentrations. The system consists of 4 main components: 1) a Beseler 45 MX, unmodified photographic enlarger; 2) a spot densitometer circuit developed in our laboratory using a PIN silicon photodiode; 3) a Z-80 based microcomputer system with analog to digital converter and floppy disks; and 4) computer software written in Fortran IV. The densitometer sensor is placed on the base board of the enlarger. The film is positioned on the negative stage of the enlarger and moved manually to position the desired area of the image over the sensor, and a foot switch is used to signal the computer to initiate a read cycle.

The system has been used in our laboratory for over 1 year for the quantification of local cerebral glucose utilization and quantitative receptor autoradiography. In addition to its relatively low cost and rapid operation there are two other significant advantages to this system: 1) the image is not obscured by the sensor as is the case with most commercial spot densitometers; and 2) this system allows the image to be magnified in order to read small structures with greater accuracy.

Supported by grants from NINCDS (NS-15655) and The United Cerebral Palsy Research and Educational Foundation, Inc.

161.7 BENZODIAZEPIN RECEPTORS IN GOLDFISH RETINA. Y. Y. T. Su, J.-Y. Wu, and D. M. K. Lam. Cullen Eye Institute and Cell Biology, Baylor College of Medicine, Houston, Texas 77030

It is very well established that GABA receptor, benzodiazepine receptor and chloride ionopore are part of a functional unit. The GABAergic pathways in goldfish retina have been identified by autoradiographic and immunocytochemical methods. Here we like to report our recent studies of benzodiazepine receptors in goldfish retina. Goldfish retinas were homogenized in 0.32 M sucrose solution. The homogenate was centrifiged at 1000 x g for 15 min and the supernatant was further centrifuged at 45000 x g for 45 min. The pellet, P_2, was resuspended in the same solution and spinned again at 45000 x g for 45 min. The rinsed P_2 preparation was stored at -20 C overnight, resuspended in cold water and spinned at 45000 x g for 45 min. The pellet, crude membrane preparation, was suspended in a small volume of 0.05 M Tris-citrate buffer (pH 7.1). The reaction mixture for the receptor binding experiment contained 320 µg of crude membrane preparation and 5 to 50 nM of ^{3}H-flunitrazepam in the presence (nonspecific binding) or absemce (total binding) of 40 µM of clonopine. The reaction mixture was incubated at ice water bath for 30 min. The reaction was terminated by centrifuged at 45000 x g for 15 min. After quickly rinsed twice with cold distil water containing 10 µM of clonopine the pellet was dissolved in tissue dissolver. The activity was measured in liquid scintillation counter. Scatchard analysis of the binding indicates one binding site with an apparent affinity constant , K_d=2.6 nM. This value is in the range of that of mouse or rat brain membrane preparations. The localization of the receptor and it relation with GABAergic neurons will be discussed.

161.8 CYTOPLASMIC PROGESTERONE RECEPTORS IN THE FEMALE RAT BRAIN: IN VIVO EFFECTS OF ESTROGEN AND PROGESTERONE. T. W. Jasper and V. D. Ramirez. Department of Physiology and Biophysics, University of Illinois, Urbana, IL 61801.

We have used the tritiated synthetic progestin, ^{3}H-R 5020, to measure cytoplasmic progestin receptors (PR) in the brain and pituitary of ovariectomized (ovx) and ovariectomized-adrenalectomized (ovx-adrex) rats treated in vivo with estrogen (estradiol benzoate; E) or estrogen plus progesterone. Scatchard analysis of ^{3}H-R 5020 binding in the hypothalamus-preoptic area and pituitary revealed high affinity (Kd < 1.0 nM) and limited capacity binding for progestin. Subcutaneous implantation of silastic capsules containing E (10 mm length, 1.6 mm width id, 235 µg E/ml oil) into ovx rats doubled hypothalamic-preoptic area PR within 2 days and maintained elevated PR levels for at least 12 days. In ovx E-primed rats, in vivo administration of progesterone (1.0 mg/0.1 ml oil sc) reduced hypothalamic-preoptic area PR levels within 1 h and maintained the depressed PR levels for at least 6 h. E stimulation of PR levels in the pituitary was similar in ovx and ovx-adrex rats, but the PR levels were greater in the hypothalamus-preoptic area of ovx-adrex versus ovx rats following E stimulation. In both the hypothalamus-preoptic area and pituitary, PR levels were greater after E stimulation than in the unstimulated controls.

The regional specificity of PR stimulation by E, and its depletion following in vivo progesterone (1.0 mg sc, 1 h before decapitation), was examined in ovx rats. Silastic E treatment elevated PR levels in the medial basal hypothalamus, preoptic area-septum, and pituitary, but not in the cerebellum, parietal cortex, of hindbrain of ovx rats. Progesterone administration reduced PR levels in all tissues examined, regardless of the presence or absence of E pretreatment.

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161.9 LAMINAR ANALYSIS OF OPIATE RECEPTOR DISTRIBUTION IN RAT CEREBRAL CORTEX. M. E. Lewis*, A. Pert, C. B. Pert and M. Herkenham (SPON: W. E. Bunney, Jr.). Biological Psychiatry Branch and Lab. of Neurophysiology, NIMH, Bethesda, MD 20205

Although rat cerebral cortex contains easily measurable quantities of opiate peptides, immunocytochemical procedures have revealed very few enkephalin-containing fibers in cortex. Since opiate receptors could be markers for "opiatergic" pathways in brain (Proc. Nat. Acad. Sci. USA, 77:5532, 1980), we carried out a laminar analysis (translaminar autoradiographic grain counting) of different cortical areas which had been labeled for "μ"-like or "δ"-like opiate receptors. These receptors were labeled by [^{3}H]naloxone or [^{3}H]D-Ala^2,D-Leu^5-enkephalin ([^{3}H]DALA), respectively, on slide-mounted rat brain sections which were then processed for autoradiography. In addition, we compared the distribution of binding sites in some areas to thalamocortical projections as determined by [^{3}H]amino acid transport.

The laminar distribution of grains differs markedly across areas with the pattern depending upon which radioligand is used as a receptor label. We also observed correlations between the laminar distribution of "μ"-like binding sites and thalamocortical projections to different regions. For example, the distribution of both [^{3}H]naloxone sites in frontal cortex and [^{3}H]amino acids transported from the mediodorsal nucleus shows a sharp decline in density from the most superficial part of layer I to the border of layer II, and a second peak in layer III. [^{3}H]DALA sites, in contrast, show a shallower layer I peak and a broad peak extending from layers III to VI. The medial frontal, cingulate and retrosplenial areas all show layer VI peaks of [^{3}H]naloxone binding; however, the layer III binding peak disappears in posterior retrosplenial cortex. The insular and entorhinal areas are densely labeled in layer VI by [^{3}H]naloxone; piriform cortex is sparsely labeled throughout. Insular and piriform cortex are the only limbic cortical areas to show more overall [^{3}H]DALA than [^{3}H]naloxone binding. Unique to primary sensory cortices is [^{3}H]naloxone labeling in upper layer V. In addition, area 17 can be easily discriminated from surrounding area 18 by a lower density of labeling in layer VI. Because of evidence that apparent multiple opiate receptors may be functionally different conformations of the same receptor (Bowen et al., Proc. Nat. Acad. Sci. USA, in press), we suggest that laminar differences in [^{3}H]naloxone and [^{3}H]DALA binding reflect an in vitro stabilization of different receptor conformations which undergo dynamic interconversions in vivo.

161.10 VARIATIONS IN OPIATE RECEPTOR DISTRIBUTION IN THE MAMMALIAN STRIATUM. S. Moon Edley*, M. Herkenham, C. B. Pert., Lab. of Neurophysiology, and Biological Psychiatry Branch, NIMH, Bethesda, MD 20205.

The opiate receptor binding patterns in rodents, carnivores, and primates have been investigated using tritiated naloxone, dihydromorphine, and enkephalin on fresh-frozen cryostat-cut sections. Incubated sections were emulsion-dipped (Kodak, NTB-2) for microscopic study, or exposed to tritium-sensitive film (LKB) for computer analysis.

The mouse and rat striata under the appropriate allosteric binding conditions, (Bowen, et al., PNAS, in press) exhibit the same general pattern of high-density opiate receptor patches. In the other mammals examined, the general pattern is that of a ventromedial to dorsolateral gradient of decreasing alkaloid receptor binding. Embossed on this specific blanket labeling are other pattern complexities. In the cat, receptor-rich areas overlay the blanket labeling in the rostral caudate, but caudally, it is interrupted more frequently by receptor-sparse zones. In the dog striatum, the impressive feature is the ventromedial-to-dorsolateral shift in receptor density, though receptor-dense zones can be found. In the one squirrel monkey examined, a more homogeneous opiate receptor pattern is evident. However, the rhesus monkey has a strong laterally shifting decreasing density gradient, embellished by dense zones and sparse holes intermingling at the same level. The heavily labeled human striatum, obtained at autopsy, has the greatest intermingling of the above mentioned patterns of the mammals examined.

The specificity and anatomic validity of the pattern of striatal opiate receptors has been demonstrated in the rat by the precise fit of opiate-dense patches to the areas free of thalamic parafascicular terminals (Herkenham and Pert, Nature, in press). Moreover, the tissue preparation and the sensitivity of the receptor labeling method make it suitable for the study of abnormalities as well. Preliminary evidence suggests a lower opiate receptor density in the striatum of a strain of "genetically nervous" pointer dogs (brains from U. of Arkansas Medical Center, Little Rock, AK) as compared to normal pointer kennel mates. The "nervous" dogs are excessively fearful, becoming extremely cateleptic in the presence of humans or when startled, and are unable to demonstrate operant conditioning without the aid of drugs (Murphee et al., Pav. J. of Biol. Sci., '74). Their catalepsy is reversible by dopamine blockers and naloxone (Shideler, unpublished), making them a candidate for the experimental study of affective disorders. The suggestion of interactions in the striatum between opiate and dopamine systems has encouraged us to examine human brains from victims of schizophrenic disorders.

161.11 PRESENCE OF TWO BENZODIAZEPINE RECEPTORS IN THE RAT HIPPOCAMPUS. L. Volicer and T.M. Biagioni*. E.N. Rogers Memorial Veterans Hospital, Bedford, MA 01730.

Although the initial characterization of benzodiazepine receptors indicated the presence of a single class of binding sites, more recent reports suggest that multiple benzodiazepine receptors exist in the brain. This evidence is mostly indirect, based on the differential effects of GABA agonists on benzodiazepine receptors and on differential inhibition of benzodiazepine binding by various benzodiazepine analogs. We recently found that the presence of two benzodiazepine receptors can be demonstrated in the hippocampus directly by a Scatchard analysis of diazepam binding. Male Sprague-Dawley rats were sacrificed by decapitation and the brains dissected on ice. The synaptosomal membranes were prepared by differential centrifugation and kept frozen for at least 24 hrs. On the day of an assay the membranes were treated with 0.05% Triton X100. The membranes were incubated with ^{3}H-diazepam or ^{3}H-flunitrazepam in the presence or absence of 10^{-5}M flurazepam and the incubation was terminated by filtration on Whatman GF/B filters.

In experiments in which five ligand concentrations were used (0.75 to 35 nM) the number of binding sites in the cerebral cortex and cerebellum were similar for both diazepam and flunitrazepam and the affinity was 4 times lower for diazepam than for flunitrazepam. In contrast, in the hippocampus the number of binding sites was higher for diazepam than for flunitrazepam, and the affinity of diazepam was 17 times lower. Analysis of diazepam binding in the hippocampus using 0.75 to 1517 nM concentrations demonstrated the presence of two binding sites (affinities 4.58 ± 0.38 and 1077 ± 128 nM, and the number of binding sites 0.285 ± 0.056 and 6.09 ± 0.81 p moles/mg of protein for high and low affinity sites respectively, means ±S.E.M. of 5 determinations). The high affinity binding site detected in these experiments, corresponds to the flunitrazepam binding (similar number of receptors, 4 times lower affinity). The presence of two diazepam binding sites in the hippocampus explains the different relationship between diazepam and flunitrazepam binding in this brain area and in the other two areas. The pharmacological function and localization of these receptors remains to be investigated. (Supported by the Veterans Administration).

161.12 REGIONAL DISTRIBUTION OF N^6-CYCLOHEXYL[^{3}H]ADENOSINE BINDING IN RAT AND MOUSE BRAIN. M.G. Murphy* and H.A. Robertson, (SPON: J.G. Rutherford). Department of Pharmacology, Dalhousie University, Halifax, Nova Scotia, Canada B3H 4H7.

Adenosine has recently been implicated as a modulator of excitability in the central nervous system, and may be important in the regulation of convulsion activity (Dunwiddie, T.V., Epilepsia 21, 541-548 (1980)). In the initial phase of our investigation of the relationship between adenosine and seizure activity, we have examined the regional distribution of adenosine receptors in rat and mouse brain using N^6-cyclohexyl-[^{3}H]adenosine ([^{3}H]CHA). Following dissection, brain regions were pooled from 3-6 animals and were either homogenized in a physiological Tris medium, or crude synaptosomal (P_2) fractions were isolated in 0.32M sucrose prior to suspension in the same medium. All experiments were carried out with fresh membrane preparations. Following incubation with adenosine deaminase (20 units/g wet weight) to remove endogenous adenosine, membranes were washed, and incubated for 1.5 h at 25°C at 5 concentrations (1.25-20nM) of [^{3}H]CHA. Displaceable binding, which accounted for 92-99% of total binding at 1nM [^{3}H]CHA, was determined using 10 μM 2-chloroadenosine. Scatchard analyses were carried out on all preparations.

Specific binding in the P_2 fractions was 2 to 4 fold higher than in homogenates of the corresponding regions. Considerable variation was observed in adenosine-receptor density (B_{max}) in the P_2 of rat brain; density was highest in cerebellum and lowest in amygdala.

Brain Region	Bmax (fmol/mg Protein	K_D (nM)
Cerebellum	940.6	1.90
Hippocampus	714.2	1.66
Cortex	668.4	1.91
Striatum	351.3	2.81
Hypothalamers	225.5	2.55
Amygdala	165.6	2.37

In contrast, density of binding sites in mouse brain was higher in cerebral cortex (B_{max} 447.6) than in cerebellum (B_{max} 310.8). Preliminary studies have been carried out to determine the effects of pentylenetetrazol-induced seizures on adenosine binding in different regions of rat brain. Results indicate that following convulsion, there is a selective decrease in the number of [^{3}H]CHA binding site in the hippocampus. (Supported by the Medical Research Council of Canada).

161.13 CHARACTERIZATION AND REGIONAL DISTRIBUTION OF ACIDIC AMINO ACID BINDING SITES IN RAT BRAIN. D.T. Monaghan, A.C. Foster, G.E. Fagg, E.E. Mena, and C.W. Cotman (SPON: T.J. Cicero). Dept. of Psychobiology, Univ. of Calif. Irvine, Irvine, Calif. 92717.

Ligand binding studies have demonstrated L-[3H]-glutamate binding sites in rat brain synaptic membranes. Previous work from this laboratory has demonstrated the presence of a Ca++ dependent L-Glu binding site which has a similar pharmacology to that of the lateral entorhinal perforant path-granule cell evoked response as determined by use of the phosphonic acid derivatives 2-amino-3-phosphonopropionic acid (APP), 2-amino-4-phosphonobutyric acid (APB), and 2-amino-5-phosphonovalerate (APV). These compounds were a generous gift of Dr. J.C. Watkins. Both the binding of L-Glu in the presence of Ca++ and the synaptic response of the perforant path are inhibited by APB, and less so by APV and APP.

To further clarify the role of this Ca++ dependent, APB sensitive L-Glu receptor, binding of L-[3H]-Glu (50nM) to rat synaptic plasma membranes was measured using a microfuge assay, and inhibition constants were determined: APP 1000uM; APV 39uM; L-APB 5uM; and D-APB 75uM, which are similar to the apparent Ki's determined from the antagonism by these compounds of perforant path evoked responses (Koerner and Cotman, Brain Res., in press). Interestingly, APB appears to inhibit (competively) only the Ca++ dependent L-Glu sites. Thus, APB appears specific for a subpopulation of glutamate receptors.

In view of these observations, it is important to determine the regional distribution of the APB sensitive-Glu receptor. Nine rat CNS regions were dissected: spinal cord, brain stem, thalamus/hypothalamus, septum, hippocampus, caudate, cortex, and cerebellum. For these binding studies, a P2/P3 pellet was obtained and washed in 200 uM Hepes 4 times. The membrane fraction was assayed for inhibition of L-[3H]-Glu binding by 0.5mM APB in the presence of 2.5 mM Ca++. This concentration of APB gave maximal inhibition of Ca++ dependent binding sites, while having no effect on the binding in the absence of Ca++.

APB sensitive L-Glu binding sites showed a regional variation; high levels were found in brain stem and hippocampus, low levels in the cerebellum, and intermediate levels in the remaining regions. The obtained regional distribution was compared to other indices of glutamate transmitter activity.

Thus, the APB sensitive sites have a widespread distribution in the CNS and are of interest as potential receptors for glutamate using pathways. (Supported by fellowships from NATO and NIH, and by grants NS 08957 and MH 19691)

161.14 DEVELOPMENT OF CHOLINERGIC MUSCARINIC RECEPTORS IN RAT CNS: AUTORADIOGRAPHIC STUDIES. L. Charles Murrin, Dept. of Pharmacology, Univ. of Nebraska Medical Center, Omaha, NE 68105

Neurotransmitter receptors have been localized anatomically in the CNS in recent years through the use of autoradiography. We have developed conditions for labeling cholinergic muscarinic receptors for autoradiography with ^{3}H-1-quinuclidinyl benzilate (^{3}H-QNB; Amersham) in tissue slices from neonatal rats, using the procedure of Young and Kuhar (Brain Res. 179: 255, 1979). We here report preliminary studies on the ontogeny of these receptors. Sprague-Dawley strain pups were used. At various ages brains were fixed via cardiac perfusion with 0.1% formalin in PBS and 3-5 mm sections of brain were frozen to cryostat chucks in liquid N_2. The brains were sectioned at 6μ and thaw-mounted onto subbed slides. Binding was carried out in 170 mM Tris, pH 7.4 at 20°C for 60 min, which we found sufficient to reach equilibrium. Initially, ^{3}H-QNB was used at 3 x 10^{-9}M with 10^{-6}M atropine added to generate blanks. Using these procedures specific to non-specific binding ratios of 40:1 were obtained. The tissues were washed for 10 min in buffer at 20°C, then dipped briefly in distilled water to remove salts. ^{3}H-QNB was found to dissociate so slowly that there was no significant loss of specific binding with this procedure. Coverslips dipped in NTB-3 were apposed to the slides and exposed for two weeks. The slides were then developed, the tissue stained with Pyronin Y and examined by light microscopy. At birth autoradiographic grains were found in most areas having high concentrations of cholinergic muscarinic receptors in adult brain. The nucleus olfactorius of the olfactory bulb was heavily labeled. The cortex was labeled throughout but this labeling was uniform with no variation between layers. The striatum was also densely labeled with an uneven distribution of the labeling beginning about A8000 (Konig and Klippel, 1963) and continuing caudally. The least dense areas did not appear to correspond to fiber bundles. This uneven distribution was not as discrete as is seen with opiate receptors in striatum. By day 5 receptor levels in layer IV of the cerebral cortex were higher than in other layers. Density of binding had visibly increased in all labeled areas and continued to increase to day 21. In the striatum by day 11 fiber bundles could be seen while the uneven distribution of grains was still present. By day 21 the uneven distribution was gone while the striatum was clearly mottled by the fiber bundles passing through. In cortex at day 21 grain densities resembled those seen in adults with higher levels in layers 2,3,4 and 6. While much work remains, it is clear that this approach will be of great value in understanding the ontogeny of cholinergic systems in the CNS. Supported by the March of Dimes Birth Defects Foundation, MH 33390 and BNS 79-21105.

161.15 ESTROGEN MODIFICATION OF β-ADRENERGIC RECEPTOR DENSITY IN THE RAT HYPOTHALAMUS. H. Ryan Wagner and Elena Yablonskaya-Alter*. Dept. Neurology, College of P+S, Columbia Univ., NY, NY 10032.

Several lines of evidence suggest that estrogens modify the activity of noradrenergic neurons in the brain. Based on this, we have investigated the possibility that estrogen-induced changes in adrenergic function may be reflected at the receptor level. Hypothalamic membrane binding of the β-adrenergic radioligand, ^{3}H-dihydroalprenolol (DHA), was compared between male and cycling female rats and between age-matched female rats ovariectomized at least four weeks prior to the study. We also compared hypothalamic binding of ^{3}H-DHA in ovariectomized female rats following estrogen replacement and in pre-(25 day) and postestrous (60 day) female rats.

Hypothalamic ^{3}H-DHA binding was significantly elevated ($p \leq .01$) in female (F) rats relative to male (M) rats. Scatchard analysis showed a higher maximum receptor density in female animals (F, Bmax=89 fmols/mg; M, Bmax=80 fmols/mg) with no difference in ligand affinity (F, Kd=4 nM; M, Kd=5 nM). Binding capacity in ovariectomized female rats (O) was also significantly elevated ($p \leq .01$) over levels found in male rats (O, Bmax=96 fmols/mg) but did not differ significantly ($p < .1$) from levels in age-matched females without ovariectomy; ligand affinity was again unchanged (O, Kd=5 nM).

Assessing ^{3}H-DHA hypothalamic binding at a single ligand concentration (6 nM), we found no apparent age difference in binding between female rats at 25 and 60 days post partum (Day 25= 57±10 fmols/mg; Day 60=57±7 fmols/mg). Age was also not found to be a significant factor in male rats (Day 25=43±6 fmols/mg; Day 60=43±6 fmols/mg) although, ignoring age, binding capacity between male and female rats was again significantly different (F= 57 ±6 fmols/mg; M=43±4 fmols/mg). Treatment of ovariectomized female rats with estrogen (E) for four consecutive days (100 μg/kg, s.c.) increased hypothalamic ^{3}H-DHA binding capacity over levels found in vehicle injected control (C) rats (E=60+4 fmols/mg; C=51+3 fmols/mg). Results suggest that estrogens may elevate β-adrenergic receptor density in the rat hypothalamus, that such elevations occur before the 25th day post partum and that such changes, once present, are relatively permanent.

161.16 CHARACTERIZATION OF BENZODIAZEPINE BINDING AND ACTION ON RAT ANTERIOR PITUITARY CELLS. L. Grandison, Dept. of Physiology and Biophysics, Rutgers Medical School, College of Medicine and Dentistry of New Jersey, Piscataway, New Jersey 08854

As previously shown by this laboratory the benzodiazepines are potent inhibitors of prolactin secretion (Fed. Proc. 40:415, 1981). One observation demonstrated that diazepam blocked haloperidol induction of prolactin release. Since haloperidol stimulates prolactin release by binding to pituitary dopamine receptors to block the tonic inhibitory action of dopamine the above effect of diazepam suggests that benzodiazepines may act directly at the pituitary. This study sought to examine actions of benzodiazepines directly on the pituitary by determining the existence of benzodiazepine receptors in pituitary tissue and secondly by assessing the effects of benzodiazepines on prolactin release from dispersed pituitary cells maintained in tissue culture. Anterior pituitaries from male rats were enzymatically dispersed and maintained in culture for three to five days. For ligand binding studies the cells were kept in suspension culture. Using ^{3}H diazepam as the ligand specific binding to intact pituitary cells was found. This binding was saturable and scatchard analysis indicated one binding site with dissociation constant of approximately 10 nM and a binding maximum of 25 pmoles/10^6 cells. Displacement studies using unlabeled benzodiazepine analogues indicated a K_I of approximately 5 nM for RO5-4864 and a K_I of greater than 1 μM for clonazepam. For studies examining benzodiazepine action on prolactin release, dispersed pituitary cells were plated onto petri dishes and allowed to attach over a period of three days. During a 2 hour incubation the addition of diazepam to the medium over the dose range 10^{-9} - 10^{-6} M had no significant effect on spontaneous prolactin release. However, preincubation of the cells with diazepam for 24 hrs altered the responsiveness of the cells during a subsequent incubation. Benzodiazepine pretreatment potentiated the inhibition of prolactin release produced by GABA or apomorphine. These data suggest that benzodiazepines can act at the pituitary to alter hormone secretion. (Supported by NIAMDD Grant AM 26661).

162.1 BOMBESIN: CENTRAL NERVOUS SYSTEM (CNS) ACTION TO INCREASE GASTRIC MUCOSAL BARRIER IN RATS. Y.F. Taché. Pediatric Research Center, Ste-Justine Hospital, Université de Montréal, Montreal, Quebec, H3T 1C5.

We have previously shown that intracerebrally applied bombesin (1 µg dose) prevents gastric hemorrhagic lesions caused by stress and completely suppressed gastric acid secretion (Taché et al., Life Sci. 24: 1719, 1979; Taché et al., Proc. Soc. Natl. Acad. Sci. 77: 5515-5519, 1980). We report here that bombesin induced a CNS mediated increase in gastric mucosal barrier which may also contribute to explain its protective action against stress ulceration.

Saline or peptides were injected intracisternally (i.c.) in 24 h fasted male rats under light ether anesthesia and the pylorus was ligated. Conscious rats were decapitated two hours post injection (except in time-course study experiments) and the stomachs were removed. The estimation of mucosal barrier adhering to the everted glandular portion of the stomach was done using a dye binding procedure described by Corne et al. (J. Physiol. 242: 116, 1974). Bombesin (1 µg, i.c.) enhanced by 80%, whereas the mucolytic agent N-acetyl-L-cysteine (300 mg, per os) reduced by 89% the gastric mucosal barrier. The action of bombesin given i.c. is rapid in onset (22% significant increase is observed 15 min post injection), long acting (50% elevation is still observed 3h post injection), dose-dependent (minimal effective dose less than 50 ng), specific (as shown by the inactivity of β-endorphin, somatostatin, norepinephrine, serotonin, or dopamine 5-10 µg dose, i.c.) CNS mediated (since the peptide is inactive when infused into the jugular vein at 100 ng dose/min for 2 h), and not mimicked by other gastric acid inhibitor like atropin (2 mg/kg, i.v.). Indomethacin pretreatment (5 mg/kg, per os -1 h) did not affect bombesin induced increase in mucosal barrier indicating that peptide action is not mediated through prostaglandin release.

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162.2 ANALGESIC ACTIVITY OF NON-OPIATE NEUROPEPTIDES FOLLOWING INJECTIONS INTO THE RAT PERIAQUEDUCTAL GRAY MATTER. T. L. Sullivan* and A. Pert. NIMH, Bethesda, MD 20205

Injections of opiates into the periaqueductal gray matter (PAG), which is high in opiate receptors and opiate peptides, produce profound analgesia in the rat. Besides endorphins, the PAG also contains relatively high concentrations of other neuropeptides. Since this region of the brain appears to be critical for pain perception, it was of interest to ascertain whether any other neuropeptides might also have analgesic properties following direct microinjections. Rats were prepared with chronic indwelling cannulae guides through which various neuropeptides could be injected into the PAG. Analgesia was assessed with both the tail-flick and hot-plate procedures.

Of the opiates, β-endorphin exhibited the most potent analgesic effects in both the hot-plate and the tail-flick test. Both procedures revealed it to be approximately ten times as potent as either morphine or D-Ala^2-D-leu^5-enkephalin (DADL). D-Ala^2-met-enkephalin, on the other hand, appeared to be approximately one-third as potent as either morphine or DADL.

Bombesin, VIP and neurotensin all exhibited analgesic effects. Bombesin was the most potent non-opiate peptide analgetic in the hot-plate test, while VIP was the most potent in the tail-flick test. In general, all of these peptides were approximately three times more potent than morphine following PAG injections.

β-endorphin-induced analgesia was readily antagonized by naloxone. Analgesia induced by either bombesin, neurotensin or VIP, on the other hand, was not reversed by naloxone. Injections of β-endorphin and morphine into the mesencephalic reticular formation (MRF) produced considerably less analgesia than direct injections into the PAG. Injections of bombesin, VIP and neurotensin into the MRF also increased hot-plate latencies but had no effect on the tail-flick response. Injections of TRH, somatostatin, cholecystokinin 26-33 (non-sulfate) and bradykinin had no appreciable analgesic activity following PAG injections. Contrary to some reports, substance P was not found to have any substantial analgesic properties from a dosage range of 0.001-27 µg. Only the highest doses produced a very transitory (5 min post-injection) elevation of hot-plate and tail-flick latencies.

It is clear that there is considerable redundancy in the neurochemical coding of the endogenous pain suppression mechanisms at the level of the PAG and MRF. Experiments are underway to determine whether each system may respond selectively to different inputs.

162.3 CAPSAICIN-INDUCED ANALGESIA: CHARACTERIZATION AND A SITE OF ACTION. M.S. Miller*, S.H. Buck, I.G. Sipes* and T.F. Burks. Department of Pharmacology, University of Arizona Health Sciences Center, Tucson, Arizona 85724.

Capsaicin (8-methyl-N-vanillyl-6-nonenamide), the principle pungent component of hot peppers, has been reported to produce an acute, localized nociceptive response in rats following subcutaneous administration. The acute response is followed by a long lasting desensitization to chemogenic nociceptive stimuli on all body surfaces and depletion of the putative peptide neurotransmitter substance P (SP) from the dorsal spinal cord (DC) and dorsal root ganglia (DRG). In this report we have characterized the sensory effects of capsaicin (C) and investigate the site of action for C-induced sensory deficits in the guinea pig, a species more sensitive to the actions of C than the rat. Hartley guinea pigs of either sex (300-400 g) were used in all studies. The sensory effects of C were investigated in animals treated with a single dose of C (50 mg/kg s.c.). Significant alterations in non-nociceptive heat, nociceptive heat and nociceptive chemical sensory modalities were detected. Pressure, cold and vibratory sense were unaltered. SP levels were significantly decreased in both the DC and DRG as determined by RIA in the absence of detectable changes in DRG somatostatin content. To investigate the site of action for C, animals received a single injection (8µg) of the radiolabeled C analog ^{3}H-dihydrocapsaicin (DHC) in the right front footpad. At various times after treatment (4 hrs-10 days) both forefeet were tested for thermal analgesia. Immediately following analgesia testing, right and left DRG (C_4-C_7) and footpad skin were collected. DRG SP levels, DRG DHC equivalents (DHCe), skin DHCe and skin unextractable DHCe content were then determined in tissues from the treated side and contralateral vehicle-injected control side of each animal. Treated feet demonstrated analgesia within 4 hrs with the effect lasting in excess of 10 days. Levels of SP and DHCe in DRG from treated sides were not different from contralateral controls throughout the experiment. Within 24 hrs of dosing, levels of DHCe in treated skin were not different from those in contralateral control skin. Levels of unextractable DHCe in treated skin were consistently 3-4 fold greater than levels in contralateral control skin throughout the 10 day experiment. It is concluded that in the guinea pig C produces chemogenic and thermal analgesia through a mechanism which does not involve SP depletion from the DRG or non-specific actions of C at the DRG. The site of action for C-induced analgesia may be peripheral to the DRG, possibly involving covalent binding of C to peripheral terminals of primary afferent thermal and chemogenic nociceptors. Depletion of SP from DRG appears to be mediated at a site more central to that which mediates C-induced analgesia. (Supported by USPHS NS15420, DA02163 and NIEHS ES82130).

162.4 INCREASED DRINKING RESPONSE TO INTRAVENOUS ANGIOTENSIN II AFTER LOW-POWER MICROWAVE EXPOSURE. D.L. Hjeresen* and J.B. Simpson. Department of Psychology, NI-25, Univ. of Washington, Seattle, WA. 98195 (Spon: R.D. Phillips, Battelle N.W., Richland, WA.)

Several recent research reports suggest increased permeability of the mammalian blood-brain barrier (BBB) following exposure to low power density (10 mW/cm^2). These studies noted reversibly increased permeability to tracers such as mannitol, Na-fluorescein, and horseradish peroxidase. We have infused a behaviorally active peptide following microwave exposure and determined the behavioral response.

The octapeptide Angiotensin II (AII) is dipsogenic following intravenous as well as intraventricular administration. It does not, however, enter the cerebrospinal fluid from the blood. We determined that 30 min exposure to 1 mW/cm^2 of continuous wave microwaves (918 MHz) enhances the drinking response to intravenous AII, perhaps by permitting its penetration into the brain

Male Long-Evans rats with indwelling jugular catheters were tested in one of four groups: 1) 30 min microwave exposure followed by 4 hr of AII infusion (2 ng/min/10 ul), N=8; 2) 30 min microwave exposure followed by isotonic saline infusion, N=16; 3) 30 min sham microwave exposure followed by AII infusion, N=9; 4) 30 min microwave exposure followed by no infusion, N=4. During the 4 hr post-exposure infusion, water consumption was measured at 30 min intervals and after 24 hr. These values were compared to an averaged daily (24 hr) baseline value taken over 5 days and a 4 hr baseline value taken after 30 min of sham microwave exposure while being infused with isotonic saline. The rats were exposed to 918 MHz, CW microwaves at an incident power density of 1 mW/cm^2 in individual, circularly polarized waveguides.

Both microwave exposure and AII affected 24 hr water consumption. Microwave/AII rats drank 14.9% above baseline consumption ($p < 0.05$, paired t) whereas sham-exposed/AII rats drank only 4.5% above baseline. Rats exposed to microwaves and infused with saline decreased their consumption 8.6% while microwave/non-infused rats differed from baseline by less than 1%. Four-hr consumption values were significantly higher than baseline for all groups except the microwave/non-infused control. However, at the end of 24 hr only the AII microwave group remained significantly elevated.

One interpretation of these data is that microwave exposure permitted penetration of the peripherally infused AII into brain ventricles and/or neuropil by opening the BBB to circulating AII. If this were true the AII might gain access to additional dipsogenic receptors, not accessible to blood-borne AII. However this conclusion awaits direct AII measurement in brain.

162.5 CENTRAL PRESSOR AND PERIPHERAL DEPRESSOR EFFECTS OF NEUROTENSIN IN CONCIOUS RATS C. Sumners*, M.I. Phillips and A. Camacho*. Dept. of Physiology, University of Florida, Gainesville, FL 32610

The tridecapeptide neurotensin (NT) elicits hypotension in rats when injected intravenously (IV) (Carraway et. al. J. Biol. Chem. 248: 6854, 1973), and is also known to have centrally mediated actions such as stimulation of hypothermia and antinociception. In order to examine whether the peripheral depressor effect of (NT) contains a central component, we have compared the effects on blood pressure of NT applied either i.v. or intracerbroventriculary (i.c.v.) in concious rats. Male 250g Sprague Dawley rats were implanted with a permanent lateral ventricle cannula under chloral hydrate anesthesia, and after a recovery period of 3-4 days both the femoral artery and vein were catheterised (under ether) for recording of blood pressure and i.v. injections respectively. The catheters were tunnelled under the skin, exteriorised through an incision on the back, and cemented into position. Rats were used the following day. Neurotensin (Sigma) injected i.v.t. (1 - 30 µg, in 1 - 3 µgl) caused dose related increases in arterial blood pressure ranging from 5-28mmHg (n=12 animals). The response latency was 30 secs and duration was up to 10 mins. Similar doses injected i.v. (in 0.1ml) caused a dose related depressor effect of 15 - 50 mmHg (n= 5 animals), with a duration of up to 90 min. The presser effect of i.v.t. NT was antagonised by i.v.t. phentolamine (10 µg), but not by the potent angiotensin II antagonist sar^1-ala^8-AII (10-20 µg). It was also shown that phentolamine (10 µg) antagonises the pressor effect of i.v.t. angiotensin II (100 ng). Results indicate that i.v.t. administered NT can cause a rise in blood pressure as distinct from its peripheral depressor action, and that this rise is mediated via an alpha adrenergic mechanism. Angiotensin receptors seem to play no role in this NT pressor response.

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162.6 ANTIPYRETIC EFFECT OF CENTRAL AND PERIPHERAL ACTH (1-24) IN ADRENALECTOMIZED RABBITS. J.A. Zimmer* and J.M. Lipton. Physiology and Neurology Departments, University of Texas Health Science Center, Dallas, TX. 75235.

Intracerebroventricular (ICV) administration of ACTH (1-24) reduces fever induced by injection of leukocytic pyrogen (LP) in normal rabbits. This result suggests that central release of ACTH may normally limit fever (Glyn & Lipton, in press). However, because intra-hypothalamic injections of corticosteroids also reduce fever (Chowers, et al, 1968; Willies and Woolf, 1980), the antipyretic effect of central ACTH might be indirect and require stimulation of the adrenal cortex. To test this possibility ACTH (1-24) was injected ICV in 13 bilaterally adrenalectomized (ADX) rabbits made febrile by IV injections of LP. ACTH (250 ng) reduced fever when given simultaneously with LP in seven ADX rabbits and when given 30 min after LP in six others. This dose had a small hypothermic effect on body temperature when given to the same animals when they were afebrile. In order to evaluate the possibility that central ACTH can normally inhibit fever it was important to know if smaller non-hypothermogenic amounts of the peptide can reduce the febrile response. In experiments on seven ADX rabbits doses of ACTH averaging 50 ng reduced fever induced by IV LP, and 25 ng was antipyretic in some animals. Peripheral ACTH also reduces fever in man and rabbits (Kass and Finland, 1950), but it may be that this antipyresis depends upon an action of corticosteroids on the brain. To examine this possibility ACTH (2.5 ug) was given IV to ADX animals made febrile by IV LP. Fever was reduced when ACTH was given both simultaneously with and 30 min after LP injections. This dose of ACTH had no effect on body temperature when the animals were afebrile. The antipyretic effect of ACTH (1-24) is not peculiar to the rabbit since ICV injection of the peptide also lowered fever induced by IV administration of bacterial endotoxin in squirrel monkeys. From these results we conclude that (1) the release of adrenal corticosteroids and their subsequent action on the brain is not required for the antipyretic effect of ACTH (2) peripheral ACTH can enter the brain in quantities sufficient to reduce fever and, (3) the antipyretic effect of ICV ACTH (1-24) also occurs in primates. The findings suggest that release of endogenous central ACTH, and entry into the brain of the circulating ACTH known to increase in fever, limits the magnitude of the febrile response by influencing central temperature controls. (Supported by National Institute of Neurological Communicative Disorders and Stroke grant NG 10046.)

162.7 EFFECTS OF CENTRAL ADMINISTRATION OF VASOPRESSIN AND OXYTOCIN ON BODY TEMPERATURE OF THE RABBIT. J.R. Glyn* and J.M. Lipton. (SPON: H. Feit). Physiology and Neurology Departments, University of Texas Health Science Center at Dallas, TX. 75235.

The presence of vasopressin and oxytocin within brain sites outside the pituitary has evoked questions about their roles in extra-pituitary mediation of physiological functions. Previous research on these peptides suggested that they may influence or participate in central control of body temperature (Lipton & Glyn, 1980). In these earlier experiments intracerebroventricular (ICV) injections of arginine vasopressin (AVP) produced rapidly-developing hyperthermias which lasted 2.5-3.0 hr. Oxytocin produced hyperthermias of lower amplitude and longer duration. To examine further the nature of the central influence of these peptides, vasopressin and oxytocin were administered ICV to rabbits in cold (10°C) and hot (30°C) environments. Doses of 1.25-5.0 ug caused only small (0.2°C maximum) increases in body temperature in the cold; thus these peptides do not raise the central setpoint of temperature control. When 5 ug of AVP was given in the heat all rabbits showed significant increases in body temperature, and three had to be removed to a 23°C environment to prevent lethal hyperthermia. When these three animals were reintroduced into the 30°C environment their body temperatures again rose to life-threatening levels. In additional experiments AVP given centrally with 0.5 ug PGE_2 prolonged prostaglandin-induced hyperthermia. These combined results indicate that AVP and oxytocin inhibit heat loss and therefore have little effect on body temperature in the cold in which heat loss is already maximal.

Knowledge of the additivity of effects of centrally administered substances on body temperature can be used as an indication of whether the substances share common receptor sites (Glyn & Lipton, in press; Goodman & Gilman, 1975). Vasopressin and oxytocin (1.25 ug each), when combined in a single ICV dose, elicited a rise in body temperature greater than that produced by 2.5 ug of either peptide alone. This supra-additivity indicates that vasopressin and oxytocin act at different sites in the CNS temperature control pathways in the production of hyperthermia. Thus, the two peptides tend to raise body temperature when given centrally, without raising the central setpoint, by acting at least partially on different receptor sites. These findings open the possibility that endogenous central AVP and vasopressin are involved in CNS control of body temperature. (Supported by National Institute on Neurological and Communicative Disorders and Stroke Grant 10046).

162.8 RAPID INDUCTION OF TOLERANCE TO THE HYPOTHERMIC EFFECT OF β-ENDORPHIN (βE), BOMBESIN (BN), AND NEUROTENSIN (NT) AND LACK OF CROSS-TOLERANCE TO THE HYPOTHERMIC EFFECT OF ETHANOL (E). John Wenger*, Jeffrey Yusim*, Floyd Bloom and Marvin Brown. (SPON: Joseph Rogers). Peptide Biology Laboratory and Arthur Vining Davis Center for Behavioral Neurobiology, The Salk Institute, La Jolla, CA 92037.

Hypothermia results from the intracerebroventricular (icv) administration of βE, BN and NT. Peripheral E also causes hypothermia and tolerance develops to this effect. Studies were performed to determine whether tolerance can be induced to the hypothermic effects of these peptides given icv, and whether such tolerance would affect the hypothermic effect of E (3 g/kg i.p.).

Lateral ventricular cannulae were placed in male rats. Two days afer surgery they were then 1) tested for responsiveness to the hypothermic effect of a particular peptide, 2) if responsive, infused for at least 5 hours with that peptide to induce tolerance to it, 3) tested for tolerance to that peptide, 4) if tolerant, tested for tolerance to ethanol-induced hypothermia. Baseline temperatures were measured and injections and infusions given in a 22°C room. Following tolerance-test injections, the animals were placed immediately in a cold-room (4°C) for 1 hr.

βE (30 µg/10 µl) initially reduced rectal temperature (RT) to 33.78° C, a fall of 4.33 ± 0.34 °C (mean ± s.e.m.). Following at least 5 hr infusion with βE (3 µg/µl/hr), βE (30 µg/10 µl) resulted in a decrease in RT to 38.82, a fall of 0.06 ± 0.43°C thereby demonstrating tolerance. E then reduced RT to 34.22°C, a fall of 4.52 ± 0.43°C. E given to drug-naive controls reduced RT to 34.13 ± 0.41° C indicating cross-tolerance to E had not occurred.

BN (1 µg/10 µl) initially reduced RT to 34.35 ° C, a fall of 4.00 ± 0.20 ° C. Following an 18 hr infusion of BN (1 µg/µl/hr), BN (1 µg/10 µl) resulted in a decrease in RT to 37.13°C, a fall of 1.10 ± 0.28°C thereby demonstrating tolerance to BN. Immediate injection of E reduced RT 2.85 ± 0.36° C to 34.28 ± 0.15°C indicating cross-tolerance to E had not occurred.

NT (30 µg/10 µl) initially reduced RT to 35.84, a decrease of 2.56 ± 0.42°C. The same dose of NT following a 13 hr NT (3 µg/µl/hr) infusion resulted in a decrease of RT to 37.44, a decrease of 0.75 ± 0.33°C, thereby demonstrating tolerance to NT. Immediate injection of E reduced RT 3.00 ± 0.34°C to 34.44 ± 0.21°C indicating cross-tolerance to E had not occurred.

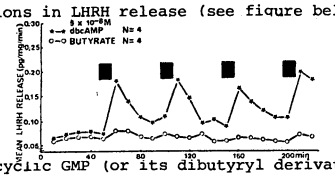
Animals tolerant to hypothermia induced by βE, BN and NT are not tolerant to the hypothermic effect of E. This suggests that ethanol-induced hypothermia is not dependent upon endogenous βE, BN or NT (Supported by ARC grant AA 03504).

162.9 DIBUTYRYL CYCLIC AMP INDUCES CYCLIC RELEASE OF LHRH FROM SUPERFUSED MALE RAT HYPOTHALAMI. Daryl E. Hartter* and V. D. Ramirez. Dept. of Physiology and Biophysics, Univ. of Illinois at Urbana-Champaign, Urbana, IL. 61801.

It is widely accepted that cyclic nucleotides (cyclic AMP and cyclic GMP) are intracellular messengers implicated in a variety of neuronal functions. The present study compares the effect of dibutyryl cyclic AMP (dbcAMP) and dbcGMP infusions on LH-releasing hormone (LHRH) release from male rat hypothalami superfused in vitro.

Two adult male Holtzman rats (per chamber) were killed after light ether anesthesia by heart bleeding; their mediobasal hypothalami (MBHs) were gently placed in a 500-µl. volume chamber kept at 37°C and superfused with a Kreb's Ringer phosphate medium (pH 7.40) with glucose (10mM), bacitracin (0.1mM) and bovine serum albumin (0.1%) at a flow rate of 60-70 µl/min. Theophylline (1mM) was added to medium. Ten-min. samples were collected on ice and later assayed for LHRH content by radioimmunoassay.

The pattern of in vitro LHRH release was clearly dependent on the mode of dbcAMP administration. A continuous infusion of 5 X 10^{-8}M dbcAMP for 2 hrs. produced a slow, steady increase in LHRH release, reaching eight-fold preinfusion values by the end of the infusion. Thirty- or 60-min. infusions were also effective in increasing LHRH release rate, producing irregular bursts in LHRH output. More strikingly, 10-min. infusions of dbcAMP given in an on-off pattern (at either 10-, 20- or 40-min. intervals) were extremely effective in generating a cyclic pattern of LHRH release from these MBH neurons; butyrate did not produce such cyclic variations in LHRH release (see figure below).



Furthermore, cyclic GMP (or its dibutyryl derivative) and the cAMP metabolite 5'-AMP did not modify LHRH release.

These experiments demonstrate that the LHRH neural release apparatus of superfused male rat hypothalami can be activated by cAMP but not cGMP, and, more importantly, that the pattern of LH-RH release is dependent on the mode of cAMP administration. Thus, it is tempting to speculate that cyclic release of LHRH is dependent on a regular generation of cAMP which periodically activates the LHRH release apparatus.

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162.10 Central release of arginine vasopressin (AVP) in rabbit brain. Q.J. Pittman, T.J. Malkinson*, W.L. Veale & K. Lederis*. Depts of Pharmacology & Physiology, Univ. of Calgary, Calgary, Canada.

In addition to its hormonal actions on kidney water retention and peripheral vasomotor tone, recent observations indicate that AVP may be a putative neurotransmitter within the brain. Immunocytochemical studies have established the presence of immunoreactive AVP in neurons in the hypothalamus and in neural processes throughout widespread areas of brain. When injected into the brain, AVP has potent effects on many behavioral and physiological processes. We have sought evidence for AVP release within brain tissue in response to a stimulus which also activates pituitary secretion of this peptide into the circulation.

Male New Zealand white rabbits were fitted with stereotaxic headplates and chronic jugular catheters. Following a 5 day recovery period during which time the animals were habituated to the experimental surroundings, a push-pull cannula was lowered through the headplate so its tip was positioned in either the lateral septum or the amygdala. A sterile physiological solution was perfused through the cannula at 35µl/min for three 30 min periods; the perfusate was collected on ice and then frozen immediately at -70°C. After the first 30 min sample was collected, and concurrent with the beginning of the second collection period, 8 ml of sterile 3M NaCl was infused for 10 min into the jugular catheter. This amount of NaCl raises the blood osmolality to approximately 345 mOsmoles and constitutes a potent stimulas for AVP release from the pituitary. After termination of the second push-pull perfusion a period of 30 min elapsed before the third and final perfusion was started.

Perfusates were assayed for AVP using a sensitive radioimmunoassay which shows little cross-reactivity for other related peptides. In control perfusates, AVP levels varied from undetectable (<1.5pg/ml) to 135pg/ml. Following systemic injection of hypertonic NaCl, AVP levels in the second push-pull perfusate rose by 316% in the lateral septum (N=3) and by 299% in the amygdala (N=5). In the third and final perfusates AVP levels were still elevated above control levels.

Circulating AVP in physiological concentrations is not believed to cross the blood-brain barrier. Therefore, evidence for AVP release within rabbit brain is in keeping with the view that AVP could be a neurotransmitter in the brain. Further work is required to establish the specificity of the release in response to various stimuli and to explore other areas of the brain for their ability to release AVP.

Supported by MRC and AHFMR

162.11 NEUROCHEMICAL EFFECTS OF CAPSAICIN TREATMENT IN ADULT MALE WISTAR-KYOTO (WKY) AND SPONTANEOUSLY HYPERTENSIVE (SHR) RATS. Robert M. Virus*, Dennis Q. McManus* and G.F. Gebhart. Dept. of Pharmacology, University of Iowa, Iowa City, IA 52242

The effects of capsaicin (8-methyl-N-vanillyl-6-nonenamide) on the contents of substance P (SP) in central and peripheral nervous system tissues were investigated in adult male WKY and SHR rats. Capsaicin was subcutaneously administered in 3 successive daily 50 mg-kg^{-1} doses to lightly ether-anesthetized subjects. Subjects were sacrificed by decapitation 15 days after the last vehicle or capsaicin injection; brains, spinal cords and sympathetic ganglia were rapidly removed, frozen on dry ice, and stored at -80°C for subsequent assay of SP contents. SP contents were determined using a specific radioimmunoassay (Immuno Nuclear Corporation, Stillwater, MN). Selected brain nuclei were microdissected from 300 µm thick frozen coronal sections using the punch technique of Palkovits et al. (Brain Res., 77: 137-149, 1974; Brain Res., 79: 443-450, 1974; Brain Res., 80: 237-249, 1974).

Capsaicin treatment significantly increased the SP contents in the periventricular preoptic hypothalamic and medial amygdaloid nuclei only in rats of the WKY strain. SP contents of the raphe magnus, periventricular preoptic hypothalamic, and medial amygdaloid nuclei of vehicle-treated SHR rats were significantly greater than the SP contents of vehicle-treated WKY rats. Capsaicin treatment significantly reduced the content of SP only in the medial amygdaloid nucleus of SHR rats. No significant strain or treatment differences were observed in the SP contents of the solitary tract, locus coeruleus, and periaqueductal central gray.

The SP contents of spinal cords of vehicle-treated SHR subjects were significantly greater than those of vehicle-treated WKY subjects and capsaicin treatment significantly reduced spinal cord SP contents in rats of both strains. The amount of SP in both superior cervical and celiac sympathetic ganglia of vehicle-treated WKY rats was significantly greater than the SP content of these ganglia in vehicle-treated SHR subjects. Capsaicin treatment significantly reduced the SP contents of both ganglia in both strains.

These results indicate that differences between the WKY and SHR strains exist in the sensitivity to capsaicin-induced alterations in SP contents in a variety of central and peripheral nervous system structures. (Supported by NS-12114 and MH-15172).

162.12 HORMONAL REGULATION OF HIPPOCAMPAL SYNAPTIC PLASMA MEMBRANE PROTEIN PHOSPHORYLATION. Linda A. Dokas, Henk Zwiers* and Willem H. Gispen* Depts. of Biochemistry and Neurosciences, Medical College of Ohio, Toledo, Ohio 43699 and Inst. of Mol. Biol. & Rudolf Magnus Institute for Pharmacology, Utrecht, The Netherlands.

Hippocampal synaptic plasma membranes (SPM) were prepared in 10mM sodium acetate-10mM magnesium acetate-1mM calcium acetate, pH 6.5 and incubated in vitro for 20 sec with 7.5µM γATP^{32}. Under these conditions the major phosphorylated protein is of molecular weight 48,000. Addition of 10^{-5}M ACTH to the phosphorylation assay caused inhibition of SPM protein phosphorylation, including a 33% decrease in the phosphorylation of the 48K protein. Thus, hippocampal SPM contain an ACTH-sensitive protein kinase and its substrate protein, B-50. Hippocampal SPM were prepared from normal, sham-operated and adrenalectomized (ADX) rats. At all times following surgery, there was no difference in SPM protein phosphorylation between normal and sham-operated rats. At 3 and 4 days post-adrenalectomy (post-ADX), hippocampal SPM showed diminished protein phosphorylation in vitro. At 4 days post-ADX the decrease in B-50 phosphorylation was -36.0±2.9% compared to normal levels of phosphorylation. No difference was seen in hippocampal protein phosphorylation between normal and 14-day ADX rats. The time course of the effect following adrenalectomy suggests the inhibition of hippocampal SPM protein phosphorylation is not dependent upon the lack of adrenal steroids.

Comparison of SPM protein phosphorylation from the hypothalamus of normal, sham-operated and ADX rats showed no differences at either 3 or 14 days post-ADX. Hypophysectomy did not abolish the decrease in hippocampal SPM protein phosphorylation seen at 3 days post-ADX. The time course of the effect of adrenalectomy on hippocampal SPM protein phosphorylation and its regional specificity are consistent with the observation of Van Dijk et al (J. Endocr. 88:243-253, 1981) that endogenous levels of ACTH in the hippocampus are decreased at 3, but not 14 days, post-ADX. Previous work of Zwiers et al (Mechanism, Regulation and Special Functions of Protein Synthesis in the Brain, pp. 267-272, 1977), has shown that administration of ACTH in vivo leads to subsequent stimulation of incorporation of labeled phosphate from γATP^{32} in vitro into a SPM protein with the molecular weight of B-50 from subcortical (including hippocampal) tissue. In the present case, lowered ACTH levels in vivo would result in increased amounts of endogenous phosphate groups on SPM proteins, leading to decreased incorporation of P^{32} in a subsequent in vitro assay. Thus, our observation that adrenalectomy decreases hippocampal SPM protein phosphorylation most probably reflects a transient decrease in endogenous ACTH in the hippocampus following adrenalectomy. Supported in part by BRS RR 05700.

163.1 HIGH MOLECULAR WEIGHT SOMATOSTATIN SECRETION BY CULTURED BRAIN CELLS. Robert A. Peterfreund* and Wylie Vale. Department of Neurosciences, UCSD School of Medicine and Peptide Biology Laboratory, The Salk Institute, La Jolla, CA 92037.

Somatostatin-28 (SS-28) is a 28 residue, N-terminally extended form of the tetradecapeptide somatostatin-14 (SS-14). SS-28 has been characterized in brain and gut extracts and found to have biologic activities greater than or equal to the widely distributed SS-14. In order to clarify any possible extracellular role for SS-28, it is essential to determine whether intact cells secrete this peptide. We have investigated the chromatographic and immunologic behavior of the somatostatin-like peptides secreted by primary cell cultures of rat cerebral cortex and hypothalamus. Hypothalamic or cerebral cortical tissues from 18 day rat fetuses are dispersed with collagenase and maintained in a serum supplemented medium. The cells from both tissues exhibit a basal secretion of 100-300 pg of somatostatin as measured by radioimmunoassay with antibody S201. Depolarizing agents elicit a calcium dependent secretion of up to 1 to 4 nanograms of somatostatin per 10^7 cells. Hypothalamic cultures secrete somatostatin in response to administration of 8-BrcAMP or IBMX. Both drugs have lesser effects on secretion by cerebral cortical cells. The secretory product elicited by high potassium medium was collected in acid plus protease inhibitors, heated for five minutes at greater than 95° C, lyophilized, redissolved in 6 M guanidine-HCl in 3 N HOAC and applied to a Sephadex G50 column eluted with 3 N acetic acid at room temperature. For both types of cultures, two main peaks of somatostatin were detected by RIA using antiserum S201 which is directed against the center of SS-14. These peaks comigrated with synthetic SS-28 and SS-14 respectively. Radioimmunoassay with antiserum S39 which reads the amino terminus of SS-14 and does not detect synthetic or native SS-28 failed to detect the peak corresponding to SS-28. For cortex cultures, 23% of the recovered somatostatin comigrated with the SS-28 peak. Twenty-two percent of the hypothalamic product migrated as SS-28. Both hypothalamic and cortical elution profiles had a third, minor peak near the void volume. This peak could correspond to an even larger form of somatostatin. When considered with the high potency of SS-28 in the central nervous system, these results indicate that SS-28 or a somatostatin-like moiety of similar size may be a significant secretory product of cerebral cortical and hypothalamic cells.

Supported by NIH grants GM07198, AM20917, AM26741 and a grant from The March of Dimes Birth Defects Foundation.

163.2 BRAIN TSH AND GH: EVIDENCE FOR SEPARATE POOLS OF BRAIN AND PITUITARY IMMUNOREACTIVE AND SYNAPTOSOMAL ASSOCIATION OF BRAIN GROWTH AND THYROID STIMULATING HORMONES. S. Hojvat*, G. Baker*, L. Kirsteins* and A. M. Lawrence* (SPON: I. Held). Biochem. Neuroendocrinol. Lab., VA Hospital, Hines, IL 60141, and Biochem. Dept., Loyola Univ. Stritch Sch. of Med., Maywood, IL 60153.

Pituitary-like peptides with immuno- and biologic activity similar to, if not identical with, hormones from the pars distalis have been shown to be present in discrete areas of the rodent and primate central nervous system (CNS), particularly within the hypothalamus and limbic system. These include ACTH, luteinizing hormone (LH), thyroid-stimulating hormone (TSH), and growth hormone (GH)-like peptides. Several lines of evidence indicate these are not of pituitary origin. Levels of these peptides remain unchanged following hypophysectomy. Dispersed brain cells produce these materials in tissue culture. Studies reported here, utilizing differential ultracentrifugation, demonstrate TSH and GH-like immunoreactivity associated with synaptosomal fractions of whole brain homogenate. Whereas significant changes in the concentration of GH and TSH in the brain and in the pars distalis can be evoked by removal of the thyroid and gonads or by the administration of supraphysiologic doses of thyroxine, these changes, in general, vary inversely. For example, rat brain GH levels rise significantly following thyroidectomy while pituitary and serum levels drop significantly. Pituitary and serum TSH rise dramatically after thyroidectomy; there was no change in brain TSH. Pituitary and serum TSH dropped significantly when supraphysiologic doses of thyroxine were administered while TSH in the brain remained unchanged. Castration caused a rise in hypothalamic TSH and a significant fall in pituitary levels. Whereas TSH and GH rise during development of the pituitary, from prepartum values to levels at puberty, brain GH and TSH rise dramatically 24 hours before birth, drop precipitously and reappear at the time of puberty. Conclusion: Immunoreactive GH and TSH-like peptides in the brain are synaptosomally associated; changes following target gland ablation or after administration of supraphysiologic amounts of thyroxine show a pattern quite different from that observed in serum and pituitary. Unique profiles in the development of fetal, neonatal, young adult GH and TSH in the brain and in the pituitary indicate their apparent independence from each other. This data suggests these brain-based peptides play no role in regulation of pituitary hormone secretion, but because significant and consistent changes followed thyroidectomy and castration, it may still be that these brain peptides are involved in overall neuroregulation of the organism's endocrine physiology.

163.3 VASOPRESSIN CONTENT OF INTRA AND EXTRAHYPOTHALAMIC NUCLEI OF RAT BRAIN. D.M. Dorsa* and M. Raskind* (SPON: W. Crill). GRECC, VA Medical Center, Seattle, WA 98108.

Although extrahypothalamic projections of vasopressin (AVP) containing fibers have been visualized using immunocytochemical techniques, successful quantitative measurements of AVP contents of these brain nuclei have not been reported. Since AVP has been shown to influence conditioned avoidance behavior when locally applied to areas in the septum, thalamus, and locus coeruleus, we have established methods for quantitation of AVP in discrete microdissected brain nuclei to further examine the possible involvement of vasopressin containing neuronal systems in CNS processes.

Male Sprague-Dawley (SD) rats (n=7-11) and homozygous (HODI, n=5) and heterozygous (HEDI, n=5) Brattleboro rats (hereditary diabetes insipidus) were killed by decapitation and their brains and neurointermediate lobe (PNI) of the pituitary were rapidly removed and frozen on dry ice. Selected nuclear brain regions were "punched" according to the method of Palkovits, frozen, sonicated in 0.1N HCl at 4°C, then centrifuged, aliquoted and lyophilized. A highly sensitive and specific radioimmunoassay was performed on these samples using an antiserum to AVP kindly provided by Drs. D.A. Fisher and H. Artman. Pituitary and brain nuclear contents in μU/μg protein for the 3 groups were as follows:

Area	SD	HEDI	HODI
median eminence	122.9±19.1	51.2±16.9	N.D.
supraoptic N.	12.87±4.23	3.74±0.59	N.D.
suprachiasmatic N.	3.75±0.76	2.25±1.12	N.D.
paraventricular N.	1.16±0.21	1.38±0.51	N.D.
OVLT	0.63±0.18	0.32±0.03	N.D.
ventral tegmental N.	0.31±0.13	0.29±0.07	N.D.
medial preoptic N.	0.19±0.14	0.14±0.01	N.D.
lateral septum	0.10±0.02	N.D.	N.D.
locus coeruleus	0.06±0.006	0.17±0.05	N.D.
frontal cortex	N.D.	N.D.	N.D.
PNI (n=1)	1005.5	576.6	N.D.

N.D. = not detected

These data confirm quantitatively the presence of vasopressin in low but significant amounts in extrahypothalamic behaviorally sensitive brain nuclei. The lack of immunoreactivity in HODI PNI and brain indicates the specificity of these measurements for AVP and supports the contention that AVP deficiency in HODI underlies their behavioral deficits. Quantitative measurements of AVP content of specific brain nuclei will allow us to examine the regulation of activity of these neurons and their physiologic importance in CNS function.

163.4 COMPARATIVE STUDIES OF PLASMA AND BRAIN ANGIOTENSINOGEN IN THE RAT. Anthony Vitto and Morton P. Printz*. Division of Pharmacology (M-013), Department of Medicine, Univ. Calif., San Diego, La Jolla, CA 92093.

There is much evidence that distinct renin-angiotensin systems exist in the plasma and the brain of a variety of animal species. Angiotensinogen (renin substrate), the precursor protein from which the angiotensin peptides are derived, is a key element in this system. We present studies that distinguish plasma angiotensinogen from brain angiotensinogen in the Sprague-Dawley rat. A rapid and efficient purification of stable renin substrate was carried out using high-salt precipitation, hydrophobic interaction chromatography (Phenyl-Sepharose), and gel filtration (Ultrogel AcA 34). Essentially one protein-staining band was observed on native polyacrylamide gel electrophoresis. Plasma and brain forms of angiotensinogen are similar in molecular size as determined by gel filtration and dodecyl-sulfate polyacrylamide gel electrophoresis. However, whereas a molecular weight in the 60,000 dalton region of migration was observed on polyacrylamide gels, a molecular weight of 100,000 was observed on gel filtration. This apparent overestimation of molecular weight suggested the presence of carbohydrate. The glycoprotein nature of angiotensinogen was confirmed by differences in heterogeneity on isoelectrofocusing polyacrylamide gels that could be altered by treatment of renin substrate with neuraminidase prior to electrofocusing. However, neuraminidase failed to convert the plasma and brain forms of angiotensinogen to a single identical form. Distinctions between plasma and brain angiotensinogen were also made on the basis of differential binding to various lectin-gel columns. Differences in primary sequence, if any, remain to be determined. Current projects underway involve the production of antibody directed toward each form of renin substrate and the immunohistochemical localization of angiotensinogen within various brain regions.

163.5 PROPERTIES OF NEW HYBRID CELL LINES. T. Amano and Y. Kudo, (SPON: H. Kawamura) Mitsubishi-Kasei Inst. of Life Sci., Minamiooya, Machida-shi, Tokyo, Japan 194.

New hybrid cell lines between mouse neuroblastoma N115TG3 and rat glioma C6Bul (NG115-301, NG115-401), hybrids between neuroblastoma N18TG2 and pheochromocytoma PC12 (NP-1A, NP-3A, NP-4), and hybrids between PC12 and C6Bul(PG-1D, PG-2A, PG-3A) were made by using polyethlene glycol #1000. Hybrids were selected in HAT medium and identified by the presence of both marker chromosomes of the parents. Some of the hybrids exhibited high choline acetyltransferase activities independent of low activities of parent cell lines. NP series hybrid cells had both dense and clear vesicles resembling to the parent PC12 cells under electron microscope. On the other hand PG-1D cells had only clear vesicles characteristics to acetylcholine.

NG115-301 cells responded to dopamine (1-100 μM) by elevating intracellular cyclic AMP to 30-fold. NG115-401 cells responded to dopamine by inhibiting intracellular cyclic AMP accumulation. NG115-401 cells had 100 pmoles/min/mg protein of choline acetyltransferase activities together with significant amount of substance P like immunoreactivity(SPLI) ranging 500-1,000 pg/mg protein. Acetic acid extract (0.1 M) of the cells showed SPLI in fractions corresponding to 26K molecular weight by Biogel column (H. Hatanaka and T. Amano, Brain Res. in press). Acid/acetone extract revealed SPLI corresponding to the fractions of authentic substance P. The extracts showed biological activities by contracting guinea pig ileum in the presence of atropine (10^{-6} M) and chlorpheniramine (10^{-6} M). Electron micrographs showed dense cored vesicles suggesting secretory activities of the cells. Rat spinal ganglions derived from 30-day-old Lewis rat as reported substance P positive tissues also had low but significant amount of choline acetyltransferase activities (10 pmoles/min/mg protein). These findings provide the evidences of the presences of the activities of substance P and choline acetyltransferase in a new neuronal cell line. These cell lines may provide usefull models for the physiological study and biochemical processing of substance P.

163.6 CHARACTERIZATION AND DISTRIBUTION OF MOTILIN-LIKE IMMUNOREACTIVITY IN THE RAT CENTRAL NERVOUS SYSTEM. T. L. O'Donohue*, M. Beinfeld,* W. Y. Chey,* and D.M. Jacobowitz. Lab. of Clin. Sci., NIMH, Bethesda, MD 20205 and The Genesee Hospital, Rochester, N.Y. 14607.

A number of reports have suggested the presence of motilin immunoreactivity in the central nervous system of various species. The distribution and identity of motilin immunoreactivity in rat brain was investigated in this study. Using two C-terminally directed motilin antisera, approximately 1.5 ng and 0.4 ng of motilin were detected in the rat forebrain and cerebellum, respectively. The rat brain immunoreactive material, as characterized by Sephade G-50 chromatography, consisted of a large high molecular weight peak eluting near the void volume and a smaller lower molecular weight peak eluting near the elution volume of synthetic porcine motilin. Further characterization of the low molecular weight peak by reverse phase high pressure liquid chromatography indicated that this peptide was distinct from synthetic porcine motilin. Furthermore, one N-terminally directed antiserum poorly detected immunoreactive material in brain while another N-terminal antisera failed to recognize brain motilin. Therefore, chromatographic and immunological data distinguish the immunoreactive material in brain and synthetic porcine motilin.

The distribution of motilin-like immunoreactivity was investigated by immunofluorescence and radioimmunological procedures. Nerve terminals containing motilin immunoreactivity were primarily distributed throughout the hypothalamus with the greatest density in the median eminence. Perikarya-containing motilin immunoreactivity were also primarily localized in the mediobasal hypothalamus. Moderate concentrations of immunoreactive motilin were also contained in extrahypothalamic regions. The data reported here demonstrate that a peptide distinct from, but perhaps related to, motilin is present in neurons in rat brain.

163.7 SECRETIN IN RAT BRAIN: LOCALIZATION, CHARACTERIZATION AND BEHAVIORAL EFFECT. C.G. Charlton,* T. L. O'Donohue,* R. L. Miller,* J.N. Crawley and D. M. Jacobowitz (SPON: A. M. Laties). Lab. of Clinical Science, NIMH, Bethesda, MD 20205 and Dept. of Pharmacology, Coll. of Med., Howard Univ., Washington, D.C.20059.

Secretin is a basic polypeptide of 27 amino acids. Secretin-like bioactivity in extracts of porcine brain has been reported. This report will further describe the localization and characterization of brain secretin and its possible role as a neuroregulator.

Secretin-like immunoreactivity (SLI) has been identified and characterized in acid extracts of rat and pig brain utilizing a highly specific radioimmunoassay and fractionation on a reverse phase high pressure liquid chromatographic system. SLI of rat brain coeluted precisely with SLI of rat duodenum, but slightly ahead of SLI of pig brain and duodenum and synthetic porcine secretin. The difference in elution pattern suggest that secretin in the rat is slightly different from secretin in the pig.

Gross dissection revealed a wide distribution pattern of SLI throughout the rat brain. The highest concentrations were observed in the pineal and pituitary glands, followed by the thalamus, hypothalamus and olfactory bulb. Lower concentrations were observed in the cerebellum, midbrain, septum, striatum and hippocampus. The medulla-pons and the cerebral cortex contained the lowest concentrations.

Intraventricular injection of secretin (5μg) reduced open field activity and decreased the number of novel-object-approaches in rats. Secretin also decreased ventilation rate in anesthetized rats and increased defecation in awake rats.

The detection of SLI in the rat brain, combined with the fact that intraventricular injections of the peptide produced behavioral and physiological changes, may indicate that secretin is another of the growing number of neuroregulatory peptides.

163.8 STUDIES ON AN α-MSH ACETYLATING ENZYME IN THE RAT AND FROG. M.C. Chappell,* T.L. O'Donohue,* Y.P. Loh, R.L. Miller* and D.M. Jacobowitz. Lab. of Clin. Sci., NIMH, Bethesda, MD 20205 and Dept. of Pharmacology, Howard University, Washington, D.C. 20059.

Both deacetylated and N-acetylated α-MSH are present in proopiomelanocortin neurons in the rat and human brain and rat pituitary gland and the N-acetylated form is much more behaviorally active than the deacetylated peptide (Neuroscience Abstracts, 1981). Since the N-acetylation reaction appears to be a key step in the regulation of the behavioral activity of α-MSH, the enzymatic process regulating this conversion was investigated.

The enzyme assay involved incubating an 8000xg tissue supernatant with acetyl-(^{3}H)-coenzyme A and deacetylated α-MSH. The enzymatically synthesized (^{3}H)-α-MSH was isolated by a combination of ion exchange and high pressure liquid chromatography and counted. The enzyme was distributed unevenly with a 50-fold gradient between the highest and lowest activity in the pituitary gland and brain.

Highest activities were found in the pituitary gland. Similar activities were found in the anterior lobe (1.7 pmole/5 min/mg wet wt) and the neurointermediate lobe (0.96 pmole/5 min/mg wet wt). Enzyme concentrations in the brain were, on the average, about 30-fold lower than that found in the pituitary gland. The relative activities were olfactory bulb > hypothalamus, septum, cerebellum > striatum, cortex > thalamus, midbrain, brain stem > hippocampus. The regional distribution of enzyme activity did not parallel the distribution of α-MSH concentration in brain and pituitary. It is therefore unlikely that the enzyme is solely restricted to α-MSH synthesizing cells of the pituitary gland.

To determine if the α-MSH N-acetylating enzymatic activity is linked to α-MSH biosynthesis, enzyme activities were determined in pituitary glands of frogs (Xenopus laevis) housed in either chronic light to suppress α-MSH synthesis or chronic dark to induce α-MSH synthesis. Frogs housed in chronic darkness had significantly higher acetylating enzyme activities in both the anterior and neurointermediate lobes of the pituitary than did frogs housed in chronic light. The results of these studies indicate that although the α-MSH acetylating enzyme may not be solely localized to α-MSH synthesizing cells, it does appear to play an important role in the physiology of the α-MSH neuron or cell as 1) physiological alteration of α-MSH biosynthesis also alters acetylating enzyme activity and 2) N-acetylation markedly potentiates the behavioral actions of α-MSH.

163.9 REGIONAL CONCENTRATIONS OF INSULIN IN THE RAT BRAIN.
D. G. Baskin*, D. M. Dorsa*, and D. Porte, Jr.* (SPON: S. D. Hauschka). Depts. of Biological Structure, Pharmacology, and Medicine, Univ. Washington School of Medicine, Seattle, WA 98195, and Veterans Administration Medical Center, Seattle, WA 98108

Insulin has been found in extracts of rat brain, but the concentrations of insulin within various regions of the brain have not been clearly established. Therefore, we measured the concentrations of immunoreactive insulin (IRI) in different regions of the brain of fed, adult male Wistar rats (300g). Brains were removed after decapitation and dissected into various portions, which were frozen on glass slides in contact with CO_2 ice. The brains (N = 4) were perfused with Krebs Ringer phosphate prior to decapitation. Frozen brain tissue was homogenized in 10 vols. of ice cold 0.2M HCl/75% ethanol and shaken overnight at 4°C. Aliquots (5 and 10 µL) were taken for protein determination by the Lowry assay. The homogenates were centrifuged at 30,000 g for 20 min. at 4°C. Supernatants were evaporated to 5% of original volume at room temperature. The concentrates were resuspended in 5 vols. 0.05M ammonium carbonate containing 1% BSA, and neutralized with ammonium hydroxide. The samples were then centrifuged at 30,000 g for 20 min. at 4°C and the supernatants were lyophylized and frozen until radioimmunoassay. The sensitivity of the radioimmunoassay was 0.04 ng per tube. The results from 5 rats are shown in the Table ($\bar{x} \pm$ sem). Measurements from a single unperfused brain gave comparable values and are included.

Region	Total IRI (ng)	Total protein (mg)	IRI/protein (ng/g)
Amygdyla	0.394 ± 0.018	6.4 ± 0.5	65.3 ± 9.1
Hypothalamus	0.168 ± 0.009	4.9 ± 0.4	36.5 ± 4.2
Hippocampus	0.258 ± 0.009	7.0 ± 0.3	37.4 ± 2.6
Olfactory bulb	0.188 ± 0.011	4.0 ± 0.5	49.2 ± 4.3
Cortex	0.458 ± 0.041	5.9 ± 0.5	78.8 ± 4.8
Midbrain	0.829 ± 0.027	18.7 ± 1.5	46.5 ± 5.6

The results indicate that insulin concentrations are very low in these portions of the brain. Although the amount of insulin per ng protein varied slightly among the regions examined, the total amount of insulin detected in each region appeared to reflect the relative size of the extracted portion. Compared to neuropeptides that are known to be a product of brain neurons and exhibit large regional differences in concentration, insulin is more diffusely and evenly distributed in the brain. The results suggest that most of the insulin in the rat brain is probably not produced by local neuronal systems but very likely originates from the endocrine pancreas. (Supported by NIH Grant AM 17047 and the Veterans Administration).

163.10 DISTRIBUTION OF SUBSTANCE P IN EMBRYONIC RAT BRAIN.
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Neurons containing Substance P(SP) are present in many regions of brain, but the physiological functions under their regulation remain to be defined. In this study we have examined the regional content of SP during prenatal and postnatal development.

Radioimmunoassay (RIA) was used for all SP determinations. Antibodies were raised in New Zealand white rabbits, injected subcutaneously with SP coupled to bovine albumin (BSA-SP) at doses equivalent to 50 µg SP per rabbit. SP antiserum was also obtained as a gift from Dr. William Campbell, University of Texas. SP was iodionated with ^{125}I Bolton Hunters reagent. RIA was carried out in phosphate buffer containing saline (PBS) at pH 7.4; and the bound and free SP were separated by the Dextran-charcoal method.

Brain tissue was dissected and homogenized in 500 µl of 20 mM HCl. An aliquot of homogenate was removed for protein assay by the Bradford method and the remaining homogenate was extracted for 10 minutes in a boiling water bath, cooled and centrifuged at 10,000 rpm for 10 minutes. The supernatant fluid was lyophilized and stored at -20°C until assay.

SP was present in brain by the 15th day of gestation at a relatively low level; 233 ± 50, 430 ± 50, 348 ± 120 pg/mg protein (pg/mg p) for the forbrain, cerebellium, and midbrain respectively. The levels for comparable regions on the 20th-21st day of gestation were: 438 ± 100, 972 ± 200, and 1454 ± 100 pg/mg p; and 1034 ± 200 pg/mg p for hypothalamus. However, 1 day after birth the levels appeared to decrease.

We conclude that SP neurons are significantly developed by the 15th day of gestation and continueously develop until birth. We are presently completing a more detailed study of the developmental pattern and the function of SP in rat brain.

163.11 FEVER-INDUCED CHANGES IN CENTRAL α-MSH CONCENTRATIONS IN THE RABBIT.
W.K. Samson,* J.M. Lipton, J.A. Zimmer* and J.R. Glyn*. (SPON: Sami I. Said). Physiology and Neurology Departments, University of Texas Health Science Center, Dallas, TX. 75235.

Body temperature in fever rarely exceeds 41.1°C even though man and other homeotherms have the capacity to raise temperature even higher (DuBois, 1949). The regulatory mechanism that underlies this phenomenon may depend upon central α-MSH, a peptide which reduces fever when administered centrally (Glyn & Lipton, in press). To test this idea leukocytic pyrogen (LP) was injected into the lateral ventricle of adult NZW rabbits. Once maximal fever was reached (~41.0°C), the animals were decapitated, and brains quickly frozen on dry ice. Control animals did not receive LP but were similarly handled and sacrificed. Discrete brain regions were microdissected and extracted in 1 ml iced, 2.0 N acetic acid containing 500 KIU trasylol. Antiserum to synthetic α-MSH (Drs.C. Oliver and R.L. Eskay) employed for RIA at an initial dilution of 1:3,000 generally bound 30% of total iodinated trace and provided a range of assay sensitivity from 2.0-500 pg α-MSH. Aliquots of tissue extracts from all areas where α-MSH was detected displayed dose displacement curves that parallel that of the synthetic peptide. Additionally, extracts of rabbit pituitary, median eminence and septum comigrated with synthetic α-MSH on column chromatography (G-25, 0.9 x 96.0 cm). Immunoreactive α-MSH (ir α-MSH) recovered in these column fractions also displayed parallel dose displacement RIA curves. No significant differences in ir α-MSH content ($\bar{x}$ ± SEM, pg/ug protein) were detected in pyrogen-injected rabbits (n=9) versus controls (n=17) in the following areas: Paraventricular area of hypothalamus (2.94 ± 0.42 vs 3.13 ± 0.40), median eminence (5.41 ± 0.82, 4.90 ± 0.63), midbrain central grey (2.46 ± 0.28, 2.32 ± 0.19), arcuate nucleus (7.15 ± 1.07, 10.33 ± 1.29), preoptic/anterior hypothalamic area (6.36 ± 0.76, 6.78 ± 0.93), pineal (7.87 ± 1.95, 7.71 ± 1.26) or whole pituitary (86.5 ±10.0, 89.5 ± 11.9 ng/ug protein). Less than 0.05 pg ir α-MSH/ug protein was detected in cerebral cortex and thalamus. A significant difference ($p < .01$) did exist in the septal extracts of control rabbits (0.54 ± 0.04) when compared to pyrogen injected animals (0.81 ± 0.10). These punches of rabbit septum were unilateral and included portions of both the medial and lateral divisions. The finding that LP-induced fever was associated with an increase in levels of a CNS-peptide (α-MSH) that has been implicated in the central control of thermoregulation lends support to the theory that CNS peptides act as neuromodulators at discrete CNS sites to control this vital physiologic function. (Supported by NINCDS Grant 10046).

164.1 TRIMETHYLTIN PRODUCES SOMATOSENSORY DYSFUNCTION. R. S. Dyer, W. E. Howell and T. J. Walsh. Neurotoxicology Division, US Environmental Protection Agency, Research Triangle Park, NC 27711.

Trimethyltin (TMT) is a neurotoxic alkyltin which produces selective neuronal damage within the limbic system (Brown et al., 1979) and a unique behavioral syndrome characterized by spontaneous seizures, hyperreactivity, and tail mutilation (Dyer et al., 1980). Observed tail mutilation suggested somatosensory disturbances. To study this, the following tests were performed: (1) heat pain threshold; (2) peripheral mixed nerve threshold and conduction velocity; and (3) the somatosensory evoked response (SER). Male Long-Evans hooded rats were implanted with ground, reference, and somatosensory cortex electrodes. Prior to receiving an i.p. injection of either 0 (saline vehicle) or 7 mg/kg TMT chloride, animals were tested on each of the three tests, and re-tested on days 1 and 4 following dosing. Sensitivity to heat was measured by placing each animal on a hot plate (51.0°C) and recording the latency to lick the hind paw. Animals were then anesthetized and placed on an apparatus permitting immersion of the tail in warm paraffin oil (37.5°C). Prior to immersion three needle electrode pairs (two stimulating-one recording) were inserted near the left dorsal caudal nerve. The tail was allowed to equilibrate to bath temperature before testing was begun (5 min). Mixed nerve conduction velocity was determined by supramaximally stimulating (5 Hz, 0.1 msec) proximal and distal electrode pairs (100 mm separation) and recording the compound nerve action potential at a distant tail location. The SER was elicited by stimulating the proximal electrode pair (1 Hz, 0.1 msec) at an intensity sufficient to produce a maximal compound action potential from the nerve. A mean of 64 responses was obtained for each animal and the peak-to-peak amplitudes and latencies of the resulting averaged response were measured. TMT exposure produced a significant increase in the latency to hind paw lick, thus indicating impairment in the somatosensory system. The absence of TMT-induced alterations in conduction velocity and mixed nerve threshold suggested that the deficit was not due to peripheral nerve dysfunction. Analysis of the SER data revealed that TMT induced a statistically significant increase in P2 latency. The absence of alterations in late SER peaks, and the absence of any amplitude changes implies that the dysfunction reflects timing of somatosensory information. Whether this timing dysfunction results from impaired central conduction, synaptic function or synchronization will be addressed in future studies.

164.2 2-DEOXYGLUCOSE MAPPING OF THE GUINEA PIG AUDITORY PATHWAY USING STIMULI WHICH ELICIT TINNITUS IN HUMANS. J.S. Kauer, L.Babitz* J.W. Nemitz, C.T. Sasaki*. Sections of Neurosurgery, Neuroanatomy and Oto-Rhino-Laryngology. Yale University School of Medicine, New Haven, CT 06510.

We have used the 14C 2-deoxyglucose (2DG) technique to examine changes in metabolic activity in the auditory pathway of 52 guinea pigs after removal of the cochlea, removal of the middle ear ossicles, and after treatment with acetylsalicylic acid (aspirin). Three experiments were performed. In the first series we examined the levels of neuronal activity at 2½ hrs to 42 days after asceptic uni- and bilateral cochlear ablations. In this series we observed a spontaneous increase in activity which appeared at 2-14 days in those regions having major connections with the ablated cochlea when compared with the background levels of activity seen when tested immediately after surgery.

In the second series we performed asceptic uni- and bilateral removal of the middle ear ossicles. These manipulations, in contrast to cochlear removal, caused little or no spontaneous increase in activity on the side connected to the lesion when examined at either one day or 21 days. This suggests that the increased activity seen several days after cochlear removal was due to the process of deafferentation per se and not to the deafness which was caused by both the cochlear and ossicle removals. These findings are consistent with the possibility that the spontaneous increase in metabolic activity seen after cochlear removal may be a reflection of the subjective sensation of tinnitus experienced by humans after cochlear damage or VIII nerve section.

In the third experimental series we administered acetylsalicylic acid 30-50 mg/da for 3-7 days to animals which had had uni- or bilateral ossicle removals 9-21 days prior to 2DG testing. This experiment was designed to examine whether moderate levels of a salicylate known to produce tinnitus in humans, could elicit increased activity in those animals which would normally show no spontaneous return of neuronal activity after experiencing a conductive hearing loss. In the three animals examined to date under these conditions an increase in 2DG uptake was observed after aspirin administration. These preliminary data are consistent with the findings seen after cochlear ablation and further suggest that this increased activity may reflect a tinnitus-like phenomenon in this experimental animal. We believe the findings from these three sets of experiments may form the basis for the development of an experimental animal model for the study of a profound otologic problem about which there is virtually no pathophysiological information. Supported by the Deafness Research Foundation and USPHS grant NS 16288

164.3 DIFFERENTIAL EFFECT OF ISCHEMIA ON ACTIVE UPTAKE OF DOPAMINE, GABA AND GLUTAMATE. Jesse Weinberger* and Gerald Cohen. Dept. Neurology, Mount Sinai School of Medicine, N.Y., N.Y., 10029

Unilateral cerebral ischemia was induced in the Mongolian gerbil by left carotid ligation. Energy-dependent, active uptake of ^{3}H-dopamine, ^{3}H-GABA and ^{14}C-glutamate was measured in isolated synaptosomes as a paradigm of neuronal membrane function in ischemia. Synaptosomes were prepared by differential centrifugation in 0.3M sucrose. Uptake was carried out in Krebs-Ringer-phosphate buffer, pH 7.4, at concentrations of 5×10^{-9}M for dopamine and 8×10^{-6}M for GABA and glutamate. Synaptosomes were separated from the incubation medium by filtration through.65 micron Millipore filters for subsequent scintillation counting of radioactive tracer within the synaptosomes. The uptake at 10 min into the ischemic (Left) and control (Right) hemispheres was expressed as the L/R ratio.

Significant decrease in uptake did not occur until 16 hours after carotid ligation. No change was evident at 1, 4 and 8 hours. The extent of reduction of uptake at 16 hours is shown in the following table (mean ± SEM):

	Dopamine	GABA	Glutamate
Ischemic L/R Uptake	.152 ± .048	.280 ± .059	.475 ± .035
Sham Control L/R Uptake	.956 ± .063	.991 ± .095	.977 ± .068

The greater reduction in uptake of dopamine compared to glutamate was significant ($p < .001$).

Potassium stimulated release was evaluated by adding KCl to the medium to a final concentration of 60 mM and measuring the radioactive tracer remaining in the synaptosomes after 3 min. Release, expressed as a % of the amount taken up, was not affected even when uptake was markedly reduced. This indicates that uptake took place into normally functioning synaptosomes and that the decrease in uptake was probably due to loss of nerve terminals.

In separate experiments, uptake was studied in striata dissected from ischemic and control hemispheres to evaluate whether the difference between dopamine and glutamate was due to regional variation or intrinsic differences in the nerve terminals. The L/R uptake ratio in the striatum at 16 hours was .347 ± .140 for dopamine and .649 ± .142 for glutamate ($p < .02$).

These findings indicate that loss of active uptake of putative neurotransmitters after ischemia is a delayed process and that the dopaminergic synapse is more sensitive to ischemic damage than the glutamate synapse.

164.4 REDUCED SYMPATHETIC NERVOUS SYSTEM ACTIVITY IN RATS WITH DIETARY INDUCED OBESITY. B. E. Levin, J. T. Triscari*, M. B. Finnegan*, A. C. Sullivan*, Dept. Neurosci., N.J. Med. Sch., Newark, NJ 07103, VA Med. Ctr., E. Orange, N.J. 07019, and Dept. Pharm. II, Hoffmann-LaRoche Inc., Nutley, NJ 07110.

The sympathetic nervous system plays an important modulatory role in the control of many metabolic systems which function abnormally in obesity. The genetically obese Zucker rat has abnormal sympatho-adrenal function (Levin, B. E., et al., Pharm. Biochem. Behav. 13: 107, 1980), although it is uncertain if this is the cause or effect of obesity. We therefore fed 4-5 mo old, male Sprague-Dawley rats on a highly palatable diet consisting of rat chow admixed with 10% corn oil and condensed milk for 3 mo. These rats over-ate and developed diet-induced obesity (DIO) with a 97% greater weight gain than chow-fed controls. DIO was associated with a 31% increase in cardiac tyrosine hydroxylase activity and decreased cardiac (18%) and interscapular brown adipose tissue (27%) dopamine-β-hydroxylase activity. DIO rats also had reduced norepinephrine (NE) levels in their adrenals (25%), hearts (46%), aortas (68%) and epididymal white adipose tissue pads (38%). Turnover rates for NE, assessed by tyrosine hydroxylase inhibition with α methyl-p-tyrosine, were also significantly reduced in the hearts (73%), aortas (50%), pancreases (46%), and white adipose depots (51%) of DIO rats. No changes occurred in NE levels or turnover in brown adipose tissue of DIO rats, however. Therefore, while the mechanism is uncertain, DIO is associated with decreased peripheral sympathetic function which may contribute to the reduced ability of obese animals to expend calories as heat.

164.5 STRIATAL ^{3}H-SPIPERONE BINDING AND SPONTANEOUS PERIORAL MOVEMENTS IN THE RAT DURING AND AFTER WITHDRAWAL FROM 6 MONTHS PHENOTHIAZINE TREATMENT. John L. Waddington, Stephen J. Gamble* and Rachel C. Bourne*. Division of Psychiatry, M.R.C. Clinical Research Centre, Northwick Park Hospital, Harrow, Middlesex. HA1 3UJ, U.K.

It is commonly believed that the pathophysiology of 'tardive dyskinesia' resides in the functional supersensitivity of dopamine (DA) receptors in the basal ganglia induced by neuroleptic drugs. We have investigated the incidence of spontaneous perioral movements during and after withdrawal from 6 months continous phenothiazine treatment in rats and have sought to relate these phenomena to in vitro binding indices of DA receptor function.

Rats were treated with fluphenazine decanoate (FPZ-D) by i.m. injections or with trifluoperazine HCl (TPZ) via drinking water. After 6 months of FPZ-D injections (5 mgm at 2-3 week intervals), but not at 7 days after a single FPZ-D injection, there was an excess incidence of spontaneous jaw movements (70%) in comparison with controls (12.5%). Injections of FPZ-D were then terminated and over the next 6 month period these incidences ranged from 50-80% in FPZ-D animals and 17-50% in controls. Striatal ^{3}H-spiperone binding was unaltered after 6 months of withdrawal. After 6 months but not 1 week of oral TPZ treatment (3.8 mg/kg/day) there was a 40% incidence of such movements (16% in controls). At 2½ months after TPZ withdrawal the incidence was 83% (18% in controls) yet striatal ^{3}H-spiperone binding was unaltered.

6 months phenothiazine treatment was associated with an excess incidence of spontaneous perioral movements, enhancing a pre-existing disposition. The pathophysiology did not appear to involve those DA receptors exemplified by striatal ^{3}H-spiperone binding.

164.6 PHENYLKETONURIA (PKU), TETRAHYDROISOQUINOLINES (TIQS) AND NEUROLOGICAL DAMAGE. M. Druse-Manteuffel, M.A. Collins, D.Tonetti*, C.Waddell* & P.Patel*. Dept. Biochem. & Biophys., Loyola Med.School, Maywood, IL 60153

Recently Lasala & Coscia(Sci.203:283,1979)reported that the TIQ, desoxynorlaudanosoline-1-carboxylate (DNLCA), an endogenous product of dopamine and phenylpyruvate, was elevated in brains of adult hyperphenylalaninemic rats and in urines of children with PKU. Based on DNLCA distribution and enzyme inhibitory studies, a neuropathological role for the TIQ was implied. To test this idea we examined the effects of DNLCA on myelin proteins and the biogenic amines in the brains of normal neonatal rats.

DNLCA(in sterile saline)was given intracerebrally to rats on days 6(20 µg), 8, 11, 14 & 16(10 µg/day). Controls received saline only. On day 18, rat pups were decapitated. Some brains were used for the preparation of purified myelin and separation of myelin proteins by SDS polyacrylamide gel electrophoresis(Druse et al. Brain Res.76:423,1974). Other brains were used to quantitate brain region content of biogenic amines by reverse phase HPLC(with electrochemical detection). Repeated injections of DNLCA produced a slight (6%) reduction in brain weight and did not affect brain protein content or the composition of myelin proteins. However, DNLCA exhibited a potent and perhaps selective toxicity toward the serotonin(5-HT)system and was found to be toxic to CNS myelination. Both the content and concentration of myelin protein were significantly reduced (41% & 39%, respectively). Levels of 5-HT in the striata of TIQ-treated 18-day-old pups were ∿80% below normal, but dopamine and DOPAC levels were unchanged. This effect on brain 5-HT resembles that seen earlier with another carboxylated TIQ (Hannigan & Collins, Drug Alc.Depend.4:235,1979). It is well-known that the two processes, myelination and biogenic amine dynamics, are disrupted in the brain during PKU. Several other phenylalanine metabolites (i.e. phenylacetate) have toxic effects on myelination, but effects on the biogenic amines are not prominent. Our data in neonatal rats indicate that a more complex metabolite, a TIQ alkaloid, has selective actions on both processes which certainly could be important in the neuropathology of PKU.

We thank Dr. Coscia for the DNLCA. Supported by Loyola BRSG and USPHS.

164.7 PLATELET 5-HYDROXYTRYPTAMINE CONCENTRATIONS IN PSYCHIATRIC PATIENTS AND CONTROL SUBJECTS. H.L. Jackman and H.Y. Meltzer, Dept. of Psychiatry, University of Chicago and the Illinois State Psychiatric Institute, Chicago, Illinois.

The role of 5-hydroxytryptamine (5-HT) in the etiology of schizophrenia and the affective disorders has been studied by examining 5-HT in blood platelets. Several investigators have reported increased 5-HT levels in platelets from schizophrenic patients. In this preliminary study, we report the concentration of 5-HT in platelets from 59 psychiatric patients and 30 control subjects.

Five ml of venous blood from controls or patients was put into polypropylene tubes containing ACD anti-coagulant. Platelet-rich-plasma (PRP) was prepared by centrifugation at 600 g for 2.5 minutes and the number of platelets per ml of PRP was determined electronically. A platelet pellet was formed by centrifugation, frozen at -40°C, and assayed within two weeks. The platelet 5-HT was assayed spectrofluorometrically and reported as ng 5-HT per 10^8 platelets (U). Patient diagnoses were established by the Research Diagnostic Criteria.

The number of patients in each diagnostic group and their mean platelet 5-HT concentration ± SD are as follows: 11 paranoid schizophrenics, 91 ± 38 U; 7 undifferentiated schizophrenics, 87 ± 39 U; 5 schizoaffective depressed (mainly schizophrenic), 95 ± 13 U; 8 unipolar depressed, 53 ± 13 U; 12 bipolar depressed, 62 ± 27 U; 7 schizoaffective depressed (mainly affective), 62 ± 23 U; 5 bipolar manics, 58 ± 17 U; 4 schizoaffective manics (mainly affective), 82 ± 28 U. The concentration of 5-HT in platelets from 30 control subjects was 62 ± 16 U. A two way ANOVA was performed on the data and revealed a significant diagnostic effect; paranoid schizophrenics have a significantly higher platelet 5-HT concentration than controls ($P < .01$). The 5-HT in platelets from schizoaffective depressed (mainly schizophrenic) and undifferentiated schizophrenics also tended towards higher concentrations than that found in platelets from controls. No sex effect or interaction between sex and 5-HT content was detected. Neuroleptic treatment produced no significant effect on platelet 5-HT levels.

While a great deal of overlap in platelet 5-HT concentration values exists, our data is in agreement with previous findings of a greater than normal concentration of 5-HT in platelets from some schizophrenic patients. This finding supports the hypothesis that an abnormality in 5-HT metabolism may contribute to the pathophysiology of schizophrenia.

Supported in part by USPHS MH 25116 and MH 30938.

164.8 ^{3}H-GABA BINDING IN GALACTOSAMINE-HCl INDUCED HEPATIC ENCEPHALOPATY IN RAT: A DENERVATION SUPERSENSITIVITY PHENOMENON. M.Baraldi*, M. L. Zeneroli*. (SPON:F VARGAS). Istituti di Farmacologia e di Semeiotica Medica°, Modena University, 41100 Modena, Italy.

Fulminant hepatic failure (FHF), characterized by massive necrosis of the liver, is associated with unclear brain changes resulting in hepatic encephalopathy (HE). Our approach to study HE was to investigate GABA receptor characteristics. FHF was obtained in rats by the injection of Galactosamine-HCl and the degree of HE was evaluated with visual evoked potentials. The kinetics of Na^+-independent ^{3}H-GABA binding of fresh or frozen three times Triton X-100 treated P_2 membranes from brain of controls and comatose rats were studied. Scatchard analysis of the saturation curve in freshly prepared membranes revealed only one receptor with low affinity for GABA ($GABA_1$) in both situations; hovewer the affinity of GABA to membranes from comatose rats (K_d=122±3 nM) was significantly increased in comparison with that of controls (K_d=193±7 nM). In frozen, Triton treated and repeatedly washed membranes, two receptor components, one ($GABA_1$) with low affinity (K_d=192 nM, B_{max}=7.6 pmol/mg prot.) and one ($GABA_2$) with high affinity (K_d=20 nM, B_{max}=2.1 pmol/mg prot.) were found in control while in membranes from brain with HE only the high affinity site ($GABA_2$) was present (K_d=25 nM, B_{max}=1.8 pmol/mg prot.). This result was confirmed performing the same ^{3}H-GABA binding studies in different brain areas.

The relative increased affinity found in the freshly prepared membranes from rats with HE could be interpreted as a supersensitivity phenomenon due to a degeneration of Gabaergic neurons associated with a decrease of endogenous inhibitors of GABA binding. Hovewer it is impossible to completely evaluate this phenomenon in fresh membranes because of the still present endogenous GABA and endogenous protein inhibitor (GABA modulin). The treatment with Triton seems to completely remove the endogenous inhibitor from the HE membranes and to leave only the high affinity binding sites.

The present study provides evidence that $GABA_1$ and $GABA_2$ receptors are two different entities and that the mechanism of HE due to FHF could be related to an induced $GABA_1$ receptor degeneration.

164.9 PROLINE-LEUCINE-GLYCINAMIDE (PLG) PREVENTS NEUROLEPTIC DRUG-INDUCED DOPAMINE RECEPTOR SUPERSENSITIVITY IN STRIATUM. Ram K. Mishra*, S. Chiu* and C.S. Paulose*, Neuropharmacology Lab., Depts. of Psychiatry and Neurosciences, McMaster Univ., Hamilton, Ontario, L8N 3Z5, Canada. (SPON.: E. Werstiuk).

The potential anti-Parkinsonian and antidyskinetic properties of PLG have recently been recognized. There is evidence in the literature suggesting that neuropharmacological profile of activity of PLG may be mediated through interacting with central dopamine receptors. In the present study we have examined the effect of PLG on neuroleptic drug-induced supersensitivity in rats. Various groups of animals received: PLG (10 mg/kg), Haloperidol (3 mg/kg), Chlorpromazine (20 mg/kg), Haloperidol + PLG, and Chlorpromazine + PLG. The control group received the vehicle solution. The drugs were administered subcutaneously for 3 weeks. After 5 days of last injection, the animals were sacrificed and striata dissected out for ^{3}H-spiroperidol binding assay. The spiroperidol binding to tissue preparation was carried out as previously described (Brain Res. 170, 381, 1979). As reported earlier, neuroleptic drugs increased the spiroperidol binding sites significantly in the striatum, Haloperidol caused a mean increase of 58% in the B_{max} whereas chlorpromazine produced a mean increase of 67%. PLG at two different doses (10 mg/kg and 20 mg/kg) prevented neuroleptic drug induced increase in spiroperidol binding sites. PLG by itself had no significant effect on binding sites. These results suggest an interaction of specific PLG receptors with dopaminergic mechanisms in the CNS and may explain the beneficial effect of PLG in tardive dyskinesia. We have recently reported the presence of specific PLG receptors in various discrete regions of brain tissue obtained from several mammalian species including humans (Fed. Proc. 39, 625, 1980). The interaction between PLG and neuroleptic dopamine receptors and modulation of dopaminergic system by PLG remains an interesting topic for future research. (This work was supported by the Ontario Mental Health Foundation).

164.10 TETRABENAZINE MODEL OF DEPRESSION: CHANGES IN CEREBRAL FLUID DYNAMICS AND REVERSAL BY AMITRIPTYLINE. T. Kent*, S. Preskorn, R. Glotzbach*, and G. Irwin*. Depts.of Psychiatry & Pharmacology, Univ. of Kansas Sch. of Med., Kansas City, KS 66103.

The central adrenergic vasoregulatory hypothesis postulates that this neuronal system has as one of its functions the regulation of brain permeability (PS) and blood flow (CBF). We found that the blood:brain barrier becomes more permeable to compounds, such as water, at high $PaCO_2$. This finding is consistent with the hypothesis: high $PaCO_2$ stimulates an increase in the firing rate of central adrenergic neurons which should lead to the observed increase in PS. Moreover, treatment with amitriptyline(AMI)--an indirect acting adrenergic agonist--doubled the increase in PS observed at high $PaCO_2$ (Table I).

In this experiment, the effect of tetrabenazine (TBZ)--a catecholamine depleting agent--was studied on the increase in PS observed with increasing CBF. Using a dual label radioistope technique previously described, changes in the cerebral extraction of ^{3}H-water (E_w), brain permeability to water (P_wS), and CBF were measured in rats. The animals were anesthetized, paralyzed, and passively ventilated.

TBZ is a standard screen for potential antidepressant drugs. 20mg/kg of TBZ administered (i.p.) produces autonomic and behavioral changes. These effects occur within 45 mins., persist for over 3 hours, and coincide with drug-induced depletion of central catecholamines. Reversal of these effects is a predictor of antidepressant activity in man. In our hands, these behavioral effects were produced by TBZ and were reversed by AMI. We tested the effect of TBZ on the apparent adrenergic-mediated, $PaCO_2$-induced increase in PwS. (Note: The effect of experimental treatments can be expressed either as a change in the slope of PwS vs. CBF or vs. $PaCO_2$.) At 45 min., coincident with maximal central catecholamine depletion, TBZ essentially abolished the $PaCO_2$-induced increase in PwS (Table I). AMI reversed the effect of TBZ (Table I).

Group	PwS versus CBF	r	n	Dose
Controls	$.8\bar{x}+0.28.$	.98	3	
*Controls	$.9\bar{x}+0.34$	.98	12	
*AMI	$1.83\bar{x}+0.01$	.96	15	17.5mg/kg i.p.
TBZ	$.24\bar{x}+1.67$	.65	13	20mg/kg i.p.
TBZ+AMI	$.76\bar{x}+1.03$	.87	17	

*from Preskorn, et al., Sci. 1981

These results support the central adrenergic vasoregulatory hypothesis. Moreover, a functional change occurred in central adrenergic innervation of the blood:brain barrier in this pharmacological model of affective illness--a disease long thought to be related to disturbances in central adrenergic mechanisms. *(Supported by MH-00272 and NS-17252).

164.11 IN VIVO ASSESSMENT OF PRESYNAPTIC ADRENERGIC α-2 RECEPTOR FUNCTION IN RHESUS MONKEYS AND HUMANS. D. S. Charney*, G. R. Heninger, D. S. Sternberg*, and R. H. Roth. Yale University School of Medicine, New Haven, CT 06508.

There is considerable evidence that presynaptic α-2 adrenergic receptors regulate the release of norepinephrine (NE) through negative feedback mechanisms. The present investigation was undertaken to develop methods for assessing in vivo the sensitivity of this receptor because of the potential for providing important new information on the regulation of the NE system in intact experimental animals and humans. Alpha-2 adrenergic antagonists and agonists increase and decrease, respectively, brain NE turnover and 3-methoxy-4-hydroxyphenelethyleneglycol (MHPG) production. Plasma-free MHPG had been found to reflect brain MHPG in a variety of experimental situations (Elsworth, J.D. et al, Neuroscience Abstracts 6:140, 1980). In the present study, the effect on plasma-free MHPG of an α-2 adrenergic antagonist (yohimbine) and agonist (clonidine) were studied in rhesus monkeys, normal humans, and in depressed psychiatric patients. Plasma was obtained from blood samples drawn through an indwelling intravenous catheter, both before and at a variety of time periods following drug administration. Plasma MHPG was determined by gas chromatography and mass spectrometry. In four rhesus monkeys tested twice, clonidine (3 μg/kg IV over 5 min.) reduced plasma MHPG by 36% ($P < .01$), and yohimbine (1 mg/kg IV over 5 min.) increased MHPG by 78% ($P < .01$) with the maximum effect for both drugs occurring 2 hrs. following the dose. The mean difference between the 1st and 2nd test in each monkey was less than 1/3 the mean change produced by either yohimbine or clonidine. In 12 normal human volunteers, clonidine (5 μg/kg p.o.) decreased plasma MHPG by 14% ($P < .05$) and in five other volunteers, yohimbine (10 mg p.o.) increased plasma MHPG by 31% ($P < .01$), with the maximum effect for both drugs occurring 4 hrs. following administration. Clonidine (5 μg/kg p.o.) was administered to 15 depressed patients before and during chronic treatment with desipramine (N = 10) and amitriptyline (N = 5). Desipramine, but not amitriptyline, significantly attenuated the effect of clonidine on plasma MHPG and blood pressure. This is consistent with findings in laboratory rats, where chronic desipramine, but not amitriptyline attenuates the effects of clonidine on brain MHPG. These results indicate that this method could be of considerable utility in the study of adrenergic α-2 receptor regulation of the NE system in neurologic and psychiatric illness. (Supported by MH-18949, MH-25642, and MH-30929).

164.12 BIOCHEMICAL DEMONSTRATION OF REDUCED DOPAMINE TURNOVER WITH LOW-DOSE APOMORPHINE IN SCHIZOPHRENIC PATIENTS.
N.R. Cutler,* D.V. Jeste,* F. Karoum, N.H. Kalin,* S.C. Risch,* and R.J. Wyatt, Adult Psychiatry Branch, National Institute of Mental Health, Washington, D.C. 20032.

Apomorphine, a partial dopamine agonist, has been previously demonstrated to improve psychotic symptoms in some chronic schizophrenic patients (Tamminga, C.A. et al., Science 200: 567, 1978). The antipsychotic effect of apomorphine has been attributed to a reduction in central dopamine turnover through an autoreceptor action. Yet, to our knowledge, there has been no direct demonstration in man of such an hypothesized reduction in dopamine turnover with low-dose apomorphine. In a double-blind study, we evaluated behavioral, biochemical, and neuroendocrine effects of apomorphine, 0.005 mg/kg, in schizophrenic patients.

METHODS: Five young chronic schizophrenic patients, three males and two females, diagnosed by the Research Diagnostic Criteria, participated. Subjects had been maintained on stable doses of neuroleptics (mean daily dose equivalent to 1067.5 mg of chlorpromazine) for at least one month. Subjects were given apomorphine and placebo subcutaneously on two different days, in a random order. All subjects were at bedrest the night before and during the study. Brief Psychiatric Rating Scale (BPRS) Score, temperature, and blood pressure were assessed and blood samples collected 20 minutes before the injection and every 20 minutes thereafter for a total of two hours.

RESULTS: Analysis of the total BPRS scores showed marked but temporary clinical improvement in two patients. Overall, the most significant changes were noted in ratings of depression and anxiety. Plasma homovanillic acid (HVA) concentrations, measured by mass fragmentography, showed significant decrease following apomorphine as compared to placebo ($t=5.1$; $p<.009$). All five patients had reduced plasma HVA concentrations as early as 20 minutes following apomorphine; in some patients the plasma HVA concentrations had not returned to their baseline values, even at 100 minutes. Plasma cortisol and human growth hormone concentrations did not change significantly.

CONCLUSIONS: A small dose of apomorphine (.005 mg/kg) given to medicated schizophrenic patients produced a significant drop in plasma HVA concentrations. Studies by Bacopoulos, N.G. et al. (Eur. J. Pharmacol. 56: 225, 1979) and Swann, et al. (Life Sci. 27: 1857, 1980) suggest that drug-induced changes in plasma concentrations of dopamine metabolites reflect changes in dopamine metabolism. Our findings, therefore, indicate that a low dose of apomorphine significantly reduces dopamine turnover in schizophrenic patients. This is consistent with the postulated action of low-dose apomorphine on dopaminergic autoreceptors. Further implications of our findings will be discussed.

164.13 EFFECTS OF CHRONIC HYPERTENSION ON ALPHA-ADRENERGIC, SEROTONERGIC AND HISTAMINERGIC RECEPTORS IN ISOLATED CEREBRAL ARTERIES. C. Estrada*, M.V. Conde*, B. Gomez*, and S. Lluch*, (SPON: R.E. McCaman), Department of Physiology, Fac. de Medicina, Universidad Autonoma, Madrid, Spain.

Direct measurements of cerebral blood flow in unanesthetized chronic hypertensive goats have shown an increase in cerebrovascular resistance during the hypertensive state. In order to study the mechanisms underlying this phenomenon, the responsiveness to vasoconstrictor agents acting on different vascular receptors was tested in isolated cerebral vessels from normotensive and hypertensive goats. Chronic hypertension was induced by unilateral renal artery constriction. Values for mean arterial pressure were 102±2 mmHg in normotensive goats and 136±3 mmHg in hypertensive ones. The middle cerebral arteries from 20 normotensive and 7 hypertensive goats were dissected out and isometric contraction of 5 mm length segments was measured in a standard way. Cumulative concentration-response curves for norepinephrine (NE, 10^{-8} - 10^{-4} M), tyramine (TYR, 10^{-6} - 10^{-3} M), serotonin (5HT, 10^{-8} - 3×10^{-6} M), and histamine (HIS, 10^{-8} - 3×10^{-5} M) which were obtained for arteries from both groups of animals were compared. The maximum effect (E_{max}) of NE and TYR was increased in vessels from hypertensive goats (150% of normotensive control), but the EC_{50} values were unchanged. The concentration-response curve to 5HT was shifted to the left in arteries from hypertensive animals, indicating an increase in sensitivity (EC_{50}, 3×10^{-7} M in normotensive goats versus 10^{-7} M in hypertensive ones), but the E_{max} was not significantly different. The responsiveness to HIS was similar in arteries from both groups of animals. The increase in response to NE and 5HT, transmitters thought to be present in nerve endings in the cerebral vessels, may explain the increased cerebrovascular resistance seen during chronic hypertension. The responses obtained after stimulation of alpha-adrenergic, serotonergic and histaminergic receptors were altered in different ways during hypertension. This suggests that the increased cerebrovascular responsiveness involves specific changes at the receptor level. Supported in part by Comisión Asesora Cientifica y Técnica.

164.14 PLATELET ALPHA-2 RECEPTOR BINDING BY ^{3}H-YOHIMBINE IN NORMAL HUMAN VOLUNTEERS AND PSYCHIATRIC PATIENTS. S.M. Stahl, D.J. Woo*, P.A. Berger and R.D. Ciaranello. Lab. of Developmental Neurochemistry, Stanford U. Med. Ctr., Stanford, CA. 94305

Alpha-2 adrenergic receptors on intact human platelets were characterized by ^{3}H-yohimbine, a potent alpha-2 adrenergic antagonist. ^{3}H-yohimbine binds specifically and with high affinity to alpha-2 adrenergic receptors of human platelets. We have adapted a rapid and reliable method for determining K_D and B_{MAX} of platelet alpha-2 receptors in 30 ml of blood from human donors. This method employs intact human platelets rather than platelet membranes, as well as the Corrash method for isolating the full population of intact platelets. ^{3}H-yohimbine had the same binding characteristics in Corrash-isolated platelets as in platelets isolated after a single centrifugation. Total binding was 2-5% of total ^{3}H-yohimbine added. Non-specific binding using phentolamine (1.5×10^{-6}M) varied between 10-20% of total binding. The association and dissociation kinetics were rapid and linear at 30°, and the binding is of a single order with the k_{-1}/k from pooled normal human volunteers of 0.51 nmolar. Tissue binding curves showed that at least 1.5×10^8 total platelets per tube were needed for linear kinetics.

^{3}H-yohimbine binding displaced by phentolamine was determined in 10 medicated and 12 unmedicated patients of various psychiatric diagnoses, and in 14 normal volunteers. Scatchard analysis showed that K_D for ^{3}H-yohimbine binding in normal human volunteers was 2.00 ± 0.49 nM and B_{MAX} was 107 ± 42 binding sites per platelet. The results of K_D and B_{MAX} values as a function of psychiatric diagnosis and psychotropic medication will be discussed.

165.1 CORRELATION OF FIRING THRESHOLD TO MEMBRANE POTENTIAL IN SCIATIC AXONS. Kenneth J. McLeod and Stephen A. Raymond. Research Laboratory of Electronics, MIT, Cambridge, MA 02139.

The three phase variation in excitability that follows impulses in axons (Raymond, J. Physiol. 290:273, 1979) was compared to intracellularly recorded afterpotentials to ascertain the relationship between membrane potential and firing threshold.

Action potentials and afterpotentials were recorded in excised sciatic nerve of frog using silanized microelectrodes. Axons selected for study maintained a resting potential of at least -65 mV for a period of at least one hour, and produced action potentials of at least 70 mV peak amplitude.

Following impulse bursts of 1 to 1000 spikes at 5 ms intervals all axons exhibited a brief afterhyperpolarization of 3 - 15 mV. Short bursts were marked by a subsequent depolarizing wave that decayed over 200-500 ms. A lingering hyperpolarization could be seen after this wave that was larger and persisted longer in proportion to the duration of the conditioning burst. Halftimes for this hyperpolarization, PTH, depended strongly on the activity, typically ranging from 0.5 sec after bursts of 16 impulses to 2 min. after bursts of 1000 impulses at 5 ms intervals. The total time course of the afterpotential variations in each axon corresponded closely to the total time of the combined refractory, superexcitable and depressed phases for any given stimulus pattern as measured concurrently using threshold hunting. Membrane voltage changes of less than 1 mV were observed under conditions where threshold had changed by a factor of 2 or more. Superexcitability was consistently seen during the first few hundred ms following a spike or a burst whether or not the membrane was hyperpolarized. After short bursts or single spikes the depolarizing wave resulted in a net depolarization. As conditioning activity was increased, the wave appeared on top of a more and more dominant PTH. After a very long tetanus the depolarizing wave could not be seen in the superexcitable phase.

These results are consistent with the idea that superexcitability is not mediated by the membrane potential but by the direct influence of the raised extracellular K concentration immediately following activity. Depression appears to be strongly correlated to intracellular loading with Na.

Additional experiments using steady depolarizing and hyperpolarizing currents indicated that resting threshold levels are logarithmically related to the membrane potential. This observation and the results showing the dependence of threshold on ion concentration suggest that firing threshold may be mediated by a surface reaction.

165.2 A MECHANISTIC INTERPRETATION OF INTERMITTENT RESPONSIVENESS TO REPETITIVE STIMULATION OF MYELINATED FIBERS. Stephen A. Raymond and Dinah Sah*. Research Laboratory of Electronics, MIT, Cambridge, MA 02139.

Studies of the relation between impulse activity and threshold have shown why fibers subjected to long trains of repetitive electrical stimuli respond in bursts and gaps ('intermittent responsiveness'), but the ionic and molecular basis of the changes in threshold produced by activity remains obscure. The following hypothesis has been useful in efforts to find a mechanistic interpretation of the empirical relations: superexcitability is mainly caused by the aftereffects of an impulse on external K, and depression is proportional to the rate of sodium pumping.

Here we mention several findings bearing on this hypothesis. Diffusion of K away from the external surface of nodal membrane was modeled by assuming that the myelin sheath is impermeable to K and that a net 7.5×10^{-13} mmoles of K are exchanged per impulse. We assumed that the diffusion constant for K in the matrix is that in H_2O. Kinetics of K concentration taken at the membrane surface showed a high correspondence in relative amplitude and time course to the superexcitability following single spikes and bursts. Both build up over bursts and show a significant increase that lasts over 1 s.

Longitudinal diffusion within the axon of Na accumulating at the node was also calculated. For the 2 sigma boundary of excess post-impulse Na to extend through the internode (1000 μ) would require more than 10 minutes. Recovery from depression is known to be prolonged by extending the duration of activity-induced depression (Raymond, J. Physiol. 290, p. 294, 1979). We suggest the prolongation occurs because the sodium pump current remains augmented while pump sites located at the nodes extract the extra Na that has diffused into the internodes during the long exposures to repetitive activity. Cohen (Neurosci. 6: 301, 1980) has found that at any given firing rate fibers having the greater degree of depression appear to have a greater rate of sodium pumping. One expects such fibers to exchange more quickly the Na accumulated during a burst and to show a faster recovery from depression. We analyzed oscillatory firing patterns during intermittent responsiveness in frog axons, and found that more depressible fibers showed more strongly periodic response with briefer recoveries.

Though the diffusion kinetics match superexcitability nicely, the present experiments do not indicate that the diffusing substance is K. Afterpotentials change less dramatically than the threshold (McLeod, these proceedings) and do not have a Nernstian relation to K ratios. As suggested by R. Grossman (J. Physiol. 295: 307, 1979), K may have a direct effect on excitability without altering membrane voltage.

165.3 PHASE LOCKING, PERIOD DOUBLING BIFURCATIONS AND CHAOS IN PERIODICALLY DRIVEN NEURAL AND CARDIAC OSCILLATORS. Leon Glass* and Michael R. Guevara*. Department of Physiology, McGill University, 3655 Drummond St., Montreal, Quebec H3G 1Y6.

A simple mathematical model for the periodic pulsatile stimulation of spontaneously active neural and cardiac oscillators displays rich dynamics as a function of the frequency and amplitude of the periodic stimulation. The autonomous oscillator may become phase locked to the periodic stimulus in a variety of regular periodic patterns (i.e., 1:1, 3:2, 4:3, 4:2, 2:2, where N:M represents N periodic stimuli to M action potentials of the driven oscillator). In addition apparently aperiodic patterns which are not phase locked are found in numerical simulations of the model system. The irregular aperiodic dynamics observed in the numerical studies are analogous to "chaotic" dynamics observed in finite difference equations (Li, T.-Y, Yorke, J.A., Am. Math. Monthly,82:985, 1975; May, R.M., Nature, 261:459, 1976). Over limited ranges of stimulus amplitude one observes the following sequence of phase locking patterns as the stimulation frequency is increased 1:1, 2:2, 4:4, ..."chaos". This sequence arises as a consequence of period doubling bifurcations similar to those observed in physical systems (Physics Today, March, 1981, p. 17). The implications of this work for the experimental and clinical observation of period doubling bifurcations and chaos will be discussed. (Supported by the National Science and Engineering Research Council of Canada and the Canadian Heart Association).

165.4 SUPRACHIASMATIC NUCLEUS ABLATION: EFFECTS ON RAT BRAIN NEUROTRANSMITTER RECEPTOR RHYTHMS. M. S. Kafka, R. Y. Moore and P. J. Marangos. NIMH, Bethesda, MD 20205 and Dept. of Neurology, Sch. of Med., SUNY, Stonybrook, NY 11794

Circadian rhythms in the number of α-adrenergic, β-adrenergic, muscarinic acetylcholine (ACh), and benzodiazepine (BDZ) receptors have been demonstrated in the brains of rats entrained to a light:dark cycle (12:12). The rhythms (measured October, 1979) persisted after 48-72 hours in continuous darkness (Kafka et al., Brain Res. 207: 409-419 [1981]). As the suprachiasmatic nuclei (SCN) of the hypothalamus appear to regulate a number of circadian rhythms in the rat (Moore, Frontiers in Neuroendocrinology 5: 185-206 [1978]), the effect on the receptor rhythms of suprachiasmatic nucleus ablation was studied (June, 1980).

SCN lesions were made electrolytically and the rats permitted to recover for 17 days in a controlled light:dark cycle (L:D, 12:12). After 36 h in continuous darkness (D:D) 8 lesioned rats and 8 sham-operated controls were sacrificed at 4-hour intervals over a 24-hour period. Forebrains (all brain rostral to the cerebellum except striatum) were dissected and quickly frozen. The number of α-receptors was measured in these brains by the specific binding of [^{3}H]WB4101; β-receptors, by [^{3}H]dihydroalprenolol; ACh receptors, by [^{3}H]QNB; and BDZ receptors, by [^{3}H]diazepam.

The 24-hour mean number of each receptor was unaltered by ablation of the SCN suggesting that the SCN do not regulate the total number of a receptor, but only the diurnal changes in its magnitude. Controls exhibited an apparent circadian rhythm (i.e., with a period of approximately 24-hours) in BDZ receptor and α-receptor number, whereas the rhythms in β-receptor and ACh receptor number appeared ultradian (i.e., with a period less than 24 hours), the periods of each approximating 12 hours. Only control BDZ receptor and α-receptor rhythms (D:D) were circadian in both October and June. The β-receptor and ACh receptor rhythms in brains from rats with SCN lesions were similar to those in brains from controls. In contrast, the rhythms in BDZ receptors and α-receptors in brains from rats with SCN lesions were distinctly different from rhythms in controls. The circadian rhythm in BDZ receptor number was either markedly damped or absent, and the circadian rhythm in α-receptor number was clearly absent, with ultradian rhythms manifest in the number of both receptors. In this study, SCN lesions appear to affect selectively rhythms in brain receptor numbers that are clearly circadian in control animals, whereas rhythms in receptors that are ultradian in brains of controls are unaffected by SCN lesions.

165.5 ACTIVITIES OF INHIBITORY INTERNEURONES IN FICTITIOUS SCRATCHING. R. Cueva-Rolón* and E. J. Muñoz-Martínez. Dept. Physiol. Facultad de Medicina. Universidad de Guadalajara. Guadajalara, Jal. MEXICO and Dept. Physiol. & Biophys. Centro de Investigación y de Estudios Avanzados. Apdo. Post. 14-740, México 14, D.F. MEXICO. (SPON: G. Massieu).

Motoneurones activities during the scratching reflex begin with a prolonged flexor discharge followed by cycles of discharges of the flexor and the antagonistic extensor motoneurones. Flexor activity coincides with extensor inactivity and vice versa. In the present experiments we investigated: a) whether the initial event in the generation of scratching activity consists of selective flexor excitation or whether the extensor motoneurones are also subliminally excised, b) whether the periods of inactivity result from deactivation or from inhibition and c) whether Renshaw cells or Ia inhibitory interneurones participate to determine the inactivity periods.

Fictitious scratching was produced in descerebrated cats. Electroneurograms were recorded from the tibialis anticus and from a filament of the medial gastrocnemious nerve. In some experiments recordings were taken from spinal neurons. Reflex excitability of motoneurones was tested by stimulation of the deep peroneal and the triceps surae nerves. Also, extensor neurones were subliminally activated in some experiments by passive stretch of the triceps surae muscles.

Our results showed that extensor motoneurones were subliminally activated before the initial flexor discharge and always inhibited during the periods of flexor activity. Correspondingly, flexor motoneurones were inhibited during the periods of extensor activation. Intracelullar recordings revealed membrane hyperpolarizations during the inhibition periods. Antidromic stimulation of flexor and extensor motor axons did not significantly changed the scratching discharges; thus, Renshaw cells do not apparently contribute to the generation of rhythmic discharges. Passive stretch of the extensor muscles may reduce the duration of the flexor discharges or supress the fictitious scratching; similar effects were obtained by electric stimulation of low threshold muscle afferents. Stimulation of cutaneous afferents caused prolonged flexor discharges and disappearance of extensor activity. Our results indicate that Ia inhibitory interneurons participate in the generation of scratching reflex.

165.6 EFFECT OF DIBUTYRYL CYCLIC-AMP ON ELECTRICAL EXCITABILITY, $^{45}Ca^{2+}$-UPTAKE AND β-ENDORPHIN SECRETION FROM AtT-20/D16-16 PITUITARY CELLS. M. Adler, S. Sabol*, M. Jackson*, N. Busis and M. Nirenberg. Lab. Biochem. Genetics, NHLBI, Lab. of Biophys., NINCDS, NIH, Bethesda, MD 20205, and Lab. of Preclinical Studies, NIAAA, Rockville, MD 20852.

We have recently shown that clonal AtT-20/D16-16 (D16) mouse anterior pituitary cells can generate Na^+ and Ca^{2+} action potentials and secrete β-endorphin and ACTH. Although all well-impaled control cells were electrically excitable, many cells were injured by microelectrode penetration (3 M KCl, 40-80 M Ω). This was indicated by findings of low and unstable resting potentials. Spontaneous action potentials were rarely observed with untreated D16 cells, but were found frequently with cells exposed to 1 mM dibutyryl cyclic-AMP (Bt_2cAMP) for $\geq$ 9 days (treated). Since treatment of D16 cells with Bt_2cAMP also resulted in increases in the resting potential, input resistance, and cell volume, the higher incidence of spontaneous activity in treated cells could reflect either a reduction in microelectrode-induced damage or a change in ion channel properties. To distinguish between these possibilities, experiments were performed using extracellular microsuction pipettes (3-5 M Ω). With extracellular recordings, 19 of 20 Bt_2cAMP-treated cells and an almost equal number of control cells (18 of 20) were spontaneously active. The spontaneous action potentials were abolished in control cells when intracellular impalement was attempted, whereas in treated cells spontaneous activity persisted after impalement. The effects of Bt_2cAMP also were examined on $^{45}Ca^{2+}$ uptake and β-endorphin secretion from D16 cells. In both control and treated cells $^{45}Ca^{2+}$ influx increased rapidly when the K^+ concentration was changed from 5 to 80 mM and attained equilibrium within 30 sec. K^+ dependent $^{45}Ca^{2+}$ uptake by control and Bt_2cAMP-treated cells were 2.0 and 0.9 nmol/0.5 min/mg protein, respectively. β-Endorphin and β-lipotropin secretion was determined by radioimmunoassay employing an antiserum against β-endorphin. Rates of secretion of immunoreactive β-endorphin were 0.60 and 0.23 pmoles/min/mg protein from control and 21-day treated cells, respectively, in medium containing 5.4 mM KCl. Upon addition of 80 mM KCl, the rate of release increased 33-fold in control cells during the first two min of exposure, then returned to the basal rate. Treated cells underwent a 23-fold depolarization-dependent increase in β-endorphin secretion before returning to the basal rate. These results indicate that Bt_2cAMP treatment of D16 cells depresses $^{45}Ca^{2+}$ uptake and hormone secretion but does not alter specific ion conductances.

165.7 DOSE-RELATED EFFECTS OF INTRACELLULAR CYCLIC ADENOSINE-3':5'-MONOPHOSPHATE INJECTIONS IN IDENTIFIABLE ARCHIDORIS NEURONS. P.E. Hockberger and J.A. Connor. Dept. of Physiol. & Biophys., and Neural & Behav. Biol. Program, Univ. of Illinois, Urbana, IL 61801.

Liberman et. al. (Biofizika 20(3):451, 1975) reported that iontophoretic injection of cyclic adenosine-3',5'-monophosphate (cyclic AMP) into unidentified Helix neurons resulted in a transient depolarization in a considerable number of cells. In addition they noticed that this response was sometimes followed by "packets of pulses"-i.e. bursts of action potentials, in otherwise silent neurons. We report here that iontophoretic or pressure injections of cyclic AMP into members of a group of fourteen identifiable Archidoris neurons resulted in similar membrane potential changes. Injections of cyclic AMP into 131 cells elicited an instantaneous depolarization in 130 with subsequent bursting in 37 of these cells. Comparable injections of adenosine-5'-monophosphate (5'-AMP) were without effect in 15 of 17 cells.

Although these responses were independent of cell type, they appeared to be related to the amount of cyclic AMP injected. Iontophoretic injections lasting several minutes often transformed a transient depolarizing response into a sustained depolarization. After a delay of approximately ten to fifteen minutes, this sustained depolarization frequently included bursts of action potentials. Such bursts were preceded by a sudden, progressive increase in size of subthreshold membrane oscillations. Bursting and oscillatory behaviors were not present prior to cyclic AMP injections. Once these behaviors were induced, they persisted for hours.

A quantitative estimate of the dose-related nature of these responses was attempted using a dye injection procedure. This involved monitoring the optical absorbance of a cell during pressure injections of buffered solutions (pH=7.4) containing 0.2M cyclic AMP and 10 mM arsenazo III. Cells injected in this manner (n=32) exhibited transient depolarizations following doses between 50 and 500 µM cyclic AMP. No single dose was found which could routinely induce bursting (or oscillatory) behavior, although some cells exhibited such an effect following multiple injections totaling less than 1000 µM cyclic AMP.

We also examined the possibility that pressure injections may result in cellular swelling and thereby alter membrane properties. Injections of either 5'-AMP or buffered internal saline which increased cell volume by 6% were found to be without effect on membrane excitability or resting potential (n=9). Saline injections greater than 20% of cell volume resulted in a transient depolarization of 10mV in two cells tested. In contrast, cyclic AMP injections which caused 10mV depolarizations were achieved with injections totaling less than a 0.2% increase in cell volume (n=4).
Supported by PHS Grants NS-15186 and GM07143, NSF SER 76-18244.

165.8 EFFECTS OF GAMMA-AMINOBUTYRIC ACID ON HIPPOCAMPAL RHYTHMIC SLOW ACTIVITY AND ACETYLCHOLINE UTILIZATION. C.N. Allen and I.L. Crawford. Depts Pharmacology and Neurology, VA Med. Ctr. and Univ. Texas Hlth. Sci. Ctr. at Dallas, Tx. 75216.

Neurophysiological experiments suggest gamma-aminobutyric acid (GABA) is an important neurotransmitter in the medial and lateral septum. Septal GABA may be part of the phasing mechanism necessary for generation of atropine sensitive hippocampal rhythmic slow activity (RSA). Electrophysiological studies were designed to test the hypothesis that GABA in the medial septum may contribute to hippocampal rhythmic slow activity generation. Neurochemical experiments were designed to determine if GABA affected the activity of septo-hippocampal cholinergic neurons.

Rats (300 ± 25 gm) were implanted with an indwelling cannula directed at the medial septum, other rats also had bilateral intraventricular polyethylene cannulae. For physiological recording bipolar electrodes were positioned on the surface of the hippocampus, bilaterally. Animals were allowed to recover three days for neurochemical experiments and ten days for electrophysiological studies. Electrical activity recorded from awake unanesthetized rats was amplified and analyzed by a time histogram computer. For neurochemical assessment of cholinergic neuronal activity muscimol (100 ng), bicuculline (3 ng) or saline (all in 0.9% saline 0.5 µl) were injected into the medial septum; hemicholinum-3 (HC-3, 5 µg) was injected into each lateral ventricle 30 min prior to sacrifice by microwave irradiation focused on the head. Acetylcholine (Ach) in discrete brain regions was extracted in formic acid/acetone then assayed radiochemically.

Muscimol reduced (41%) the incidence of 5.9-9.1 Hz electrical activity recorded from the hippocampal surface. This decrease lasted 90 min and was antagonized by prior injection of bicuculline. Intraventricularly injected muscimol (100 ng/10 µl) had no effect on hippocampal RSA. Intraseptal saline injection was also without effect. These observations provide evidence of a direct septal gabaminergic influence on hippocampal RSA. Twenty minutes after muscimol (100 ng) intraseptally ACh levels increased (8.2 ± 2.4 to 17.3 ± 5.2 mmol/g wet wt ± S.D.) in the hippocampus, indicating a decreased utilization. Bicuculline antagonized this effect and saline was without action. These data suggest a correlation between GABA inhibition of hippocampal rhythmic slow activity and a decrease in hippocampal cholinergic neural activity.
(Supported by VA MRIS 1604 and NIH Service Award GM07062, NIGMS)

166.1 PASSIVE ELECTRICAL CONSTANTS IN THREE CLASSES OF HIPPOCAMPAL NEURONS. Thomas H. Brown, Russell A. Fricke and Donald H. Perkel. Div. of Neurosciences, City of Hope Research Institute, Duarte, CA 91010, Dept. of Anatomy, Emory University Medical School, Atlanta, GA 30322 and Dept. of Biological Sciences, Stanford University, Stanford, CA 94305.

The hippocampus contains several spatially segregated classes of morphologically distinct neurons. Using the guinea pig in vitro slice preparation, we have examined the passive electrical constants of three classes of cells: pyramidal neurons of regions CA3 and CA1 and granule cells of the dentate gyrus.

Hyperpolarizing constant current steps were injected into the cell soma and the transient and steady-state current-voltage(I-V) relationships were analyzed. There was usually a linear range in the I-V curves between the resting potential and more hyperpolarized potentials. The electrical constants were analyzed within this linear range. The input resistance (R_N) was determined from the slope of the I-V curve and the membrane time constant (τ_0)was obtained by fitting the terminal portion of the charging curve with an exponential function. The slope of the charging curve was measured and analyzed to estimate two cable-theory parameters of the neurons, based on a simple Rall model: the electrotonic length (L) of the equivalent dendritic cylinder and the conductance ratio (ρ) of the dendrites to that of the soma. Three methods (see Brown, et al. J. Neurophysiol. 45, 1-15, 1981) were used to estimate L and ρ. The four electrical constants, R_N,τ_0, L and ρ were then used in an eigenfunction expansion to generate the theoretical charging curves of the Rall model.

The experimentally derived charging functions were accurately predicted by the Rall model we used, while certain alternative models were unable to account for the data. Mean ($\pm$ S.E.) values for the four electrical constants are summarized below. There were statistically significant differences in the τ_0 values for the three classes of cells, suggesting proportionate differences in the specific membrane resistivity. We conclude that hippocampal neurons are electrically compact,and that the passive membrane constants differ markedly from those of the more extensively studied spinal motor neurons. (Supported by a McKnight Foundation Scholars Award & NIH grants NS-16576, NS-09744 & NS-12151).

Electrical Parameter	CA3 Pyramid (N = 8)	CA1 Pyramid (N = 12)	Granule Cell (N = 10)	Overall (N = 30)
R_N (MΩ)	39.0 ± 3.6	42.6 ± 3.6	38.4 ± 3.0	40.2 ± 1.9
τ_0 (msec)	19.3 ± 1.8	15.2 ± 1.2	11.6 ± 1.5	15.1 ± 1.0
L	0.93 ± 0.09	0.90 ± 0.08	0.94 ± 0.08	0.92 ± 0.05
ρ	1.2 ± 0.4	1.3 ± 0.2	1.5 ± 0.2	1.4 ± 0.1

166.2 ELECTROPHYSIOLOGICAL CHARACTERIZATION OF REMOTE CHEMICAL SYNAPSES. N.T.Carnevale and D.Johnston. Neurology Division, VAMC, Northport, N.Y.; Neurology Department, HSC T12 Rm020, SUNY, Stony Brook, N.Y. 11794; and Neurology Department, Baylor College of Medicine, Houston, Texas.

We present a new approach to the analysis of linear electrotonus, which yields practical methods for estimating the coupling between an intracellular recording site and the locus of synaptic input. This approach is free of those preconditions which limit the applicability of previous methods based on continuous cable or compartmental models, viz.: detailed knowledge of neuronal geometry or assumption of an idealized branching pattern; spatial uniformity of the electrical properties of axoplasm and cell membrane; restriction to synaptic inputs with brief time course. We require only that: membrane current depends linearly on membrane potential; the experimenter be able to elicit a pure epsp or ipsp; membrane potential can be recorded while current is injected; and, for some measurements, that the composition of the extracellular fluid can be altered.

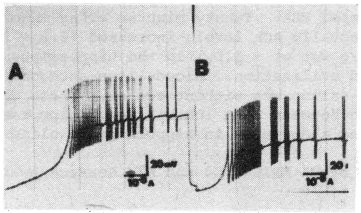
The neuron is depicted as a linear two-port network, with the location of synaptic input at one port and the site of the recording electrode (for convenience called the soma) at the other. Measurements of current, charge and potential fluctuations at the soma are related to current flow, charge entry and potential fluctuations at the synaptic region by two coupling coefficients. The first, k_{12}, defines: the steady state voltage decay from soma to synapse; charge transfer from synapse to soma; and the steady current decay from synapse to soma when the soma is voltage clamped. It can be determined empirically by measuring the change in reversal potential as a function of ionic composition of the extracellular medium. The second coefficient, k_{21}, defines: the steady state voltage decay from synapse to soma; and the charge transfer and current decay from soma to synapse. It can be estimated by driving the synaptic input to summation and measuring the potential change at the soma. A third coefficient, k^2, provides a lower estimate of the first two. It can be determined by measuring the change of synaptic potential or current amplitude as a function of membrane potential at the soma.

Our conclusions will be compared with the results of previous treatments of linear electrotonus. Typical values of these coupling coefficients will be provided, based on anatomical and electrophysiological data.

166.3 RESPONSES OF THE HYPOXIC RESISTANT APLYSIA R_{15} NEURON TO TTX, SUBSTITUTION OF EXTERNAL Ba^{2+} FOR Ca^{2+}, AND LOW PO_2's. P.E. Coyer. Lab. of Neurophysiology and Cerebrovascular Research, Dept. of Neurology and the Neurosciences Program, Univ. of Alabama in Birmingham, The Medical Center, Birmingham, AL 35294.

Using voltage-current records obtained from the neuron R_{15}, the effects of low oxygen (measured as a PO_2 of $<$ 20 torr in the suffusate) on its membrane potential and slope resistance were tested. R_{15} responded to hypoxia by increasing its membrane resistance in the non-linear portion of the anomalously-rectifying, voltage-current curves while undergoing a concomitant hyperpolarization. Figures A & B below show the effects of hypoxia (B) compared to normoxia (A).

The effects of hypoxia on R_{15} were reversible (not shown) as indicated by a return to a similar anomalously-rectifying, voltage-current curve following reoxygenation of the bath as shown in A. It can be inferred from these voltage-current records that during TTX (10^{-4}M) and Ba^{2+} (10mM) administration the events leading to an increase in the membrane slope resistance were associated with the IK(Ca) current. In other words, hypoxia may have increased the amount of inward calcium current leading to inactivation and hyperpolarization resulting from an increase in gK. Unless the membrane conductance changes were effected by a TTX-insensitive Na channel, the observations of an increased membrane slope resistance with hypoxia were present during TTX administration, a procedure which blocked spike generation but which had no effect on the voltage-current record. Substitution of Ba^{2+} for Ca^{2+}, a procedure which is known to partially block IK(Ca), produced a more linear voltage-current curve in the depolarizing direction and seemed to cause changes in the observations of R_{15}'s membrane potential and slope resistance during exposure to hypoxia. The effects of Ba^{2+} substitution on the voltage-current curves before, during, and after hypoxia are being investigated in R_{15}. Supported in part by NINCDS NS 08802 and NS 07123.



166.4 ACTIVE AND PASSIVE MEMBRANE PROPERTIES OF ISOLATED HORIZONTAL CELLS FROM THE CATFISH RETINA. Dominic Man-Kit Lam and Daniel Johnston, Neuroscience Program, Baylor College of Medicine. Houston, TX 77030

Horizontal cells in the intact retina are interconnected through a complex network of chemical and electrical synapses. The electrotonic interactions between horizontal cells make it difficult to determine the active and passive membrane properties of individual cells. In general, recordings from horizontal cells in situ have indicated extremely low input resistances and primarily passive membrane responses to current injection or light. In an attempt to investigate the active and passive membrane properties of horizontal cells, we have dissociated catfish retinae and recorded from identified isolated cone horizontal cells.

Retinae were dissociated using previously described techniques (Lam, Nature 69: 1987, 1975). Intracellular recordings were made with 30-50 megohms KCl-filled micropipettes and a 3-kHz time-share single-electrode clamp system (Johnston, Cell. Molec. Neurobiol. 1: 41, 1981). Each cell was photographed prior to recording.

Depolarizing and hyperpolarizing current pulses revealed nonlinearities in the steady-state voltage-current (V-I) relationships of most cells. The nonlinearities probably represent delayed and anomalous rectification as described in other types of neurons. Furthermore, depolarizing current pulses revealed a regenerative voltage response as defined by an increasing positive slope in the membrane potential transient. This regenerative voltage response was TTX insensitive, blocked by 10 mM Mn, Co or Ni and, therefore, is most likely calcium dependent. In order to determine the specific membrane properties of these cells, the surface area of each cell was measured from an enlarged image. The input resistance was determined from the slope of the V-I plot, and the membrane time constant calculated from small depolarizing and hyperpolarizing voltage transients. Only the cells meeting the criteria for isopotentiality were used in the analysis. For seven cells the average input resistance was 164 megohms, the average membrane resistivity ranged from about 5000-20,000 ohm-cm^2 (mean of 13,500 ohm-cm^2) and the average membrane capacity was 1.3 ± 0.3 microfarads/cm^2.

It is concluded that with the exception of an apparent lack of TTX sensitive sodium channels, the active and passive membrane properties of cone horizontal cells appear similar to those described in a variety of other neurons. {Supported by NIH grants 15772, 11535 (DJ) and EY02423 (DMKL).}

166.5 POTENTIAL DEPENDENT TRANSPORT PROCESSES IN ISOLATED RETINAL NEURONS. George S. Ayoub and Dominic Man-Kit Lam, Baylor College of Medicine, Houston, Texas 77030.

In the goldfish and catfish retinas, H1 horizontal cells which receive input predominantly from red sensitive cones, possess a high-affinity uptake system for γ-aminobutyric acid (GABA) with a K_m of about 20 μM. Lam and Steinman (PNAS 68:2777) have shown that accumulation of GABA by these neurons is enhanced by light stimulation of the retina. The cellular mechanisms controlling this stimulation-dependent process were examined by dissociating fish retinas into single cells and obtaining a fraction enriched with horizontal cell somas and axons that contain few or no synaptic endings (Lam, Nature 254:345). These cells were collected on transparent Nuclepore filters (3 μm pore size) and incubated in Ringer's solution containing radiolabeled GABA. The filters were then washed, dried, and the radioactivity measured with liquid scintillation counting. As reported earlier (Ayoub and Lam, Soc. of Neurosci. Abst. 6:54), the GABA transport mechanism remain active in these isolated horizontal cell somas. Depolarization of these cells with the Na^+ ionophore, monensin, results in a decreased accumulation of GABA, which is dependent upon the monensin concentration. To study the dependence of this GABA transport mechanism upon the membrane potential of these neurons, the permeant lipophilic cation, tetraphenylphosphonium (TPP), was employed. This ion distributes itself across the plasma membrane according to the transmembrane potential of the cell, allowing a measurement of the membrane potential of a population of cells. Using ^{3}H-TPP and ^{14}C-GABA, we were able to simultaneously determine the membrane potential and rate of GABA uptake in the same horizontal cells. The resting potentials of -70 to -90mV observed agree with intracellular recordings of these cells (Johnston and Lam, Soc. of Neurosci. Abst., 1981). The data from this double label experiment imply that GABA uptake is likely dependent upon the membrane potential of the horizontal cells in an exponetial fashion. Kinetic analysis of this transport process under hyperpolarizing and depolarizing conditions has been correlated with the rate of uptake in these states, and found to be consistent with the hypothesis that GABA uptake into somas of H1 horizontal cells increases with hyperpolarizing and decreases with depolarization. This work was supported by NIH grant EY02423 and the Retina Research Foundation (Houston).

166.6 INTERPRETATION OF VOLTAGE-CLAMP MEASUREMENTS IN HIPPOCAMPAL NEURONS. Daniel Johnston and Thomas H. Brown. Dept. of Neurology, Baylor College of Medicine, Houston, TX 77030 and Div. of Neurosciences, City of Hope Research Institute, Duarte, CA 91010

Recently it has become possible to apply voltage-clamp techniques to pyramidal neurons from in vitro hippocampal slices (Johnston, et al, Nature, 286, 1980; Johnston and Brown, Science 211, 1981), an achievement of potential importance in aiding our understanding of the synaptic physiology and active membrane properties of these cells. However, to interpret properly the voltage-clamp measurements, it is necessary to assess the influence of nonisopotentialities within the neuron on the measured current amplitude and time course. Using experimental data combined with analog simulations, we have addressed this problem for hippocampal CA3 pyramidal neurons. We were particularly interested in the mossy fiber synaptic input, which is known to be anatomically proximal to the cell soma.

We constructed an analog neuronal model consisting of nine compartments, where compartment 1 represents the cell soma and compartments 2 through 9 represent the equivalent dendritic cylinder. The values for the parallel resistor and capacitor of each compartment and for the coupling resistor between adjacent compartments were selected (Brown, et al, J. Neurophysiol. 45, 1981) so as to mimic the typical passive membrane properties and electrotonic structure of CA3 pyramidal cells (Johnston, Cell. Molec. Neurobiol. 1, 1981; Brown, et al, Neurosci. Abstr. 7, 1981). A conductance mechanism, consisting of a battery in series with an electronically-controlled variable resistance, was placed in parallel with one of the nine compartments. Two types of conductance changes were introduced into the various compartments--one was sinusoidal and the other mimicked a synaptic event. A voltage clamp was applied to the soma compartment, using a standard 30 MΩ microelectrode and a single-electrode clamp system (550 Hz frequency response). We devised several criteria for assessing the adequacy of the voltage clamp as a joint function of the frequency or waveform of the conductance change and the compartment in which it occurred.

The measurement errors associated with the various conductance changes in each of the compartments will be described. For sinusoidal conductance changes between 10 and 400 Hz and for synaptic events with realistic conductance waveforms, we find that adequate voltage-clamp measurements can be made only in compartments 1 and 2. By comparing experimental data for the mossy fiber synaptic current waveform with the results of our simulations, we can conclude that the mossy fiber synaptic input is located no farther from the cell soma than compartment 2. This particular synaptic input is therefore suitable for voltage-clamp analysis. (NS11535, NS15772, NS16576 and a McKnight Scholar's Award)

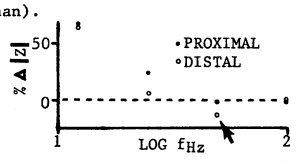
166.7 THE IMPLICATION OF THE SURFACE NEGATIVE WAVE OF CEREBRAL CORTEX EVOKED BY THE ANTIDROMIC ACTIVATION OF THE BETZ CELL. S.F. Fan* and S.L. Hu*(SPON: R.G. Butler). Shanghai Institute of Physiology, Academia Sinica, Shanghai, China.

In the previous work we have found that the cortical responses of Betz cell to the antidromic stimulation of the pyramidal tracts in medulla contains a clear surface negative component of short latency with no sign of postsynaptic activities. As judged from other criteria, however, the impulse does not travel upward to the terminal portion of the apical dendrite. Thus it seems to exist an obvious contradiction, since it is commonly held that when an impulse ceases to conduct further somewhere along the nerve tissue placed in a volume conductor, the segment further on would only serve as a current source and around which only a positive potential change could be recorded. The results presented in this paper, both from the qualitative calculation according to the volume conductor theory and from the model experiment done with peripheral nerve show that, if the membrane properties of the segment which the impulse has not invaded are somewhat different from those of the segment which the impulse has just passed through, either the membrane potential of the former is lower and/or the membrane resistivity is higher, a negative potential change could be recorded around the inactivated segment. Therefore, it is likely that the terminal portion of the apical dendrite must possess lower membrane potential and/or higher membrane resistivity as compared with the soma and the basal portion of the apical dendrite.

166.8 INTRACELLULAR IMPEDANCE ANALYSIS INDICATES RELATIVE PROXIMITY OF MEMBRANE CONDUCTANCE CHANGES. S.E. Fox and C.Y. Chan*, Dept. of Physiology and Dept. of Anatomy and Cell Biology, Downstate Medical Center, SUNY, Brooklyn, N.Y. 11203.

In identifying the synapses responsible for an event in neurons whose various afferents occupy distinct proximal/distal zones it is valuable to locate zones of changing conductance. Using cable equations, we modelled neurons to predict impedance as measured by sinusoidal current injected into the soma. The model showed that distal changes in conductance have a much smaller effect on impedance (as seen from the soma) at high frequency than do proximal changes, given the same change in impedance at low frequency (cf. fig.). This effect is due to capacitive shunting of high frequencies in the proximal region so that little current reaches the distal cellular zones.

The results from the mathematical model were verified both with an RC circuit model of a neuron and in spinal neurons in vitro. A single electrode bridge amplifier was modified to pass sinusoidal current at up to 400 Hz through electrodes of up to 100MΩ while faithfully recording the voltage drop across the cell. Measurements on the RC circuit agreed with the mathematical model. The somata of embryonic chick spinal neurons in culture were impaled with K-acetate-filled micropipets (40-80MΩ). Sinusoidal currents (0.1 to 1.0 nA p-p) were passed while recording the membrane potential. Iontophoretic application of GABA (75 to 750 nA, 200 to 700 msec) produced localized conductance increases, either on the soma or up to 200 μm out on the neurites. The figure shows the effect of GABA applied to the soma (proximal) or 50 μm out on a neurite (distal) for a cell which was typical of the six cells tested. The change in the magnitude of the impedance as a percentage of the change for DC (100 msec pulses) is plotted for the four frequencies tested, dropping more abruptly for distal conductance changes. Another fascinating effect which was also predicted by the models is the apparent increase in impedance at high frequency (arrow) with increased conductance in the distal processes. (Supported by NIH grant NS 14497 and NSF grant BNS 77-09375 to J.B. Ranck, NIH grant NS 10987 to V.E. Amassian, NSF grant BNS 80-04871 to D.H. Farb and an MDA Fellowship to C.Y. Chan).



166.9 ELECTROTONIC STRUCTURE OF THE RAT SYMPATHETIC POSTGANGLIONIC NEURONS. Barbara K. Henon, Thomas H. Brown and Donald A. McAfee, Div. of Neurosciences, City of Hope Research Institute, Duarte, CA. 91010.

The superior cervical sympathetic ganglion is often used *in vitro* as a model system for chemical synaptic transmission. We have evaluated the passive electrical constants of the postganglionic neuron in order to estimate their influence on measurements of synaptic transmission and integration.

Single microelectrode techniques were used for bridge-balanced voltage measurements of randomly selected postganglionic neurons. We measured the magnitude and time course of the voltage change across the cell membrane in response to a hyperpolarizing current step. Thus we were able to compute the input resistance R_N and the passive electrical constants described below. Within 2 ms after the onset of the current step, the voltage changed as a single exponential function and was subjected to regression analysis to determine the membrane time constant τ_0 and its coefficient C_0. In 9 of 12 cells studied the charging curves deviated from the extrapolated regression line during the first 1 or 2 ms. This deviation was peeled and fit by a second exponential function to give the first equalizing time constant τ_1 and its coefficient C_1. The time constants and their coefficients were used to estimate two cable theory parameters of the neuron based on a simple Rall model: L, the electrotonic length of the equivalent dendritic cylinder and ρ, the conductance ratio of the dendrites to that of the soma (Brown et al., J. Neurophysiol. 45:1-15, 1981). The constants R_N, τ_0, L and ρ were used in an eigenfunction expansion to generate the theoretical charging curves of the Rall model.

There was precise agreement between the actual data points and the theoretically derived charging functions. In spite of the substantial dendritic conductance indicated by our values for ρ, the value of L is relatively small and we conclude that the sympathetic neuron is electrotonically even shorter than either the hippocampal or spinal motor neurons. (Supported by Grants NS 80-12394, NS 16576 and a McKnight Foundation Scholars Award)

	R_N (MΩ)	τ_0 (msec)	ρ	L
Mean ± S.E.	109 ± 15	12.0 ± 2.1	2.12 ± .30	.670 ± .054
N	12	12	6	6

167.1 ABSENCE OF ELECTRICAL COUPLING IN DYE-COUPLED PYRAMIDAL CELLS IN THE CA1 REGION OF GUINEA PIG HIPPOCAMPUS. Paul G. Funch, W. Douglas Knowles and Philip A. Schwartzkroin, Dept. of Neurological Surgery, University of Washington, Seattle, WA.

One proposed substrate for the synchronization of hippocampal neuronal activity is direct electrical connections between pyramidal cells (PCs) via gap junctions. Others have recently used dye-coupling as evidence supporting the existence of electrical coupling in mammalian neocortex, hypothalamus, and the CA3 region of the hippocampus. We have found no evidence for electrical coupling in the CA1 region using two electrophysiological methods: 1) with simultaneous intracellular recordings from PC pairs, subthreshold current injection through one microelectrode never produced a membrane potential change measurable with the second (except when the two electrodes were in the same PC); 2) antidromic stimulation rarely activated short latency depolarizations (SLDs), and in no case were the SLDs resistant to collision with action potentials elicited by brief intracellular current injections. Nevertheless, intracellular injections of Lucifer Yellow (LY) into single CA1 PC somas usually resulted in the staining of 2 to 4 PCs. In contrast, intradendritic injections of LY rarely resulted in the staining of more than a single PC. We thus conclude that dye-coupling can artifactually overestimate the extent of electrical coupling between neurons.

167.2 DYE-COUPLING BETWEEN CA1 PYRAMIDAL CELLS IN RAT HIPPOCAMPAL SLICES. R. D. Andrew, C. P. Taylor*, R. W. Snow, and F. E. Dudek. Dept. of Physiol., Tulane Univ. Sch. of Med., New Orleans, LA 70112.

Several lines of evidence indicate that some hippocampal neurons are electrotonically coupled. In the present studies 3% Lucifer Yellow (LY), a fluorescent dye known to cross gap junctions between electrotonically coupled cells, was injected (1-3 nA hyperpolarizing current) into CA1 pyramidal cells to determine whether these neurons are dye-coupled.

In transverse slices of rat hippocampus, multiple staining of CA1 cells was observed in approximately 45% of the cases (n = 11) after dye was injected intracellularly into single cells which had action potentials $\geq$ 50 mV throughout the injection. Using the same procedures, multiple staining was never observed in neurons of sliced superior cervical ganglia (n = 11). Dye-coupling was also present in saline with 4-8 mM Mn^{2+} and 0.05-0.5 mM Ca^{2+}. Field potentials were monitored to confirm that chemical transmission was blocked. This indicates that dye transfer is not mediated by chemical synapses and strongly supports the hypothesis that dye-coupling in CA1 cells results from LY transfer directly between neurons rather than indirectly through an extracellular route.

Diffusion of LY from thin-walled micropipette tips could occasionally stain CA1 cells, even during a brief impalement. Such staining was rare and faint, but potentially could lead to an overestimation of the number of dye-coupled cells. Experiments are in progress to evaluate which factors contribute to this problem, and these results will be discussed.

Maximum separation between dye-coupled cells in transverse and longitudinal directions was measured to evaluate whether a specific orientation exists for dye-coupling within the CA1 area. Somata of coupled cells ($\sim$8 x 10 µm) were within 35 µm of each other (not corrected for shrinkage during fixation). No preferential orientation of coupling was detected.

Intracellular recordings were analyzed to determine whether the presence of fast prepotentials (FPPs) was correlated with dye-coupling. Although FPPs occurred spontaneously or to orthodromic stimulation in dye-coupled cells, they were also observed in brightly stained cells with no dye-coupling. Therefore, either some electrotonically coupled pyramidal cells are not dye-coupled, or some FPPs are not coupling potentials.

Supported by NS16683 from NIH.

167.3 PHYSIOLOGICAL EVIDENCE FOR ELECTROTONIC COUPLING BETWEEN CA1 PYRAMIDAL CELLS IN RAT HIPPOCAMPAL SLICES. C. P. Taylor* and F. E. Dudek. Dept. of Physiol., Tulane Univ. Sch. of Med., New Orleans, LA 70112.

The hippocampus is well-known for its susceptibility to seizures and synchronous afterdischarge. Physiological and anatomical evidence indicates that some hippocampal neurons are electrotonically coupled, which hypothetically could mediate synchronization of epileptiform events. Antidromic stimulation was used to determine whether some CA1 pyramidal cells are electrotonically coupled.

Transverse slices of rat hippocampus were prepared by conventional techniques. Chemical synaptic transmission was blocked with saline containing 4-8 mM Mn^{2+} and 0.05 - 0.5 mM Ca^{2+}, which completely eliminated orthodromic field potentials.

In two intracellular recordings out of 28, antidromic stimulation revealed short latency depolarizations (SLDs) which were clearly resistant to collision by action potentials evoked with current injection through the intracellular electrode. For the SLDs in these two cells the latency from the antidromic soma spike (87 and 75 mV) was 250 and < 100 µsec, and amplitude was 8.7 and 11 mV. Smaller SLDs were observed during these experiments, but could not be analyzed reliably on the falling phase of the current-evoked spike. Possible reasons for the low percentage of cells exhibiting SLDs will be discussed. Because SLDs were resistant to Mn^{2+} and collision, they were most likely electrotonic coupling potentials from spikes in other pyramidal cells.

After prolonged incubation (1-4 hr) in a solution containing 0.5 mM Ca^{2+} and 8 mM Mn^{2+}, intense stimulation of the alveus not only evoked an antidromic population spike, but also large synchronous afterdischarges of up to 30 spikes lasting up to 650 msec. Occasionally additional population bursts occurred for up to 10 sec after the stimulus. Both intracellular and extracellular recordings during stimulation of the alveus and stratum radiatum confirmed that chemical synaptic transmission was still blocked. Simultaneous intracellular and extracellular recordings during the afterdischarges occasionally revealed subthreshold depolarizations which were similar in waveform to SLDs (above) and were also synchronous with extracellular population spikes. Therefore, synchronous afterdischarges can be evoked when Ca^{2+} influx and chemical synaptic transmission are blocked with Mn^{2+}. These results provide evidence supporting the hypothesis that electrotonic coupling in the hippocampus, independent of any chemical synaptic interaction, mediates synchrony during epileptiform activity.

Supported by NS16683 from NIH.

167.4 FORMATION OF ELECTRICAL COUPLING BETWEEN XENOPUS EMBRYONIC MUSCLE CELLS IN CULTURE. I.Chow*, M.H.Chow* and M-m.Poo. Dept. of Physiology and Biophysics, Univ. of California, Irvine, CA 92717.

Low resistance pathways, detectable by electrophysiological methods, are known to be present in a variety of embryonic systems. They are generally accepted to be associated with gap junctions and have been shown to disappear at later stages of development. However, it is unclear how the cells regulate this capability for electrical coupling throughout development. The present study has investigated the mechanisms involved in the decrease of coupling with age in Xenopus embryonic muscle cells in culture. Our findings suggest that such decrease is mediated by extramembranous factors, since high coupling capability can be restored in older cells after treatment with colchicine and Ca^{++}-Mg^{++}- free medium (CMF).

Cultured Xenopus myotomal cells were manipulated into contact 12 to 72 hours after plating. After various contact intervals, electrotonic propagation of acetylcholine-induced depolarizations between the cells were recorded and the coupling ratios were determined. The results show that cells less than 24-h old in culture established measurable coupling within 30 min of contact (95% of the recorded cell pairs). The percentage of electrically coupled pairs decreased with culture age, so that by 24-36 hours 83% of the pairs were coupled. Cells older than 36 h did not establish coupling within the first half-hour of contact;however, the 2-day old cells were capable of establishing electrical coupling if left in contact for longer periods and by 2 h about half of the cell pairs were found to be coupled.

Pretreatment of 2-day old cells with colchicine (1 h), known to disrupt microtubules, and with CMF (0.5 h), which removes cell coat material, induced the onset of electrical coupling at least one-half hour earlier than in control cells. After 30 min of contact about 80% and 25% of the cell pairs pretreated with colchicine and CMF, respectively, had detectable coupling, whereas the controls were not coupled. The average coupling ratio of the pretreated cells was also higher than the control values. One and one-half hours after contact, the colchicine pretreated cells had an average coupling ratio twice that of the control pairs, and the CMF pretreated pairs had a value three times as large as the controls.

This recovery of coupling capability suggests that the decreased extent of coupling found in 2-day old cultured cells is not due to alterations in the intrinsic plasma membrane components, e. g. a loss of precursor molecules for the formation of intercellular channels.

(supported by MDA and NSF)

168 SYMPOSIUM

Cytoskeletal Determinants of Neuronal Form and Function. R.J. Lasek (Chairman, CWRU) S.L. Palay (Harvard), M.L. Shelanski (NYU), F. Solomon (MIT), R.L. Gulley (NIH).

The functional properties of a neuron are determined in great part by its general shape and by its structural details such as the presence and location of dendritic spines. What is the structural basis of the varied forms which neurons express? How can these forms be stable and yet undergo the changes which contribute to the plasticity of the nervous system? Answers to these questions and others are beginning to emerge from the study of cytoskeletons. Although most of the progress in the study of cytoskeletons has occured within the realm of cell biology, the time is ripe for this information to be integrated into neurobiology (see Lasek and Shelanski et al., Neurosci. Res. Prog. Bull. 19:1-153, 1981). The purpose of this symposium is to provide an introduction to the structural and biochemical properties of the cytoskeletal elements. The speakers will attempt to relate the cytoskeleton to the dynamic events that occur during the development of the nervous system and in specialized regions such as the synapse.

The symposium begins with Sanford L. Palay who will provide a contemporary picture of cytoskeletons in the nervous system. Our image of the cytoskeleton is a product of both the most recent electron microscopic observations as well as the classical metallic stains that depict the cytoskeleton in the light microscope. Michael L. Shelanski follows with a brief review of the molecular properties of microtubules, intermediate filaments and microfilaments. In addition he will explore the molecular interactions between these polymers and their possible roles in the integral properties of the cytoskeleton. Frank Solomon will describe his studies on intracellular determinants of cell shape. More specifically, he will explore the possibility that cytoskeletal organizing centers convey structural information from one cell generation to another and that this information can influence the shape of the progeny. Raymond J. Lasek will discuss environmental factors that may influence the axonal cytoskeleton as it moves from the cell body to the axon terminal. The final speaker, Robert L. Gulley, will analyze the recent advances in our understanding of the structure of the synapse. Emphasis will be placed on the relationship between the cytoskeleton and the plasma membrane, particularly at the synaptic junction. The presentations will be followed by an open discussion.

169 SYMPOSIUM

PARALLEL AND SERIAL PROCESSING IN VERTEBRATE VISUAL PATHWAYS. C. Enroth-Cugell (Chairman; Northwestern Univ.), R.M. Shapley* Rockefeller Univ., R.W. Rodieck, (Univ. of Washington), P. Lennie* (Univ. of Sussex, England).

In the last century, Ramon y Cajal demonstrated that the retinas in every vertebrate class contain a variety of different morphological types of ganglion cells. Recent concurrent developments in physiology and anatomy have clarified the different ganglion cell types in certain species, with regard to function, morphology and central projection. These findings point to the importance of parallel processing in the visual pathway.

R.W. Rodieck will describe the different morphological and functional types of ganglion cells that have been identified in the cat and monkey, and will trace their central destinations.

R.M. Shapley will then describe the receptive field mechanisms that characterize specific ganglion cell classes and the cells upon which they terminate in the lateral geniculate nucleus. The development of our understanding of these mechanisms has been an outgrowth of our recently increased knowledge of the physiological heterogeneity of ganglion cells.

Finally, P. Lennie will discuss possible visual roles of the physiologically distinct classes of cell. Although some psychophysical observations on man, (e.g. distinct thresholds for perceiving patterns and movement) have been thought to reveal characteristic properties of two of the major physiological classes, recent physiological observations in cat and monkey make such simple ideas less attractive.

170 WORKSHOP

THE GROWING RELEVANCE OF GAP JUNCTIONS AND ELECTROTONIC SYNAPSES TO NEUROBIOLOGY. M.V.L. Bennett (Chairman, Albert Einstein Col. of Med.), D.C. Spray (Albert Einstein Col. of Med.), R. Weingart (Univ. Bern), R.B. Hanna and T.S. Reese (SUNY Syracuse and NINCDS) J.A. Raper and C.S. Goodman (Stanford), F.E. Dudek (Tulane).

This workshop was inspired by new developments with respect to electrotonic synapses and their morphological substrate, gap junctions. 1)Junctional conductance in embryonic tissues can be readily and reversibly altered by a number of experimental manipulations. Increase in cytoplasmic H ion concentration to 0.1 μM strongly depresses conductance; much higher concentrations of Ca ion (c.1 mM) are required to produce the same effect. Thus, H ion is a more likely candidate for physiological control. Effects of other agents suggest the existance of at least two gating mechanisms. A specific junctional blocker is not yet available, but diversity of mechanisms of block may allow separation of actions on junctions from those on other cell properties. 2)Junctions in cardiac muscle exhibit a similar sensitivity to cytoplasmic H ion, and action of H ion is not mediated through Ca ion. Normal intracellular pH can reside at an intermediate point where junctional conductance can be modulated up or down by pH changes. Thus, junctional conductance provides a logical site for pharmacological and physiological actions on cardiac function. 3)High resolution techniques for freeze fracture reveal new aspects of junctional structure. The hypothesized intercytoplasmic channel and the oligomeric nature of the intramembrane particle are apparently resolved. Decreasing junctional conductance by cytoplasmic acidification produces a small change in particle structure, but no immediate change in particle spacing. 4)During neurogenesis in the grasshopper dye coupling can be transient and exhibit a high degree of spatial as well as temporal specificity. Precise mechanisms for formation and removal of junctions are presumably involved. The specificity suggests a possible role in information transfer underlying determination of cell type and navigation by growing fibers. 5)Electrotonic synapses defined by electrotonic coupling, dye coupling, and presence of gap junctions are being found in new regions of the mammalian central nervous system. Some of these synapses may synchronize neuronal firing and lead to rhythmic activity or mass release of neurohormone. The pathological synchrony of epilepsy may also be mediated by electrotonic synapses.

171.1 THE RELATIVE CONTRIBUTIONS OF CORTICAL SITE AND SIDE IN THE CONTROL OF PRAXIC AND SPATIAL BEHAVIOR. B. Kolb, R. J. Sutherland, and I. Q. Whishaw. Department of Psychology, Univ. of Lethbridge, Lethbridge, Alberta, Canada, T1K 3M4.

Studies of the localization of function in the cortex have shown that both the site as well as the side of damage are important in understanding the neural control of complex motor (praxic) and spatial behavior of humans. The frontal and parietal lobes appear to participate in certain aspects of these behaviors and may do so differently in the two hemispheres. In the present study we compared the relative contributions of cortical site (frontal vs. parietal) and side (left vs. right) in the performance of spatial and complex motor tasks by rats.

In the first experiment, rats with bilateral aspiration lesions of the medial frontal cortex, orbital frontal (sulcal) cortex, parietal cortex, or all of the neocortex (decorticate) were tested on three spatial tasks (Morris water maze, radial arm maze, Grice box) and four tasks requiring complex motor movements including: 1) opening puzzle latches, 2) traversing a narrow beam, 3) grooming, and 4) food hoarding. The results showed that the frontal and parietal cortex both participate in spatial and praxic behaviors but they do so in different ways. On the spatial tasks sulcal lesions impaired performance of the water maze and Grice box but not the radial arm maze; medial frontal lesions impaired performance of the water maze but not the other two mazes; and parietal lesions impaired performance of the radial arm maze and Grice box but not the water maze. Decortication impaired performance on all the mazes. Sulcal lesions had little effect on the motor tests whereas medial frontal lesions impaired the opening of latches and abolished food hoarding. Parietal lesions produced a mild deficit on the puzzles and a severe deficit on the beam. Decortication severely impaired all four motor tasks.

In the second series of experiments, rats with large unilateral lesions of the left or right frontal cortex or total unilateral removal of the left or right hemisphere were tested in the same spatial tasks as above, and similar rats will be tested on the motor tests. To date, the results show that removal of the left hemisphere produces a more severe deficit in acquisition of the water maze than a similar removal of the right hemisphere.

The data show that the cortical control of spatial and praxic behaviors can be dissociated in the rat and that both the site and side of lesion are important in understanding the neural control of praxic and spatial behavior in the rat.

171.2 POSTURAL ASYMMETRY IN THE NEWBORN WISTAR RAT AND IMPLICATIONS FOR BRAIN LATERALITY. G.D. Rosen*, M. Hofmann*, J. Stockler*, J.S. Gall*, D.A. Yutzey*, and V.H. Denenberg. Dept. Biobehav. Sci., Univ. Connecticut, Storrs, CT 06268.

Newborn female Sprague-Dawley rats have a rightward population asymmetry in tail position that is also a reliable predictor of later rotational asymmetry (Ross, Glick, and Meibach, Neurosci. Abs., 1980). Male pups had a non-significant bias toward the left. Other work has demonstrated a rightward bias among adult females in a rotometer as well as a two-lever operant conditioning apparatus (Glick and Ross, Dev. Br. Res., in press). Conversely, Purdue-Wistar rats from our colony have shown a leftward turning preference in the open field (Sherman et al., Br. Res., 192, 1980). If the findings of Ross et al. can be generalized to other rat strains, the prediction is that Wistar rats would exhibit a left tail bias in infancy.

Our results are summarized in Table 1. Significantly more pups exhibited a leftward tail posture in both males and females. In addition, females are more biased in the leftward direction than the males. There are also significantly more males than females who show a neutral tail position.

Thus, the differences between our results and those of Glick's group appears to be due to strain differences in postural asymmetry and consequent spatial bias, with the Wistar rat asymmetrical to the left while the Sprague-Dawley is rightward biased.

TABLE 1

Direction of Postural Asymmetry
(percentages in parenthesis)

	LEFT	NEUTRAL	RIGHT
MALES	445 (50.6)	102 (11.6)	302 (38.8)
FEMALES	473 (57.5)	58 (7.1)	291 (35.4)

171.3 EXPERIENCE INDUCED ASYMMETRIES AFFECT THE RETENTION OF A TASTE AVERSION IN RATS. M. Hofmann*, J.A. Garbanati*, G.F. Sherman*, G.D. Rosen*, D.A. Yutzey*, and V.H. Denenberg (SPON: E.B. Thoman) Dept. Biobehav. Sci., Univ. Connecticut, Storrs, CT 06268.

Previous research has found that stimulation in infancy can produce an asymmetrical brain organization in the rat. In addition, Denenberg et al.(Brain Res. 200:123, 1980) have shown that a conditioned taste aversion (CTA) may be asymmetrically processed by the intact rat brain. Following CTA induction, rats received unilateral hemidecortication. When tested subsequently for retention of the aversion, animals with a left hemidecortication (L) in general retained the aversion longer than those with right hemidecortication (R) or intact brains (I). In this study we wish to test the capacity of a single intact hemisphere to form and retain a CTA both with and without the benefit of extra stimulation in early life.

Rats were handled (H) or left undisturbed (NH) for the first 20 days of life. For the next 30 days, half of each group were either housed in an enriched environment (EE) or a standard lab cage (LC). At approximately 110 days of age, animals were given a R, L, sham lesion (S), or were not lesioned (C). At approximately 200 days of age, animals were introduced to a sweetened milk solution followed by an injection of an isotonic LiCl solution. For the next 25 days, animals were presented the sweetened milk for 30 min. to test the acquisition and retention of the aversion.

A preliminary analysis revealed no differences between the S and C groups, so they were pooled (I). In the NH-LC group, both R and L differed from I over trials ($p<0.01$), while not differing from each other. In the NH-FE group, only the L vs. I comparison was significant over trials ($p<0.01$). No individual comparisons are significant in the H-LC condition, whereas in the H-FE condition, L was significantly different from I ($p<0.01$) and R ($p<.01$) over trials, while the I and R groups did not differ.

These data indicate that stimulation in early life can produce an asymmetrical brain organization. Unilateral hemidecortication in normally reared laboratory rats produces an increased retention of a CTA regardless of the laterality of the lesion. With respect to the I group, H decreases the retention of R and L groups, and EE decreases the retention of the R group without demonstrating a clear asymmetry between the hemispheres. However the combination of H and EE acts to reduce the retention of the R, while not affecting the retention of the L group. These results support and extend the prior results on the effects of early experiences and cerebral asymmetry in rats with regard to activity, muricide, and CTA.

171.4 RECIPROCAL CONNECTIONS OF LATERAL ORBITOFRONTAL CORTEX WITH BASAL FOREBRAIN, HYPOTHALAMUS AND BRAINSTEM IN CAT. S.J. Wiegand and J.L. Price Dept. Anat. and Neurobiol., Washington University School of Medicine, St. Louis, Mo. 63110

The prefrontal cortex is involved in the regulation of complex behaviors and autonomic responses requiring interaction with diverse subcortical structures. This study demonstrates extensive direct connections between lateral orbital portions of frontal cortex and the basal forebrain, hypothalamus and brainstem in the cat. Cortical areas on the medial bank of the presylvian sulcus and posterolateral gyrus proreus were stereotaxically injected with 100 nl of HRP conjugated to wheat germ agglutinin (1%) and/or ^{3}H-leucine (50μCi/μl). Electrophysiological recording was used to guide the placement of injections.

Within the ipsilateral basal forebrain labeled neurons are present in the nucleus of the diagonal band and nucleus basalis. In the hypothalamus, retrogradely labeled neurons are present in the lateral and dorsal hypothalamic areas and the perifornical region at the level of the tuber cinereum, but not more rostrally. Caudally labeled cells are found in the tuberomammillary and supramammillary nuclei and the adjacent lateral and posterior hypothalamic areas. Labeling of caudal hypothalamic structures is more extensive in experiments in which cells of the mediodorsal nucleus of thalamus are heavily labeled, compared to cases where retrograde thalamic labeling is predominant in the submedial or ventromedial nuclei. Within the brainstem labeled cells are usually found in the central tegmental area, medial and dorsal raphe and central gray. Occasional labeled neurons are located in the substantia nigra, locus coeruleus, parabrachial nucleus or reticular formation. Light retrograde labeling of the contralateral hypothalamus and brainstem is frequently observed.

Anterograde connections are demonstrated by both HRP and autoradiographic methods. In the basal forebrain anterograde label is present in the ipsilateral diagonal band. More caudally label is frequently observed in the far lateral hypothalamus, bordering the ipsilateral cerebral peduncle. In experiments in which the submedius nucleus of thalamus is heavily labeled, bilateral anterograde label is noted in the medial ventral part of lateral hypothalamus. Within the brainstem anterograde label is present in the ipsilateral ventral tegmental area extending dorsolaterally over the substantia nigra, and bilaterally in the medial pontine nuclei and ventrolateral central gray.

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171.5 A RE-EXAMINATION OF THE ORGANIZATION OF THALAMIC-PREFRONTAL PROJECTIONS IN MONKEY. M.L. Weber-Levine and J.S. Stamm. Depts. of Psych., Morehouse College, Atlanta, GA 30314 and SUNY at Stony Brook, Stony Brook, NY 11790.

Early investigations of prefrontal lobe functions in monkeys demonstrated the existence of two distinct areas in this cortex, an orbitofrontal and a dorsolateral region. Anatomically these regions were shown to receive projections from three distinct zones in the nucleus medialis dorsalis (MD) in thalamus. Later behavioral studies have shown the existence of more than one functional region within the dorsolateral convexity. The region of sulcus principalis has been shown to be important for tasks involving a spatial delay, whereas the surrounding non-principalis cortex is related to tasks in which the animal must respond in a direction opposite to the cue in order to obtain a reward. On the basis of the behavioral data this study was undertaken in an attempt to determine if there exists a scheme of projections from MD which would lend anatomical support to the functional dissociations. A series of lesions involving behaviorally defined subareas of the dorsolateral cortex was made. In addition to total dorsolateral cortex ablations these lesions included the following: removal of; sulcus principalis, both complete and partial, non-principalis dorsolateral cortex, complete and partial, and frontal pole.

The resulting retrograde degeneration in MD clearly indicates a topographical arrangement of projections from MD to prefrontal cortex. The projection system suggested here differs from those of earlier studies. It is concluded that there exists in MD a dorsoventral arrangement of projections corresponding to the dorsoventral axis of the cortex. These projections form dorsoventral planes along the anterior-posterior axis in MD, with the greatest amount of projections emanating from the more anterior regions of MD. No evidence was found to support previous suggestions for the existence of a core projection from MD to the frontal pole. The variation in the density of degeneration following total or partial lesions lends support to the concept of sustaining projections from each region of MD to the different regions of cortex. This concept also helps to explain the lack of complete dissociation of function following lesions of the behaviorally defined subareas of dorsolateral cortex.

171.6 AREA 5 IS INVOLVED IN VISUAL NEGLECT IN THE CAT. Robert Sinclair* and Simon Horenstein. Departments of Neurology and Psychology, Saint Louis University, Saint Louis, Missouri 63104.

Ablation of a portion of area 5 on the mesial surface of the cat brain resulted in modification of a visually dependent learned task. Previous findings indicated that ablation of auditory area Ep in the posterior ectosylvian gyrus modified preference to double simultaneous visual stimulation (DSS). Ablation of AI and AII in the auditory cortex and SII in the somatosensory cortex did not. Clinical cases of sensory neglect are often trimodal. Therefore, a somatosensory analogue of Ep is implicated.

A common cortococortical projection point (Vss) between Ep and visual cortex exists in the mid suprasylvian gyrus. AI and AII project to each other and to Ep, but not to Vss. SI and SII likewise project to each other and to area 5 but not to Vss. Area 5 has been called a third somatosensory area, and a portion of area 5 projects to mid suprasylvian cortex (Mss), apparently overlapping Vss. Area 5 is therefore a possible analogue for the somatosensory system of auditory Ep.

The present study evaluated the functional role of area 5. Three cats were appetitively conditioned in a specially designed "Y" maze (Toga, A.W. et al., Psychological Reports, 40:1071-4, 1977) equipped for visual stimulation. On unilateral stimulation animals were required to move into the sole lighted arm of the maze and on DSS to move into either of two in order to obtain food reward delivered from a feeding cup at the end of each. Each animal established its own lateralization pattern to DSS. After training, unilateral subpial resection of area 5 in the anterior lateral gyrus and mesial surface in the longitudinal fissure was performed, and the animal tested from the 1st to 10th postoperative days. After intracardiac saline-formalin perfusion, brains were removed for anatomic study. Lesions imposed no apparent postural bias or lateral preference to unilateral stimulation. A significant decrease in turns toward the side affected by the lesion to DSS was found in one animal with lesion restricted to the mesial portion of area 5, and another animal with a lesion extending over both the mesial and lateral portion of area 5. In a third animal with lesion restricted to the lateral portion of area 5, no significant change in lateral preference to DSS was found. This suggests that the mesial portion of area 5 is critical for producing neglect, while the lateral portion is less or not at all important.

Results suggest that area 5 may indeed function within the somatosensory system in a manner analogous to that of Ep in the auditory. Ep and area 5 may integrate information about their respective sense modalities. Their projections to Vss possibly enter multimodal processing which in turn determines the direction of the orienting response.

171.7 EFFECTS OF NEONATAL SECTION OF THE CORPUS CALLOSUM ON INTERHEMISPHERIC TRANSFER OF VISUAL PATTERN DISCRIMINATIONS IN THE CAT. M. Ptito, F. Lepore, B. Cardu and M. Lassonde. Dépt. de Psychologie, Univ. du Québec à Trois-Rivières, Trois-Rivières, Québec and Univ. de Montréal, Montréal, Québec

Numerous studies have shown that early interventions in the visual system of both animals and men can result in its permanent alteration. Moreover, the interhemispheric transfer of visual pattern discriminations in the adult cat depends on the integrity of the forebrain commissures, and especially the corpus callosum, since its transection generally abolishes the ability of an animal to transfer this information across the hemispheres. The aim of the present experiment was to determine whether the neonatal section of the callosum might result in the greater utilization of the secondary commissures such that these become sufficient to mediate this type of transfer in the adult.

Six cats were used in the experiment: two control cats underwent the mid-line transection of the callosum and optic chiasma when they were adults; two had their callosum sectioned at 6 weeks post-natally, namely, after callosal myelinization but before the end of the critical period when the visual system is still susceptible to modifications; the callosum of the other two subjects was sectioned at 21 days of age, when myelinization is still supposedly incomplete. These 4 subjects were then raised in the animal colony until they were 7 months old, at which time their chiasmas were sectioned, followed by behavioral testing. This consisted of learning various pattern discriminations in a Thompson-like two-choice box with one eye and testing for transfer using the other eye.

Results indicated that the control group, as expected, did not transfer, requiring as many trials to learn the discrimination with the second eye as with the first. This was also the case with the subjects whose callosum was split at 6 weeks. The subjects whose callosum was sectioned at the earlier age (21 days) did not show complete transfer, although they did show some saving on a number of pattern discriminations. The results, though inconclusive, do point to some possible greater utilization of the extra-callosal commissures in early operated animals.

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172.1 GANGLIOSIDES ENHANCE MEMORY CONSOLIDATION IN RAT PUPS. S.E. Karpiak and M.M. Rapport. Div. of Neuroscience, New York State Psychiatric Institute and Depts. of Psychiatry and Biochemistry, College of Physicians and Surgeons, Columbia University, New York, N.Y. 10032.

Injection of antibodies to brain ganglioside into the CNS of the adult rat has been shown to inhibit consolidation phases of learning(1). Further, the injection of antibodies to ganglioside into the neonate rat pup (PN Day 5) results in subtle learning deficits in the mature animal accompanied by decreased levels of ganglioside in the CNS and alterations in spines of cortical pyramidal cells(2). The following study was undertaken to determine if the administration of gangliosides into neonatal rat pups might alter learning behavior. There is evidence that gangliosides in contact with cells in vitro are functionally incorporated into the cell membrane, and the addition of gangliosides to some neuronal cell cultures leads to more rapid maturation of the cells(3).

Rat pups from 5 to 15 days after birth were given daily sub.cu. injections of 2.5 mg of total brain ganglioside (in 25 µl of saline). Control rat pups received saline injections only. On PN Days 11,12 and 14 pups were trained on a multi-directional avoidance paradigm using 20 shock-escape trials, and latency to escape the shock was recorded (as a measure of performance). Both groups of rats acquired the task equally well on Day 11. Their performance on Day 12 improved significantly (50% reduction in the latency scores). However when the rats were tested on PN 14 (skipping one day of training) those injected with ganglioside showed no difference in performance as compared to Day 12, whereas the control animals had increased latency scores (+50% to 60%; $p<0.01$) indicating poorer retention. The results show that administration of gangliosides enhances consolidation phases of learning as determined by better retention of an acquired task. This enhancement may be due to an increase in the levels of ganglioside in neuronal membranes or to more rapid maturation of neurons. Preliminary data show some enhancement of learning behavior (retention) in these rats at maturity.

1. Karpiak & Rapport(1979), Behav. & Neural Biol., 27:146.
2. Kasarskis et al.(1981), Dev. Brain Res.,1:25.
3. Morgan & Seifert(1979), J.Supramol. Struc.,10:111.

172.2 INHIBITION OF GLUTAMATE RELEASE CORRELATES WITH AMNESTIC POTENCY OF PROLINE ANALOGS. Elizabeth Keller*, Richard M. Pico* and Joel L. Davis. Addiction Res. Lab., and Aging and Behavioral Biol. Res. Lab., VA Med. Center, Sepulveda, CA, 91343.

Van Harreveld et al., (Pharmacol. Biochem. and Behavior. 12: 533-541, 1980) have described the procedure for determining the amnestic potency of various amino acids. Briefly, chicks are injected intracerebrally with 10 µl/hemisphere of 300 mM amino acids, 1 min. after one-trial training to suppress the peck responce to a bead. Suppression was conditioned by coating the bead with an aversant (methyl anthranilate). Impaired memory was noted, 24 hr later, when animals with certain amino acids showed a higher peck rate than the control chicks when presented with an uncoated bead.

Memory processing, by correlation with spreading depression, is hypothesized to be caused by the effects of glutamate released into extracellular space. This glutamate effect is inhibited by L-proline and several of its analogs (Van Harreveld et al., Pharmacol. Biochem. and Behavior. 12: 533-541, 1980). It is proposed that proline's amnestic potency is due to postsynaptic competition for glutamate receptors on dendritic plasma membranes (Cherkin and Van Harreveld, Brain Res. 156: 265-273, 1978). Alternatively, it has been suggested that the mechanism may involve the inhibition of glutamate released from presynaptic compartments (Keller et al., J. Neurochem., in press). The following study was initiated to strengthen this hypothesis by establishing a correlation between the amnestic potency of proline analogs and their potency as glutamate release inhibitors.

To determine the effect of proline analogs on glutamate release minislices of ectostriatum from white leghorn chicks (strain B-300, 3 days old, 35 grams) were superfused with physiological salt solution containing suitable concentrations of proline analogs and depolarized with 45 mM K^+. Efflux was collected before and after stimulation and the released endogenous glutamate measured by an enzymatic assay (Graham and Aprison, Analyt. Biochem. 15: 487-497, 1966).

These studies show that D-proline has no effect on glutamate release, that L-proline inhibits release by approximately 50% and DL-3,4-dehydroproline inhibits glutamate release by about 75%. This very closely approximates the relative changes in retention scores that measure memory impairment, thus suggesting that L-proline's amnestic potency is related to inhibition of glutamate release.

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172.3 ACTIONS OF 4-OH AMPHETAMINE ON ACTIVE AVOIDANCE CONDITIONING AND REGIONAL BRAIN CONCENTRATIONS OF NOREPINEPHRINE AND DOPAMINE. Joe L. Martinez, Jr., Koichi Ishikawa*, Tracy J. Hannan*, K.C. Liang*, Beatriz J. Vasquez, Robert A. Jensen, Debra Sternberg, Cathy Brewton* and James L. McGaugh. Department of Psychobiology, School of Biological Sciences, University of California, Irvine, CA 92717, U.S.A.

Previously, we reported that 4-OH amphetamine (4-OH AMPH) enhanced retention of an inhibitory avoidance response (Neurosci. Abs., 1978, 4, 261) and that this effect was abolished by adrenal demedullation (Neurosci. Abs., 1979, 5, 320). The purpose of these experiments was to investigate the enhancing effect of 4-OH AMPH on memory in a one-way active avoidance task and to measure the effect of 4-OH AMPH on brain catecholamine concentrations.

Male ARS Sprague-Dawley rats (90 days old), either sham-operated (SHAM) or adrenal demedullated (ADMX), were given eight trials of training in an active avoidance task on Day 1 (640 µA footshock). Immediately after training, the rats received either SAL, .41, or .82 mg/kg 4-OH AMPH, i.p. A dose of .82 mg/kg significantly enhanced the number of avoidance responses made by the SHAM animals on Day 2 ($p<.02$). Neither dose of 4-OH AMPH had an effect in the ADMX rats. Thus, as in inhibitory avoidance conditioning, the enhancing actions of 4-OH AMPH on memory mechanisms are dependent on the integrity of the medulla.

In the second experiment, normal rats were administered either SAL, .82, or 8.2 mg/kg 4-OH AMPH i.p., and were sacrificed either 10, 30 or 60 min following injection. Regional brain and adrenal concentrations of norepinephrine (NE), dopamine (DA), and epinephrine (E) were determined by means of high performance liquid chromatography with electrochemical detection. A dose of .82 mg/kg 4-OH AMPH significantly reduced concentrations of DA in the amygdala ($p<.05$) and hippocampus ($p<.05$) 60 min following injection. The higher dose of 8.2 mg/kg 4-OH AMPH reduced concentrations of DA at the 60 min time point in the amygdala ($p<.01$), cortex ($p<.01$), striatum ($p<.01$), and hippocampus ($p<.01$). NE was significantly reduced in the amygdala ($p<.01$), cortex ($p<.01$), hypothalamus ($p<.01$), hippocampus ($p<.05$), midbrain ($p<.05$), and adrenal glands ($p<.05$). With the high doses of 4-OH AMPH, E was also reduced in the adrenals ($p<.01$), 60 min after treatment.

These results suggest that 4-OH AMPH affects both brain and adrenal catecholamines and that the behavioral effects of 4-OH AMPH may be due to a dual action on both central and peripheral catecholamine systems.

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172.4 RELATIONSHIPS AMONG AMPHETAMINE-INDUCED MEMORY IMPROVEMENT, AFFECT, AND MOTOR BEHAVIOR Geoff Carr* and Norman White, Department of Psychology, McGill University, 1205 Dr. Penfield Ave., Montreal H3A 1B1 Canada.

The dopamine (DA)-containing neurons with cell bodies in pars compacta have been implicated in the mediation of the retention of learned behavior, affect (reward and aversion), and motor behavior. Amphetamine (AM), a drug which facilitates DA neurotransmission, also influences memory, affect and motor behavior. Therefore, we used AM to study the relationship among these putative behavioral functions of the DA neurons. In Experiment 1 rats were given the training trial of a retention test (pairing of tone and shock) followed by an IP injection of 2 mg/Kg d-amphetamine sulphate. They were then placed into an observation cage where general activity was measured by photocells and stereotypy was measured by observation. Next day the rats were tested for retention (conditioned suppression of licking). The AM-injected rats showed significantly better retention than saline-injected or 1 hour delay, AM-injected control rats. The degree of improvement in retention produced by the AM was significantly correlated with the amount of stereotypy produced by the same injection ($n=22$, $r=0.43$), but not with the amount of increase in general activity ($r=-0.25$). In Experiment 2 water-deprived rats that drank a novel-tasting solution, were injected with AM, and their motor behavior measured. Next day the AM-injected rats drank significantly less solution than saline-injected or water-paired, AM-injected control rats. The strength of the conditioned taste aversion (negative affect) produced by the AM injection was significantly correlated with the increases in general activity produced by the same injection ($n=36$, $r=0.32$), but not with the amount of stereotypy ($r=0.07$). These data link the stereotypy-motor and the retention-improving actions of AM, and they link the general activation and negative affective actions of the same drug. AM-produced stereotypy has been associated with DA terminals in caudate, and increases in general activity with DA terminals in nucleus accumbens. To test the hypothesis that AM produced the pairs of correlated effects by acting on DA terminals in these two brain areas, rats were prepared with chronic cannulae aimed at lateral caudate or accumbens and tested for the effects of intracerebral AM (10ug in 1uL over 30 sec) in the Retention and CTA experiments. AM in accumbens produced significant increases in general activity, but only small increases in stereotypy. AM in caudate produced significant increases in stereotypy, but only small increases in general activity. Neither group showed a CTA. AM in accumbens had no effect on retention. AM in caudate significantly improved retention compared to controls, and the degree of improvement was negatively correlated with stereotypy ($n=8$, $r=-0.54$), and with general activity ($r=-0.25$). These data suggest that the pairs of linked effects we observed with peripheral injections of AM may not be mediated by the same neural mechanisms.

172.5 EFFECTS OF PHENCYCLIDINE ON SENSORY, MOTOR, MOTIVATIONAL, LEARNING, AND MEMORY FUNCTIONS. John D. Hardy* and Raymond P. Kesner (Spon: B. Grosser). Dept. of Psychol. Univ. of Utah, Salt Lake City, UT. 84112.

Phencyclidine (PCP) in humans has been shown to have marked effects on sensory, perceptual, motivational, mnemonic, and cognitive functions. Because of the paucity of data on the effects of PCP in humans and because of ethical concerns, it was deemed of importance to develop an animal model of PCP effects. Thus, the present study was designed to investigate systematically the effects of PCP on a variety of behaviors. Under the influence of different doses of PCP (4-24 mg/kg, i.p.) different groups of rats were tested for (a) ability to detect odors, visual square, touch, and pain, (b) ability to perform placing, righting, and grasping reflexes, (c) activity level, emotionality, and habituation in an open field, (d) illness effects and water intake, (e) acquisition and retention of active avoidance, and (f) performance on an 8-arm radial maze.

Data showed that low doses of PCP (4, 8 mg/kg) have profound effects on learning, memory, and activity level. Specifically at doses of 4 mg/kg there was (a) an increase in activity level which appeared to be a function of impaired habituation, and (b) an impairment in active avoidance learning. At doses of 8 mg/kg there was (a) no effect on pain threshold to shock, (b) a marked reduction in activity level, but no changes in emotionality, (c) a profound deficit in active avoidance learning, but no effect on subsequent retention with injections prior to training, immediately after training or prior to the retention test, and (d) a profound disruption of errorless performance on the radial arm maze.

Data also indicated that higher doses of PCP (12, 16, 24 mg/kg) produce disruption of sensory, motor, and motivational function. More specifically, at doses of 12 mg/kg, there were marked disruptive effects in detection of odors, visual square, and touch as well as performance of placing, righting, and grasping reflexes. At doses of 16 and 24 mg/kg there was a decrease in water intake, but no illness effects. In conclusion, PCP has a myriad of behavioral effects. Furthermore, it has been possible to demonstrate that higher-order behavioral functions (i.e., learning, and habituation) are affected by low doses of PCP, whereas detection of sensory input and motor reflexes are affected only by high doses of PCP.

172.6 THE EFFECTS OF ATROPINE AND BENACTYZINE ON SHORT-TERM MEMORY IN THE MONKEY. J.H. McDonough and D.M. Penetar*. U.S. Army Biomedical Laboratory, Aberdeen Proving Ground, MD 21010.

Cholinergic mechanisms have been implicated in both long and short-term memory (Bartus and Johnson, Pharm. Biochem. and Behav., 1976, 5, 39-46; Davis et al., Science, 1978, 201, 272-274). Scopolamine disrupts short-term memory in a dose-related fashion. Scopolamine's effects were potentiated by increasing retention intervals. The effects of other anticholinergics have not been systematically explored.

Dose-response curves for the anticholinergics atropine and benactyzine were generated on a delayed matching-to-sample task in rhesus monkeys. Subjects initiated trials by responding on a center lever for brief flashes of the sample (colors of red, blue or green) on a backlit key. Match stimuli appeared on three additional backlit keys after delays of 0, 4, 8 or 16 sec. Placement of match stimuli and delays were randomized. Session length was 200 food reinforced trials. The doses of atropine were 0.014, 0.044, 0.14, 0.25, and 0.44 mg/kg. The doses of benactyzine were 0.057, 0.18, 0.57 and 1.8 mg/kg. The preinjection times were equated for onset of central effects. Atropine was injected (i.m.) 45 minutes prior to the session while benactyzine was injected (i.m.) 15 minutes prior. Dependent variables were percent correct matches for each delay and session time.

Under baseline and saline control conditions, retention accuracy ranged from 92% at the 0 sec delay to 76% at the 16 sec delay. Atropine produced a graded effect on accuracy at all delay intervals. The highest dose tested produced severe memory deficits. Performance was reduced to 51% at the 4 sec delay, 45% at the 8 sec and 34% (chance) at the 16 sec delay. For all doses tested, animals responded steadily but at a reduced rate. Session lengths revealed dose-related increases of up to 6 hrs.

Benactyzine produced dose-related effects on short-term memory but only at the 4, 8 and 16 sec delays. Benactyzine's effects were much less severe than atropine's. The highest dose produced 66% correct responses on the 16 sec delay. Additionally, the nature of benactyzine's effects appear to be dissimilar. Benactyzine produced an initial response suppression followed by a period of responding similar to baseline performance.

At this time it is not known whether the differences between the two drugs are due to the relative differences in duration of action or possibly to differences in their anticholinergic mechanisms of action.

172.7 CENTRAL CHOLINERGIC FUNCTION AND SHORT-TERM MEMORY IN THE RAT. D. G. Spencer. Dept. of Psychology, Indiana University, Bloomington, IN 47405.

Systemic administration of scopolamine (a cholinergic muscarinic receptor antagonist) and hippocampal lesions are quite similar in behavioral effect. Performance on memory procedures is particularly sensitive to these treatments. Since the hippocampus has a high concentration of muscarinic receptors, the goal of the present study was to examine the interaction between systemically administered scopolamine and selective hippocampal muscarinic blockade on performance of a short-term memory task.

Sixteen male Sprague-Dawley rats were trained on a continuous non-matching to sample (CNM) task in which lever pressing was reinforced on 5 sec discrete trials on which the brightness of a panel light was different from that of the previous trial. Intervals between successive trials (retention intervals) varied between 2.5, 5, and 10 sec. Lever presses were analyzed for effects on the signal detection theory variables of sensitivity (performance accuracy) and bias (degree of responsiveness). Eight other rats were trained to perform on a superficially similar discrimination task in which lever presses were reinforced only during trials with one particular panel light brightness.

All rats were then bilaterally implanted with stainless-steel cannulae in one of three locations: dorsal hippocampus, ventral hippocampus, or caudate nucleus. After performance stabilized, all subjects received both systemic and central drug administration. The former consisted of i.p. injections of saline, scopolamine hydrobromide (0.125, 0.25, and 0.50 mg/kg), and d-amphetamine sulfate (0.50 and 1.0 mg/kg). The latter consisted of two 1 uL bilateral injections: one of 10 ug/uL scopolamine in Ringer's solution and one of Ringer's solution alone.

Whether given systemically or centrally in either hippocampal location, scopolamine decreased sensitivity to an equal extent on trials following all three retention intervals in both CNM and discrimination tasks. Scopolamine did not affect bias except at the highest systemic dose (0.50 mg/kg) and when injected into the caudate nucleus. In both cases bias decreased, however this effect produced by caudate injection was not accompanied by decreased sensitivity. Systemic injections of d-amphetamine at both doses increased bias and the higher dose (1.0 mg/kg) decreased sensitivity as well.

These results have two major implications. First, since scopolamine equally disturbed performance following the three retention intervals in both tasks, it interferes with performance by disrupting processes other than retention. Second, the hippocampal muscarinic receptors are specifically involved with the short-term memory effects produced by systemic scopolamine.

172.8 FOLLOWING SCOPOLAMINE INJECTIONS, SHORT-TERM AND LONG-TERM HABITUATION IS INTACT AS MEASURED BY THE DURATION OF INVESTIGATION OF A NOVEL OBJECT BY GERBILS. MaryLou Cheal. Neuropsychology Laboratory, McLean Hospital and Harvard Medical School, Belmont, MA 02178.

Mongolian gerbils allowed to investigate a novel object for one minute show significant habituation on a second trial. Habituation is shown whether the second trial occurs 60 seconds later or up to four weeks later. Thus, this paradigm, stimulus-elicited investigation, allows the study of both short-term and long-term habituation. In gerbils given scopolamine hydrobromide (0.01-10.0 mg/kg S.C.) 20 minutes before Trial 1, habituation of the duration of investigation was observed on Trial 2. Habituation was observed when Trial 2 occurred 60 seconds after Trial 1 and when Trial 2 was 24 hours later. Therefore, intact memory can be inferred.

However, by also measuring the number of approaches to the stimulus, it is possible to provide an additional interpretation of the data. The number of approaches to a cup was greater in gerbils given 0.1, 1.0, or 10.0 mg/kg scopolamine than in those treated with lower doses or vehicle. The larger number of approaches was seen on the first trial after drug injection, on a second trial 24 hours later, and in gerbils habituated to the cup before drug injection. Frequency and duration measures of 24 hour habituation did not differ across doses in gerbils given methyl scopolamine (0.1-10.0 mg/kg), indicating a central effect of scopolamine on the frequency of habituation 24 hours after treatment.

In scopolamine-treated gerbils, the number of approaches was greater with no difference in total duration, resulting in a shorter mean duration per approach. The shorter duration per approach suggests a decreased ability to maintain attention. A parsimonious interpretation of much controversial data in the literature is that animals can choose and respond to stimuli following scopolamine injections, but are unable to maintain attention long enough to do so when the stimulus requires more than a minimal amount of time and effort. Thus, in this simple paradigm of investigation of an object, habituation can be demonstrated in scopolamine-treated gerbils.

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172.9 ADMINISTRATION OF ENKEPHALIN ANALOGUES INTO THE AMYGDALA CENTRAL NUCLEUS: EFFECTS ON PAVLOVIAN CONDITIONED HEART RATE IN RABBITS. M. Gallagher, B. S. Kapp, J. P. Pascoe* and C. D. Applegate*. Depts. of Psychology, Univ. of North Carolina, Chapel Hill, NC 27514 and The Univ. of Vermont, Burlington, VT 05405.

Previous results from our laboratory demonstrated that administration of opiate agents into the amygdala central n. altered the acquisition of Pavlovian conditioned heart rate in rabbits (Soc. Neurosci. Abstr. 6, 168, 1980). Whereas opiate agonist administration impaired conditioned heart rate responding, opiate antagonist administration alone augmented conditioned responding. Since opioid peptides are highly concentrated within the central n. of the amygdala, the effects of opiate antagonist administration into this region may be due to blocking endogenous enkephalin activity. Consequently, the effects of enkephalin analogue administration into the central n. on conditioned heart rate were assessed.

New Zealand rabbits were surgically prepared with cannulae positioned at the dorsal surface of the central nucleus. Two weeks later all animals were trained using a standard Pavlovian conditioning procedure. Drug injections were delivered in a 1.0 µl volume immediately prior to the conditioning session. Two opioid peptides were used, D-Ala^2, Met^5-enkephalin (DALA) and D-Ala^2, D-Leu^5-enkephalin (DLEU).

Conditioned heart rate responses for UNOPERATED and VEHICLE-injected control groups did not significantly differ. Compared to the VEHICLE group, administration of DALA (2.0 or 1.0 µg) significantly attenuated conditioned heart rate responding. This effect of DALA (2.0 µg) was blocked by concurrent administration of the opiate antagonist naloxone. Statistical analysis performed on the conditioned response data for the VEHICLE group and the groups which received injections of DLEU (2.0 or 1.0 µg) revealed no significant differences among these groups.

Previous studies have indicated that the DLEU analogue preferentially labels delta receptors found in high concentration within the central n. of the amygdala (Chang et al., Mol. Pharm. 16, 91-104, 1979; Goodman et al., Proc. Nat. Acad. Sci. USA 77, 6239-6243, 1980). Since the effects on conditioning observed in this study following DALA administration were not observed following comparable doses of DLEU, it appears that delta receptor activity within the central n. may not contribute to conditioning processes. The possibility that mu receptor activity may play a role in conditioning processes is consistent with the present findings as well as our previous report that administration of opiate agents alters the acquisition of conditioned heart rate.

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172.10 DIFFERENT EFFECTS OF 1,4- and 1,5-BENZODIAZEPINES ON ECS-INDUCED AMNESIA IN A PASSIVE AVOIDANCE TASK. F.J. Hock*, H.J. Kruse* and U. Schindler*. (SPON: J.-P. EWERT). HOECHST AG, CASSELLA AG, D-6230 Frankfurt 80, Germany.

1,4-benzodiazepines have been reported to induce anterograde amnesia in humans. Therefore we investigated the effect of diazepam, a 1,4-benzodiazepine in an one-trial dark avoidance paradigm in mice and compared it with clobazam, a 1,5-benzodiazepine. The apparatus consisted of a light (L) and dark (D) compartment, connected by a guillotine door. During the acquisition trial (AT) an unavoidable footshock (FS, 1 mA, 1.0 sec) was delivered immediately after entering D, followed by a maximal electroconvulsive shock (ECS, 15 mA, 0.2 sec). The retention trial was run 24 hrs later. The latency time for entering D was measured during the acquisition and retention period (cut-off time: 300 sec). Drugs were administered orally 90 min prior to both AT and RT.

Diazepam at 1 and 2 mg/kg caused a decrease in latency time during the retention trial by 39 and 33 % respectively, while clobazam at 12.5 and 25 mg/kg brought about a marked increase by 187 and 196 % over control. Lower doses of both drugs had no effect.

In other tests on protection against hypoxia in mice (induced by sodium nitrite, brevatonal, or asphyxia) both drugs were active, diazepam being even more potent than clobazam. The ED50 values for the prolongation of asphyxia survival time was 3.8 mg/kg for diazepam and 22 mg/kg for clobazam after oral administration.

The results indicate that memory consolidation and/or retrieval in mice may be impaired by diazepam but not affected or enhanced by clobazam. Protective effects of tranquilizers against hypoxia may be due to sedation and/or anxiolytic activity.

172.11 PHARMACOLOGICAL PROTECTION AGAINST HYPOXIC AND ELECTRO-BRAINSHOCK DISRUPTION OF AVOIDANCE RETRIEVAL IN MICE. E. Gamzu and L. Perrone* Dept. of Pharmacology, Hoffmann-La Roche Inc., Nutley, N.J. 07110

Deficits of learning and memory are common to many clinical problems ranging from minimal brain dysfunction in children to senile dementia in the elderly. The present studies were designed to establish a model of one aspect of clinical amnesia - retrieval failure - by using two distinct disruptors, hypoxia and subconvulsive electro-brainshock (EBS), and to determine whether clinically used compounds such as magnesium pemoline (MP), piracetam (P), and vincamine (V) protected against these disruptors.

Naive male CF1 mice received 10 training trials at one minute intervals. On each trial a mouse was placed into the apparatus. After 10 seconds, an electric shock (1mA) was applied via the grid floor for 15 seconds. The mouse could either avoid or escape the shock by entering a second unshocked compartment through a small opening. Twenty-four hours later drug or vehicle was given orally 60 min. before 10 trials of retention testing. Hypoxia, EBS or sham treatment preceded the retention test by 5 min. Hypoxia consisted of 8 seconds exposure to pure CO_2 (normal air for shams). EBS (10 mA,, 10 mS, 60 Hz for 200 mS) was applied transcorneally via a DC stimulator (the power was turned off for shams).

At testing, the mean % avoidance response for shams was 80% (the means for groups of 5 ranged from 74-90%). The mean for mice receiving EBS was 24% and for hypoxia 40%. Control experiments have shown that the disruption is not the result of motor deficits, since learning is not affected.

MP and P protected dose-dependently against both types of disruption. The doses (in mg/kg) at which peak % recovery occurred after EBS and hypoxia, respectively, were MP-(30) 91%, (30) 86%; P-(1000) 89%, (1000) 92%.

The following compounds were inactive against EBS disruption and were not tested against hypoxia: amphetamine, chlorpromazine, clonidine, diazepam, hydergine, imipramine, and physostigmine. V produced a 43% recovery of function against EBS disruption at a single dose.

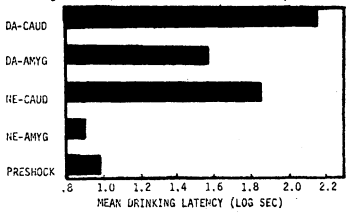
Neither stimulant nor anticonvulsant activity alone is sufficient for the effect. The procedures appear useful for evaluating potential memory enhancers.

172.12 NOREPINEPHRINE AND DOPAMINE: DIFFERENTIAL EFFECTS OF MANIPULATION OF THE AMYGDALA AND CAUDATE ON AVERSIVE INFORMATION PROCESSING. Maureen E. Ellis and Raymond P. Kesner (SPON: S.W. Miller). Dept. of Psychology, Univ. of Utah, SLC, UT 84112.

Based on substantial evidence from the electrical brain stimulation (EBS) and the lesion literature, both the amygdala and the caudate have been implicated in the processing of aversive information. EBS and lesion techniques might disrupt memory processes by influencing neurotransmitter function within the particular brain substrate. Two closely related transmitters, norepinephrine (NE) and dopamine (DA), are differentially distributed in the amygdala and caudate. Since recent evidence has also implicated the importance of central catecholamine systems in learning and memory, the present study was designed to investigate the effects of manipulation of the NE and DA systems of the amygdala and caudate on retention of a passive avoidance training experience.

Water deprived rats were trained to enter a goal box in order to drink from a tube containing water. After reaching a predetermined latency criterion, rats were given a single 3 sec, 3 mA footshock and then tested for retention 24 hr later. Immediately following footshock, all rats were injected with either NE (1 ug) or DA (1 - 10 ug) via bilateral, chronic, stainless steel cannulus implanted in either the amygdala or the caudate. Retention was evaluated as an increased latency to approach the drinking tube relative to pre-shock latency.

Rats injected with 1 ug NE in the amygdala did not demonstrate retention (amnesia) when tested in the goal box 24 hr following footshock. In contrast, rats treated with DA in the amygdala or with either NE or DA in the caudate displayed excellent retention in the 24 hr test. Furthermore, the data suggested that the DA-caudate group had enhanced retention when compared to the DA-amygdala rats. The overall results support a crucial role for the NE, but not the DA, system of the amygdala in aversive information processing. Conversely, DA, but not NE, in the caudate may have some function in passive avoidance learning.



172.13 DRUG EFFECTS ON THE DELAYED MATCH-TO-SAMPLE PERFORMANCE OF SQUIRREL MONKEYS. E. Schwam*, L. Rumennik*, J. Sepinwall, and L. Cook*, Dept. of Pharmacology, Hoffmann-La Roche Inc., Nutley, N.J. 07110

The search for pharmacological agents for treatment of human cognitive dysfunctions necessitates the development of preclinical research strategies and methods for evaluating drug effects on non-human learning and memory. Two such strategies have been to identify drugs that will either enhance performance under normal conditions or protect against induced deficits in performance. Compounds considered important to study in this regard include certain stimulants, muscarinic agonists, cholinesterase inhibitors, anti-hypoxic agents, cerebral metabolic agents and hypothalamic and pituitary peptides.

The effects of eight representative compounds were examined on the three-choice match-to-sample performance of highly trained adult male squirrel monkeys under normal conditions. Each trial of the task consisted of two successive components. First, animals were required to select the choice stimulus that matched the simultaneously presented sample stimulus. Following two consecutive correct matches all keys were darkened for a fixed interval (less than 30 sec.) after which the three choice keys only were illuminated. Responding to the correct choice key after the delay interval (delayed matching) resulted in food presentation.

All drugs were orally administered with one dosing per week. d-Amphetamine improved performance at the lowest dose tested (0.05 mg/kg) but severely disrupted performance at the highest doses tested (0.60 and 0.80 mg/kg). Increases in accuracy were seen with strychnine (at 0.25 and 0.50 mg/kg) but these were not consistent.

Diazepam and the anticholinergic scopolamine produced relatively few deviations from baseline at the lowest doses tested (0.625 and 0.0125 mg/kg, respectively) but progressively decreased matching accuracy as the doses were increased. No consistent effects have been seen thus far during a preliminary evaluation of the cholinesterase inhibitor physostigmine (0.0013-0.04 mg/kg).

The purported "nootropic compounds" piracetam (100-300 mg/kg) and Hydergine (a combination of three hydrogenated ergot alkaloids; 6.25-50 mg/kg), and the anti-hypoxic agent phenformin (1.88-7.5 mg/kg) failed to improve performance.

172.14 THE EFFECTS OF PIRACETAM, VINCAMINE, AND d-AMPHETAMINE ON MONKEY ELECTROCORTICOGRAM & VI 60 LEVER RESPONSE RATE. K.L. Keim and T. Smart*. Dept. Pharmacology, Hoffmann-La Roche Inc., Nutley, N.J. 07110.

We have shown drug-specific frequency changes in the electrocorticogram (ECoG) of unrestrained squirrel monkeys and have regarded these as measures of selective drug action (Keim et al., Neurosci. Abstr. 6: 366, 1980). Our present study compares the effect of 800, 1600, & 2400 mg/kg piracetam (PIRC; 2-pyrrolidinone acetamide) and 5 & 10 mg/kg vincamine (VINC; an alkaloid obtained from *Vinca minor*), and 0.1, 0.3, & 1.0 mg/kg d-amphetamine (AMPH) on the ECoG during an operant task. The "nootropic agents" have had clinical application in treating cerebral insufficiencies, senility, etc.; d-amphetamine is a known psychostimulant.

Drugs were administered intragastrically 60 min prior to testing food-restricted monkeys (80% ad lib wt) on a VI 60 sec schedule of food reinforcement. The session was 90 min. Telemetered ECoG activity from the anterior cortex was quantified and the 0-32 Hz range was subdivided by computer into 8 bands for spectral frequency analysis. Each monkey 2-6 per dose) was run on two consecutive days: vehicle (5% acacia) was given on Day 1 and drug on Day 2. Data are reported as the difference between drug and vehicle runs.

The 0-32 Hz wideband ECoG was not significantly altered by either PIRC, VINC, or AMPH. PIRC had little effect on the distribution of ECoG frequencies within the 0-32 Hz range, however, there was a tendency to lessen 4-8 Hz and augment 8-16 Hz activity. 10 mg/kg VINC significantly decreased 4-8 Hz and increased 20-32 Hz ECoG activity. In contrast, 1 mg/kg AMPH significantly decreased 2-4 Hz and increased 4-12 Hz ECoG activity.

PIRC enhanced and VINC attenuated VI response rate, but not significantly. Emesis was observed in 1 of 4 and in 3 of 6 monkeys following 5 or 10 mg/kg VINC, respectively. AMPH disrupted behavior following 0.3 and 1.0 mg/kg which markedly decreased VI response rate.

We found significant changes in the spontaneous EEG of primates following treatment with vincamine, but the changes were not of large magnitude. The VINC-induced ECoG alterations differed qualitatively the encephalotropic effects produced by the psychostimulant AMPH. This nondeficit animal model - shown to be sensitive to many other types of pharmacological agents - appears to be meaningful though may not be the most sensitive for evaluating the central effects of such compounds generally classified as "nootropics."

172.15 THE EFFECTS OF TRIMETHYLTIN ADMINISTRATION ON PASSIVE AVOIDANCE CONDITIONING IN RATS. E. Bostock*, T. J. Walsh, M. Gallagher, and R. S. Dyer (SPON: R. Musty). Dept. of Psychology, Univ. of North Carolina, Chapel Hill, NC 27514, and Div. of Neurotoxicology, U.S. Environmental Protection Agency, Research Triangle Park, NC 27711.

Trimethyltin (TMT) is a neurotoxic organotin compound. Human exposure to TMT has been reported to induce a variety of behavioral changes including memory deficits, hyperactivity, anorexia, visceral pain, and generalized seizure activity (Fortemps et al., Int. Arch. Occup. Environ. Hlth. 41:1-6, 1978). Recent studies of TMT toxicity report that this compound produces brain damage with a high degree of selectivity for limbic system structures (Dyer et al., in press; Brown et al., Am. J. Path. 97:59-82, 1979). The purpose of the present study was to examine the effects of TMT administration on behavioral measures sensitive to limbic system damage.

TMT was administered by gavage to male Long-Evans hooded rats. Rats were administered either 0 (n=8), 5 (n=8), 6 (n=11), or 7 (n=6) mg/kg TMT in 0.9% saline. Twenty-one days following TMT administration rats were tested on a passive avoidance task using a 0.6 mA, 1 sec footshock following the procedure described by Gallagher et al. (Sci. 198:423-425, 1977). Approximately 1 week later jump flinch shock thresholds were determined.

A Kruskal-Wallace one-way analysis of variance of passive avoidance retention latency data revealed a significant difference among groups ($p<.001$). Doses of 5, 6, or 7 mg/kg TMT produced significant retention latency deficits compared to controls (Mann-Whitney U, two-tailed, $p's<.02$). A Kruskal-Wallace one-way analysis of variance performed on jump flinch thresholds revealed no significant difference among groups.

The results of jump flinch threshold tests and information from a previous study (Dyer et al., in press) indicate that retention latency deficits are probably not produced by a change in sensitivity to sensory stimuli. The reported limbic system toxicity of TMT (Dyer et al., in press; Brown et al., Am J. Path., 97:59-82, 1979) suggests that passive avoidance retention deficits may be attributable to damage to limbic system structures. This premise is further supported by our observations that TMT treated rats exhibit deficits in radial arm maze performance (Walsh et al., Soc. Neurosci. 1981).

The effects of TMT administration on an active avoidance task will be studied to further elucidate the behavioral correlates of TMT toxicity.

172.16 ENVIRONMENT - SPECIFIC CONDITIONED ACTIVITY WITH d-AMPHETAMINE AS THE UNCONDITIONED STIMULUS. R.J. Beninger and B.L. Hahn* Dept. of Psychology, Queen's University, Kingston, K7L 3N6, Canada.

Several Studies have shown that the unconditioned increase in behavioral activity produced by the stimulant drug, d-amphetamine can be conditioned to stimuli associated with injections (e.g. Tilson & Tech, Pharmacol. Biochem. Behav. 1973, 1, 149-153). However, the precise stimuli that control this conditioned responding (for example, temporal stimuli, stimuli associated with preinjection handling, the injection itself, or stimuli from the test environment) remain unclear. The present study was undertaken to test the role of environmental stimuli in conditioned activity. Two environments (E_1 and E_2; 40 x 50 x 41 cm high), differed in floor texture, level of illumination and color (black vs. white); their floors were demarcated into 10 cm squares. Forty-eight male Wistar rats received a one hr habituation exposure to one environment on two consecutive days; half received E_1 then E_2 and half received the other order. On each of the next 5 days, each rat received two 30-min sessions; the first was an habituation session in one environment and the second was a conditioning session in the other. Again, half received one order of environments and half the other. Conditioning sessions were preceded by i.p. injection with saline (n=24), 2.5 mg/kg (n=12) or 5.0 mg/kg (n=12) d-amphetamine sulphate. On the next day, all animals received a saline injection prior to the one test session; half of each group was tested in the conditioning (same) and half in the other (different) environment. Dependent measures were number of squares entered and stereotypy rating taken during a 30 sec observation period at 5, 10, 20 and 30 min into the session by two "blind" raters. Results revealed significantly greater number of squares entered and higher stereotypy ratings in the same vs. the different environment for the d-amphetamine groups at the 10 min observation period; the saline animals showed no environment effect at this time. These results suggest that environmental stimuli play an important role in mediating conditioned activity with d-amphetamine as the unconditioned stimulus. (Supported by the MRC.)

173.1 POST-TETANIC DEPOLARIZATION OF FROG PRIMARY AFFERENT FIBERS. R.A. Davidoff and J.C. Hackman. Neurology Service, V.A.M.C. and Department of Neurology, University of Miami School of Medicine, Miami, FL 33125.

Tetanization of a frog dorsal rool (DR) results in a sustained negative shift of potential followed by a hyperpolarization of the fibers contained in an adjacent (passive) DR (Davidoff and Hackman, J. Physiol. 302:297, 1980). Our present experiments show that when sucrose gap recordings are made from the stimulated (active) DR of the isolated, hemisected frog spinal cord superfused with HCO_3^-- buffered Ringer's solution (15°C), an additional potential change is found--the sustained negative shift is succeeded by a slow (5 to 60 sec), large (5 to 12 mV) depolarizing after-potential (DAP). A long-lasting (1 to 4 min, 2 to 6 mV) hyperpolarization follows the DAP.

The DAP could be produced by tetanic electrical stimulation of a lumbar DR at frequencies ranging from 10 to 100 Hz, for durations of 5 to 30 sec provided that the DR was stimulated with voltages greater than 3X the threshold for producing a detectable dorsal root potential. Both the magnitude and duration of the DAP were monotonically related to the stimulation frequency, stimulating current strength, and tetanus duration.

The DAP originated in the cord since it was eliminated by sectioning the cord between the entrance of the DR and the primary afferent terminals. It did not result from a GABAergic effect since it was increased by GABA blockers. On the other hand, the magnitude and amplitude of the DAP were proportional to $[K^+]_o$ as measured with K^+-sensitive microelectrodes. And it was sensitive to procedures which altered $[K^+]_o$. For example, it was increased by picrotoxin and bicuculline which increase K^+ efflux, and was decreased by pentobarbital which reduces K^+ efflux; its amplitude and duration were augmented when K^+ clearance was reduced by blocking the Na^+ pump (ouabain, cooling, substitution of Li^+ for Na^+); it was increased by perfusion with Ringer's solution containing OmM K^+. That at least part of the responsible rise in $[K^+]_o$ is Ca^{++}-dependent is suggested by the finding that low concentrations of Mn^{++} (50 M) reduced or abolished the DAP.

These observations show that repetitive stimulation of primary afferents is followed by a long-duration depolarization of the stimulated fibers. The results are consistent with the view that this potential change is at least partly a consequence of a transient excess of $[K^+]_o$. (Supported by VAMC Funds, MRIS 1769).

173.2 SPINAL CORD ASYMMETRIES FOLLOWING PARTIAL DEAFFERENTATION. H. Richard Koerber, Dept. of Physiology, West Virginia Univ. Medical Ctr., Morgantown, WV 26506.

In recent years it has become apparent that quite substantial interanimal variation can be observed in the dorsal horn somatotopic map. It is very difficult, therefore, to identify any possible plastic changes in dorsal horn somatotopy following partial deafferentation when combining data from many animals. Recent studies have concluded that projections from cutaneous nerves and single cutaneous afferents from symmetrical locations on the skin project to symmetrical locations in the spinal cord laminae III and IV. Others indicate that dorsal horn cells with receptive fields from symmetrical locations on the hindlimbs are located in relatively symmetrical locations in the spinal cord.

This study was designed to examine in detail the symmetry of the postsynaptic somatotopic map, and determine what asymmetries exist following partial deafferentation. Parallel electrode penetrations were made every 250 um across the width of the spinal cord. Single units were isolated and their receptive fields recorded on one of three standard cat leg drawings. Once the receptive field was recorded the position of the electrode tip was marked by passing 1 uA of AC current for 30 sec. Once the electrode tracks had been reconstructed the dorsal horns were divided into 10 bins using decile lines drawn through the laminar boundaries. Recording sites located in symmetrical bins at the same rostrocaudal level were used as "matched pairs" for statistical tests of symmetry.

Results from unoperated animals indicate a very high degree of symmetry with respect to receptive field areas, (cm^2), receptive field positions (distance from toes, cm) and receptive field geometries (length/width ratios). In chronically deafferented animals in which L6 and L7 dorsal roots were sectioned 30 days or >150 days prior to recording experiments, asymmetries were apparent. The major contribution to the asymmetries observed was the appearance of silent areas in the denervated dorsal horn. Asymmetries between responsive areas in the spinal cord can all be accounted for simply by the loss of afferent input from the severed roots.

This work was supported by USPHS grant NS12067 to P. B. Brown.

173.3 PATTERN OF CATECHOLAMINE DISTRIBUTION IN THE LUMBAR SPINAL CORD. G. D. Peca-Vogelsang, C. J. Hodge, Jr., R. T. Stevens, A. V. Apkarian, J. Franck, and O. M. Brown* Depts. of Neurosurgery and Pharmacology, Upstate Medical Center, Syracuse, NY 13210.

Most catecholamine containing terminals in the spinal cord are from suprasegmental sources. This study was undertaken to further elucidate the pattern of catecholamine (CA) distribution in the lumbar spinal cord using both glyoxylic acid induced histofluorescence (HF) and high performance liquid chromatography (HPLC).

Control lumbar spinal cords of cats were cut serially in five sections, L3-L7. Lesions placed between L3 and L4 included unilateral and bilateral ventral quadrant section, unilateral hemisection in conjunction with a mid-lumbar sagittal section and complete transection. The spinal cord segments were analyzed by HPLC as whole and either sagittally or horizontally hemisected pieces. To observe the orientation of the CA terminals, the spinal cord was cut at various angles and examined using HF.

In control animals, noradrenaline (NA) content increased 200-300% in the more caudal lumbar segments when compared to L3. In segments halved horizontally, the increase appeared more exaggerated in the dorsal horn. There was no obvious difference with HF between rostral and caudal segments. Distinct differences are seen, however, between the laminae of the spinal grey. Bilateral ventral quadrant section caused a large decrease in NA concentration. The segment above the lesion remained relatively the same. Unilateral ventral quadrant section produced a large decrease ipsilaterally and a substantial decrease contralaterally. L3 hemisection and a more caudal sagittal section left the cord ipsilateral to the hemisection completely depleted of NA but only had a moderate decrease in NA content contralateral to the caused lesion. Transection caused a complete depletion of NA below the lesion as measured by HPLC, although HF was still able to detect small quantities of NA associated with blood vessels along the median fissure.

These results indicate that there is a higher NA content in the more caudal lumbar segments, that descending NA fibers are located primarily in the ventral quadrant, and that there is substantial segmental crossing of descending NA fibers.

173.4 DORSAL ROOT POTENTIALS ELICITED BY LOCUS COERULEUS STIMULATION - DEPENDENCE ON INTACT NORADRENALINE STORES. A. V. Apkarian, C. J. Hodge, Jr., J. Franck, R. T. Stevens, and G. Peca-Vogelsang. Dept. of Neurosurgery, Upstate Medical Center, Syracuse, NY 13210.

Nucleus locus coeruleus (LC) contains noradrenergic (NA) cells, some of which project to the spinal cord. The LC cells constitute a major source of NA terminals within the lumbar spinal cord. Electrical stimulation of the LC pontine area causes strong modulatory effects on most lumbar dorsal horn interneurons receiving cutaneous input. This study was undertaken to investigate the effects of LC stimulation on afferent dorsal root terminals.

Cats were anesthetized with chloralose, the lumbar enlargement exposed and dorsal rootlets of L7-S1 mounted on bipolar Ag-AgCl recording wires. LC was located both stereotaxically and by recording from the mesencephalic trigeminal nucleus. The brain stem stimulation parameters used were: stimulus trains of 50 msec, biphasic constant current pulses lasting 200 usec with a 100 Hz frequency and amplitudes varying from 50 to 200 uA. The LC stimulation evoked dorsal root potentials (DRP) were compared with the DRP's evoked by stimulating the hindlimb either electrically or by touch.

The brain stem stimulation evoked negative DRP's were of longest duration when the stimulating electrode was in LC as compared to other positions within the brain stem. The latency of the LC stimulation evoked DRP's was about 50 msec. Advancing the stimulating electrode deeper into pontine reticular formation gave rise to DRP's with 10 to 20 msec. latency. Hindlimb stimulation evoked DRP's had latencies of only a few milliseconds. Both IV and topical application of alpha adrenergic blockers (phentolamine and phenoxybenzamine) abolished the LC evoked DRP but not the reticular or hindlimb evoked DRP's. Application of the beta adrenergic blocker propranolol had no effect. IV injection of an MAO inhibitor (imipramine) increased the duration of LC evoked DRP with no change in the latency and without affecting the hindlimb stimulation evoked DRP.

These results suggest that LC stimulation causes NA dependent primary afferent depolarization through alpha adrenergic receptors.

173.5 CATECHOLAMINE VARICOSITIES IDENTIFIED IN CAT DORSAL ROOT GANGLION AND SPINAL VENTRAL ROOTS. R. T. Stevens, C. J. Hodge, Jr., A. V. Apkarian, and G. D. Peca-Vogelsang. Dept. of Neurosurgery, Upstate Medical Center, Syracuse, NY 13210.

During an investigation of the catecholamine (CA) innervation of spinal grey matter, dorsal root ganglia and ventral roots were examined using the glyoxylic acid histofluorescence technique. Fibers containing CA were found in both the ventral roots and dorsal root ganglion at all spinal levels but not within the dorsal roots. The CA fibers in this region were classified into two types. One form was a single, fine, varicose fiber. The other form was a bundle of intertwined fibers which collectively produced an intense fluorescence. This type was generally associated with blood vessels and was presumed to be sympathetic in origin. Both forms of these CA nerve fibers were also present in the grey matter of the spinal cord.

To examine the source of these CA varicosities, lesions were made. These included a lesion of the spinal root distal to the DRG or a total spinal transection. In both cases, CA varicosities of both types remained in both the DRG and the ventral roots. Occasionally CA fibers were observed entering the DRG accompanying blood vessels.

Catecholamine varicosities of central origin present in DRG and ventral root have been described in the rat[1], however the source and function of these fibers is, as yet, unknown.

(1) A. Dahlstrom and K. Fuxe. Evidence for the existence of an outflow of noradrenaline nerve fibers in the ventral roots of the rat spinal cord. Experientia 21:409 (1965).

173.6 POSSIBLE SOURCES OF CATECHOLAMINE TERMINALS IN THE LUMBAR SPINAL CORD OF THE CAT. C. J. Hodge, Jr., R. T. Stevens, G. D. Peca-Vogelsang, A. V. Apkarian, and J. I. Franck. Dept. of Neurosurgery, Upstate Medical Center, Syracuse, NY 13210.

The grey matter of the lumbar spinal cord is rich in catecholamine (CA) terminals in areas linked to the modulation of sensory and motor processes. Following lumbar injections, horseradish peroxidase (HRP) and Evans Blue (EB) retrograde transport were combined with glyoxylic acid induced CA histofluorescence to identify those areas of the brain stem which project to the cord and which also contain CA cell bodies. The use of EB allowed determination of those cells that both contained CA and projected to the cord.

The most rostral CA cell groups, A8, 9, and 10, which include substantia nigra, contained little or no retrogradely transported marker, and therefore do not contribute to lumbar CA innervation. The pontine areas of locus coeruleus, the subcoerulear area, the parabrachial nuclei and the Kolliker-Fuse nucleus are comprised in part of CA containing cells and cells that project to the lumbar cord. However, using the EB double labeling techniques, it was seen that most of the LC cells that project to the cord are not catecholaminergic. The more ventral areas of the subcoerulear nucleus, the parabrachial nuclei, and the Kolliker-Fuse nucleus have a much higher percentage of CA containing cells that project to the lumbar cord and likely are the major source of spinal CA.

The CA containing cells of the medulla are relatively sparse and retrograde labeling was absent or very light with the exception of the area corresponding to A1 in the rat.

These results indicate that the primary source of lumbar spinal NA is from the dorsolateral pons in the caudally projecting nuclei ventral to locus coeruleus.

173.7 A LIGHT AND EM ANALYSIS OF IMMUNOREACTIVE GLUTAMIC ACID DECARBOXYLASE (GAD) IN THE SPINAL AND TRIGEMINAL DORSAL HORN OF THE CAT. Allan I. Basbaum, Ellyn J. Glazer and Wolfgang Oertel*, Dept. of Anatomy, Univ. of California, San Francisco and Dept. of Neurology, Technische Univ., Munich, Germany.

Segmental and supraspinal control of dorsal horn nociceptors involves complex interactions among a variety of putative neurotransmitters. To analyze the contribution of GABA to this control, we have examined the distribution of immunoreactive GAD, the biosynthetic enzyme for GABA. Cats were perfused with 4% para/0.2% glut., in 0.1M phosphate buffer. To generate GAD cell body labelling, colchicine (20µg/µl) was injected 48 hours prior to perfusion, either intrathecally (10µl) or intracisternally (6µl). Sections through the lumbosacral enlargement and the trigeminal nucleus caudalis were sectioned either transversely or sagitally on a Vibratome and processed for GAD immunoreactivity with the PAP method using a sheep anti-GAD antiserum (Oertel et al, Neuroscience, 1981). Sections for LM were pretreated with 10% methanol/3%H_2O_2 to enhance antibody penetration. Sections for EM were osmicated and flat embedded in Epon-Araldite. Control sections were incubated in preimmune serum; all controls were negative.

GAD terminals are most dense in the superficial dorsal horn, laminae 1 and 2, (the substantia gelatinosa, sg). Much less staining is found in the nucleus proprius, lamina 3, 4 and 5. A dense region of GAD staining was also present in medial lamina 7 and adjacent to the central canal. GAD cell body staining was generally confined to the soma. GAD cells are found in most layers of the dorsal horn, but are most numerous in lamina 3. Many are found in the inner sg, somewhat fewer in the outer sg. A few, small fusiform cells (5x10-15µ) are found in the marginal zone. Most of the GAD cells are oval or round, measuring 5-10x10-20µ.

EM analysis reveals a variety of synaptic relationships. GAD terminals are presynaptic to dendrites in lamina 1 and 2, to somata and proximal dendrites of lamina 1 and to axons, including a population of central axonal terminals. Many of the latter are presumed to be of primary afferent origin. Occasionally, vesicle-containing GAD profiles are postsynaptic to unlabelled axon terminals.

Given the presence of numerous GAD cells, but the paucity of GAD terminals in lamina 3, this study indicates an important contribution of these lamina 3 cells to the control of superficial dorsal horn nociceptors of I and II. Moreover, despite electrophysiological similarities, lamina 3 and 4 are probably functionally distinguishable. Supported by NS 14627, 00364, DA-01949 BNS 78-247621 and the Sloan Foundation.

173.8 ELECTRON MICROSCOPIC ANALYSIS OF PACINIAN CORPUSCLE AFFERENT FIBER TERMINALS IN CAT DORSAL HORN. K. Semba, P. Masarachia*, S. Malamed*, M. Jacquin*, S. Harris* and M. D. Egger. Dept. of Anat., CMDNJ-Rutgers Med. Sch., Piscataway, N.J. 08854.

Intra-axonal injections of horseradish peroxidase (HRP) into afferent fibers from the glabrous skin of the cat's hind paw made possible observation of functionally identified terminals at the electron microscopic level (Egger et al., Brain Research, 207: 157-162 (1981)). We have now begun to characterize terminals of fibers innervating Pacinian corpuscles. Analysis of micrographs of HRP-filled boutons in Rexed's laminae III, IV and V indicated: (1) HRP-filled boutons, ranging from 1.0 to 3.5 µm in longest dimension, contained clear, round vesicles, approximately 40 nm in diameter. From lamina III to lamina V, the number of synaptic zones per bouton decreased. (2) Both axo-dendritic and axo-axonic specializations were observed, with axo-dendritic profiles predominating in laminae III and IV (about 70%), whereas axo-axonic profiles predominated in lamina V (about 60%). We observed no axo-somatic profiles. (3) HRP-filled boutons formed both asymmetrical and symmetrical synapses with dendritic profiles, with asymmetrical synapses predominating (about 80%). The longest dimension of the postsynaptic dendritic profiles ranged from 0.5 to 3.5 µm. (4) All the axons with which HRP-filled boutons had synaptic contacts contained clear, elliptical or pleomorphic vesicles, 40-50 nm in major diameter. A few of these unlabelled axons contained dense-core vesicles as well. About 40% of the axons in contact with the HRP-filled boutons were identified as postsynaptic, with the synaptic type clearly seen to be asymmetrical. The complexity of the neuropilar organization of the HRP-filled boutons was revealed by serial sectioning labelled afferent collaterals in lamina IV which included 19 boutons. One of the boutons synapsed with 5 dendrites and 5 unlabelled axons; 3 of the 5 unlabelled axons in turn synapsed with 3 of the 5 dendrites that were postsynaptic to this labelled bouton, forming a glomerulus. Some boutons were interconnected by extremely thin axon collateral segments, which in places appeared to be only 25-60 nm in diameter. HRP-filled boutons frequently gave origin to thin HRP-filled sheets which curled along the bouton, but which appeared to be distinct, --and separated by membranes,--from glial elements in the vicinity of the boutons. The predominance of axo-dendritic synapses in laminae III and IV, combined with the fact that all the HRP-labelled boutons contained clear, round vesicles, suggests that activating the Pacinian corpuscles in the glabrous skin should produce strong excitatory drive on postsynaptic dorsal horn cells. (Supported by grants BNS 78-24470 from NSF and NS 13456 from NINCDS.)

173.9 MORPHOLOGIC ANALYSIS OF NEURONS OF THE CAT SUBSTANTIA GELATINOSA INTRACELLULARLY STAINED WITH HORSERADISH PEROXIDASE.
M.A. Clendenin and G.E. Goode, Department of Anatomy, Eastern Virginia Medical School, Norfolk, VA 23501

Small neurons of the substantia gelatinosa (SG) in the lumbar dorsal horn of the cat were targets for intracellular recordings and enzyme labelling. Some cells were labelled by intracellular iontophoresis while other neurons were studied following incorporation of extracellular marker protein. No more than three "injury filled cells" appeared in our serial reconstructions of 100 micron Vibratome sections reacted with DAB, in contrast to intracellular injections when the enzyme was limited to the cell from which physiologic recordings were made. We studied the size, shape and location of the perikarya as well as dendritic and axonal shape, length, and patterns of termination. Two different cell types were labelled in the superficial zone of the substantia gelatinosa. The cells near the marginal-SG interface have small oblong or rounded soma. Their dendrites radiate to form small spherical fields within a hundred microns of the perikaryon, whereas their distal dendrites form longitudinal arrays for several hundred microns within a cord segment. Proximal dendrites are tortuous, highly branched arbors with distinct beaded dilations along their length. A variety of complexes of short spines as well as long thin pedicles with spine heads project from these proximal dendrites. The larger diameter, long longitudinal distal dendrites of these cells are relatively spine free until at their distal tips they branch into thin pedicles with multiple spine heads. One neuron in our collection may represent a subpopulation of neurons as its distal dendritic pattern was oriented from a lateral to medial direction across the SG.

The morphology of these neurons in the superficial SG is in contrast to labelled cells in lamina IV. Their larger cell bodies are stellate, their dendrites form larger spherical fields proximally, and their large distal dendrites project dorsally through lamina III, II and I into the dorsal root entry zone. These dendrites are relatively aspinous. The morphology of labelled neurons will be discussed with regards to their physiology and ultrastructure in an attempt to uncover a common denominator for the modulation of nociceptive information in the interneuronal pool of the SG. (Support by USPHS General Research Support Grant 218).

173.10 SYNAPTIC INPUTS ON THE DORSALLY DIRECTED DENDRITES OF AN HRP-FILLED LAMINA IV DORSAL HORN NEURON IN THE SUBSTANTIA GELATINOSA OF ROLANDO: AN EM ANALYSIS.
S. Gobel, G. J. Bennett, Z. Seltzer*, M. J. Hoffert and E. Humphrey*. Neurobiology and Anesthesiology Branch, National Institute of Dental Research, National Institutes of Health, Bethesda, MD 20205.

In Golgi studies of perinatal animals, Ramon y Cajal showed that the deeper parts of the dorsal horn contain neurons with either pyramidal or multipolar cell bodies which send some of their dendrites dorsally into the substantia gelatinosa of Rolando (Rexed's laminae I and II). From our Golgi studies in adult cats, it has become clear that such neurons constitute an important source of the dendrites in laminae I and II. In these Golgi studies, two kinds of neurons which send dendrites into laminae I and II have been found in Rexed's laminae III and IV. One of these has very spiny dendrites while the other one has relatively smooth aspiny dendrites. Recently, we have intracellularly filled one of these neurons with smooth dendrites in the adult cat lumbar cord with horseradish peroxidase (HRP). This neuron has its cell body close to the IV/V border. In addition to giving rise to dendritic branches in laminae III,IV, and V, this neuron sends numerous dendritic branches dorsally into laminae I and II as well as into Lissauer's tract and the dorsolateral funiculus. An EM analysis of the HRP-filled dorsal dendrites showed that numerous small dome-shaped axonal endings in lamina I, Lissauer's tract and the dorsolateral funiculus synapse on these dendrites at single short axodendritic synapses which can be either symmetrical or asymetrical. One of these endings contains a mixture of different sized small oval agranular vesicles with appreciable numbers of highly flattened ones while the other one contains only small oval vesicles. Occasional dense-core vesicles are seen in both endings. These two kinds of axonal endings resemble the D1 and D2 dome-shaped serotonergic endings respectively which were shown in other studies to take up [^{3}H]5HT and the serotonin neurotoxin 5,6 dihydroxytryptamine (Ruda and Gobel, Brain Res. 184(1980) 57-83). In their passage through laminae I and II, the HRP-filled dendrites were not observed entering any glomeruli, i.e., those structures in which the axonal endings of primary neurons are found. These observations suggest that many of the neurons in the deeper laminae of the dorsal horn which send some of their dendrites dorsally into laminae I and II may recieve appreciable input from descending aminergic axons on these dorsal dendrites but very little input from the primary axons which arborize in these laminae.

173.11 INTRACELLULAR RECORDING FROM DORSAL HORN NEURONS IN THE RAT SPINAL CORD SLICE PREPARATION.
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Utilizing transverse rat spinal cord slices and extracellular recording it has been shown that substance P (SP), somatostatin (SS) and methionine-enkephalin (ME) modify activity of the dorsal horn neurons by acting directly on postsynaptic sites (Miletić and Randić, Neurosci. Abstr., 6: 624, 1980). By using intracellular recording technique in vitro in a horizontal spinal cord slice preparation we have now investigated membrane actions of SP and SS.

Rats (5-12 days old) were anesthetized with ether and, following a laminectomy, about 1 cm long segment of lumbosacral spinal cord with attached dorsal rootlets was quickly removed and immersed in oxygenated Krebs-Ringer solution at 6°C and further sectioned with an Oxford vibratome to yield about two horizontal 300 µm-thick dorsal horn slices. The slices were incubated in Krebs-Ringer solution at room temperature for about 1 hr. Then they were transferred to the recording chamber and continuously perfused with oxygenated Krebs-Ringer solution at 37°C at a flow rate of about 2 ml/min. Intracellular recordings were performed with micropipettes filled with 1-3 M K-acetate having D.C. resistances of 60-100 MΩ. Good intracellular recordings from slices could be obtained for 6-8 hrs after isolation of the spinal cord. Electrical stimulation of the dorsal rootlets with a glass-coated platinum wire electrode was used to synaptically activate dorsal horn neurons. A high-input impedance bridge amplifier was used to inject current through the recording microelectrode. The amplitude of the recorded voltage produced by hyperpolarizing current pulses was used as a measure of the membrane input resistance. Effects of bath-applied SP and SS (10^{-4} to 10^{-6} M) were studied on spontaneous activity, membrane potentials and input resistance, and on synaptic and action potentials.

In 29 dorsal horn neurons, mean resting potential and membrane input resistance were -67.4 ± 5.9 mV, and 41 ± 12 MΩ respectively. The amplitudes of the evoked action potentials up to 85 mV were recorded. SP produced a reversible and dose-related depolarization, while SS weakly hyperpolarized the membrane of most dorsal horn neurons. Both effects appeared to be associated with decrease in membrane input resistance, although the sample of tested units is relatively small. More extensive intracellular studies of the membrane actions of SP, SS and ME and analysis of their ionic mechanisms are currently in progress. (Supported by NSF grant BNS 23871 and U.S. Dept. of Agriculture).

173.12 EVALUATION OF THE SPONTANEOUS SPINAL ELECTROGRAM AS A MEASURE OF DESCENDING SPINAL CORD FUNCTION FOLLOWING TRAUMA.
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In the study of experimental spinal cord trauma objective tests of cord function are necessary for the evaluation of the degree of injury and the efficacy of treatments. The most commonly employed method of electrophysiological assessment is the somatosensory evoked potential. Tests of descending cord pathways are less frequently employed even though these tracts represent a large percentage of the cross sectional area of the cord and it is their interruption that results in the loss of motor activity.

In this investigation the spontaneous electrical activity of the spinal cord (spontaneous spinal electrogram or SEG) was analyzed as a method to assess descending cord function. The SEG consists of low voltage background activity (25 µV) upon which occur spontaneous, random, large voltage (75-300 µV) negative potentials termed negative sharp waves (NSWs) which are 20-40 msec in duration. NSWs are inhibited by activity in descending cord pathways. This fact was used as a rationale to test the hypothesis that surgical interruption of descending pathways that remain functionally intact 48 hours following spinal cord trauma in the cat will result in an increase in the occurrence of NSWs that will inversely correlate with both the intensity of the injury and the histologically determined extent of the trauma-induced lesion.

Anesthetized cats were injured by dropping a 25 gram weight variable distances onto an impounder resting on the dura of the L2 cord. The change in momentum (impulse) of the weight was measured. 48 hours later the cat was rendered decerebrate under ether anesthesia. The SEG was recorded caudal to the site of injury. The mean amplitude of NSWs was determined and the mean number/min exceeding this value was determined. The cord was then surgically transected rostral to the trauma-induced lesion and recording continued. The average number/min of NSWs passing the previously determined threshold was determined as was the mean amplitude. The percentage of cross sectional area showing lesions was determined histologically.

The results show that there was a significant inverse correlation between percent change in count following surgical transection and both impulse and lesion size confirming the hypothesis and indicating that the SEG may be a valuable tool in assessing trauma and treatment in experimental spinal cord injury. In addition the results show that the mean pretransection count correlated positively with both impulse and lesion size suggesting that the level of SEG activity may give insight into cord functional status, an important result in terms of possible human application. Supported by Biomed. Res. Suppt Grant 5-SO7-RR05413.

173.13 SITES OF ACTION OF SEGMENTAL AND SUPRASPINAL INPUTS INHIBITING IMPULSE TRANSMISSION ALONG THE PATHWAYS MEDIATING PAD OF Ia FIBERS. P. Rudomín, I. Jiménez*, M. Solodkin* & S. Dueñas*. Centro de Investigación del I.P.N. México 14 D.F.

Impulse transmission along the pathways mediating primary afferent depolarization (PAD) may be inhibited by segmental and supraspinal stimulation. However, the site of action of these inhibitory inputs has not been yet determined. Previous work suggests that at least two interneurons are interposed in the PAD pathway to group I fibers and that intraspinal microstimulation (ISMS) produces PAD probably by direct activation of the last order interneuron. Now we are using the ISMS technique to detect inhibitory actions on this interneuron. Cats were anesthetized with pentobarbital, paralyzed and maintained with artificial respiration. PAD was inferred from the changes in the threshold current necessary to keep a constant probability of antidromic firing of single fibers. A conditioning train to group Ia fibers in the biceps-semitendinosus (BST) nerve usually decreased the activation threshold of single Ia fibers in the gastrocnemius (GS) nerve. This effect was inhibited by preceding sural (SU) conditioning. The PAD of Ia GS fibers produced by ISMS applied either in the intermediate nucleus (IN) or in the motor nucleus (MN) was not affected by SU conditioning. Stimulation of the magnocellular reticular formation (RF) could reduce the PAD of Ia GS fibers produced either by BST stimulation or by ISMS to the IN, but not the PAD produced by ISMS to the MN. It thus appears that SU inhibition acts on the first order interneuron. RF inhibition appears to act on the last order interneuron which is located within the IN and makes axo-axonic contacts with the Ia fibers ending both in the IN and in the MN.

The inhibition produced by RF stimulation on the BST induced PAD was usually abolished by sectioning the ipsi. dorso-lateral fasciculus in the thoracic spinal cord, but sometimes it was also necessary to cut the contralateral half of the cord. Not unfrequently RF stimulation reduced the activation threshold of Ia GS fibers (classified as such because SU conditioning reduced the BST induced PAD). This depolarizing action was mediated by fibers running in the ipsi. ventromedial fasciculus. This suggests that the facilitatory and inhibitory actions produced by RF stimulation are mediated by independent descending fiber systems which may be coactivated by the electrical stimulation.

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174.1 DESCRIPTIVE MEASURES OF THE INNERVATION OF THE CORNEAL EPITHELIUM IN RABBIT. A.J. Rózsa and R.W. Beuerman, Division of Ophthalmology, Stanford University Medical Center, Stanford, CA 94305

All forms of supraliminal stimulation of the cornea are perceived as noxious. Although sensory receptors of the corneal epithelium are known to be morphologically unspecialized or "free nerve" endings, there have been no adequate descriptions of the organizational design or of the density of the innervation of corneal epithelium.

Both corneas of 24 albino rabbits (2-2.5 kg) were stained with a modified gold chloride technique. Cross-sections (15 µm thick) and flat-mounts (100 µm thick) were prepared for light microscopical (400X-1000X) examination.

Cross-sections, used for determining neural density, were apportioned into 100-µm samples, and the number of axonal endings, their branching pattern and mode of termination were assessed. Averaging terminal counts from 248 samples confirmed the notion that the corneal epithelium was densely innervated. A normal rabbit corneal epithelium with a surface area of 213 mm^2 was calculated to contain about 1.4 million terminal endings. This meant that the fine filament of an esthesiometer (0.13 mm dia.), when contacting the surface of the epithelium, could stimulate approximately 100 terminal endings. A combination of serial cross-sections and horizontal flat-sections enabled the reconstruction of the three-dimensional organizational geometry of axon terminals within the epithelium. After penetration of the epithelium, axons branched in characteristic patterns of "leashes" containing 4-50 terminals. These "leashes" often coursed together for several hundred micrometers within the basal cell level of the epithelium. Vertical branches rising from the "leashes" at irregular intervals formed a dense and complex arrangement of terminal endings at all levels of the epithelium. A practical method for assessing relative changes in innervation showed that neural density at all levels in the epithelium increased as a function of the distance from the limbus to the center. This observation agreed well with the psychophysical finding that corneal sensibility to mechanical stimulation increased as its center was approached.

Based on psychophysical evidence and the results of these studies, the cornea suggests itself as an excellent model for the neurobiological investigation of experimental pain.

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174.2 PRURITUS EVOKED BY ELECTRICAL, CUTANEOUS STIMULATION, R.P. Tuckett, Department of Physiology, University of Utah, Med. Ctr., Salt Lake City, Utah 84108

Only polymodal nociceptors have been found to respond to cowhage (Tuckett, R.P., Neurosci. 6:428, 1980), a potent itch producing agent; however, the same receptors also respond to levels of heat and to chemicals that produce pain. It is possible that by producing different firing patterns, a single neuron is able to signal both pain and itch to the central nervous system.

The skin of 25 human subjects was stimulated at frequencies varying from 2 to 100 Hz, with a pulse width of 7 ms. One electrode (2x3 cm) was placed on the volar aspect of the wrist and the other either about 18 cm proximal on the forearm or on the ankle. At the beginning of the experiment, the nature of the procedure was explained and the subject's informed consent obtained, with the understanding that the subject could drop out of the experiment at any time. Subjects were randomly presented with pairs of stimuli and were asked to compare the intensity of the test to the control stimulus within the pair. The control stimulus was 10 Hz. All but two (93%) reported pruritus that increased in intensity with increased frequency of stimulation through 40 Hz. The intensity diminished and was more variable at 100 Hz, probably due to fatigue of the sensory neurons. The quality of the sensation did not change from itch to pain over the range of frequencies presented.

Afterwards, cowhage was applied to the wrist that had not been electrically stimulated. All subjects felt itching. Twenty-one (84%) said the itching was very similar to that from electrical stimulation. Their verbal description of the two experiences were also quite similar. The remainder reported the quality of itch to be different for the two types of stimuli.

In addition, discharge patterns of individual polymodal neurons in cat to heat (45-50°C) and cowhage were used to trigger the stimulator in double-blind experiments. Subjects were unable to distinguish any differences in the quality of the evoked sensations. (All reported itching.) It was concluded that the experimental evidence did not support the possibility that the quality of the noxious sensation elicited by electrical stimulation is dependent on the stimulus frequency. (Supported by USPHS Grant NS15102.)

174.3 THE ROLE OF MUSCLE AFFERENTS IN RELIEF OF PAIN FOLLOWING PERIPHERAL NEUROTRAUMA: CLINICAL AND EXPERIMENTAL STUDIES. A. Rabin* and R.A. Levy. Department of Pharmacology, University of Illinois College of Medicine, Chicago, IL 60680.

Although electrical stimulation of the skin is a common procedure for the treatment of chronic muscle pain syndromes, stimulation of muscles to relieve burning pain from skin has not yet been explored. During the course of clinical observations[1] we noticed that cutaneous burning pain and pain paroxysms following peripheral neurotrauma could be relieved by tensing of muscles having afferent inputs segmentally congruent with those of denervated skin. We found, in patients with denervation of the upper extremity, that referred sensation could be induced in the hand and forearm by stimulation of the shoulder girdle muscles. Thus, there exists a close topographical relationship in the central representation of these muscles and the distal part of the extremity. We succeeded in relieving burning pains in the hand in such patients by electrical stimulation of those muscles or by physical exercises.

Experiments in rats with full or partial section of the brachial plexus were carried out to support these observations. The animal model of autotomy (Wall et al., 1979; Wiesenfeld and Lindblom, 1979), which has been associated with painful sensation, was used to determine the role of the shoulder girdle muscles in prevention of autotomy of the paw. The degree of autotomy after denervation of the upper extremity, by section of the brachial plexus in the subclavicular area, was markedly intensified by additional denervation of the shoulder girdle muscles.

These data support the notion that activation of partially deafferented sensory centers through overlaping muscle inputs may be effective in relieving chronic pain syndromes.

Supported by NIH grant DE 5390.

[1]Clinical data obtained at City Hospital #67, Moscow, USSR.

174.4 IMMUNOHISTOCHEMISTRY OF RAT SPINAL DORSAL HORN TREATED WITH INTRATHECAL CAPSAICIN, KAINATE AND PIPERINE. T. Yaksh, P. Micevych and R. Elde (Spon. by F.W.L. Kerr), Dept. of Neurosurgery, Mayo Fdn., Rochester, MN 55905 and Dept. of Anatomy, Univ. of Minnesota, Minneapolis, MN 55455.

Intrathecal capsaicin (60 µg), a homovanyllic acid derivative has been shown to produce a significant elevation of the thermal nociceptive threshold in rats as measured by the tail flick and hot-plate assays. This thermal analgesia is coincident with a 40-70% depletion of substance P (sP) levels in the spinal cord as measured by radioimmunoassay (RIA). Intrathecal piperine (60 µg) and kainic acid (60 µg) also produce a significant depletion (50%) of spinal sP as measured by RIA but do not produce a significant elevation of thermal nociceptive threshold. Using immunohistochemistry we examined a series of rats which received intrathecal capsaicin (70 µg), piperine (70 µg), kainic acid (70 µg) or drug vehicle (50% dimethyl sulfoxide). The drug doses were sufficient to show significant depletion of spinal sP assayed in a parallel series of animals, but in the vehicle control rat spinal cord no changes were observed. The drug treated rats, vehicle controls and normal rats were allowed to survive 7 days and then sacrificed by transcardial perfusion. Ten micron cryostat sections were incubated with antibodies raised against sP, cholecystokinin (CCK), somatostatin (SOM), methionine-enkephalin (M-ENK) and serotonin (5-HT), and processed for indirect immunofluorescence. The most striking effects of intrathecal capsaicin, piperine and kainic acid were localized in laminae I, II and III (Rexed). The table below summarizes our findings on the effects of these agents on the immunofluorescence of these dorsal laminae.

	sP	CCK	SOM	M-ENK	5-HT
Capsaicin (7)	-	-	+	+	+
Kainic Acid (5)	+	+	+	-	+
Piperine (2)	-	+	+	+	+

n = ( ); + = no change from normal; - = profound reduction of immunofluorescence.

The sP, CCK and SOM in laminae I, II and III has been reported in small diameter primary afferents, ENK has been localized in intrinsic neurons and 5-HT is present in a descending system of fibers. Our observations imply that capsaicin depletes sP and CCK, and piperine depletes sP from primary afferent fibers. Kainate depletes the intrinsic ENK neurons but does not have an effect on the primary afferent fibers. None of the drug treatments affected the scattered 5-HT-immunoreactivity fibers in the dorsal horn. The kainic acid depletion of spinal sP measured by RIA has not been confirmed by this study. (NS 16541 and Mayo Fdn.)

174.5 RELEASE OF SEROTONIN AND NOREPINEPHRINE INTO SUPERFUSATES OF THE RAT SPINAL CORD FOLLOWING ELECTRICAL STIMULATION OF THE NUCLEUS RAPHE MAGNUS. Donna L. Hammond, Gertrude M. Tyce and Tony L. Yaksh, Dept. of Physiology and Neurosurgery Research, Mayo Foundation, Rochester, Minnesota 55905.

Axons of serotonergic neurons contained within the nucleus raphe magnus (NRM) terminate in the spinal cord. The present study examined the ability of electrical stimulation of the NRM to increase the efflux of endogenous serotonin (5HT) and norepinephrine (NE) into superfusates of the rat spinal cord. Male Sprague Dawley rats were pretreated with 10 mg/kg fluoxetine, anesthetized and prepared for superfusion of the spinal cord subarachnoid space. Artificial CSF was delivered at 0.1 ml/min to the caudal intrathecal space and, after superfusing the entire spinal cord, was collected at the level of the cisterna magna in iced sampling tubes. 5HT and NE in the same sample of superfusate were separated using column chromatography and their concentrations were determined using HPLC with electrochemical detection. The spinal cord was superfused for three consecutive 25 min intervals. The first sample was used to monitor the recoveries of added standard 5HT and NE. The second sample of superfusate was used to determine the basal concentrations of 5HT and NE. During the collection of the final sample of superfusate, the NRM was stimulated at 30 Hz using 0.5 msec monophasic square waves. Current intensity was varied from 150 to 250 μA, but remained constant during the period of stimulation in any one animal. This final sample of superfusate was used to determine the levels of the evoked release of 5HT and NE. The stimulation sites were localized histologically in all animals. Basal efflux of 5HT and of NE was 0.60 ng/ml (SE = 0.13) and 0.23 ng/ml (SE = 0.03), respectively. Following electrical stimulation of the NRM, levels of 5HT increased to 1.14 ng/ml (SE = 0.27) and those of NE increased to 0.41 ng/ml (SE = 0.09) ($p < 0.05$, Student's t-test; each amine). Electrical stimulation of sites located outside the NRM failed to increase 5HT and NE levels. The release of 5HT into spinal cord superfusates following electrical stimulation of the NRM is in agreement with the anatomical and biochemical data which indicate that serotonergic neurons of the NRM project to the spinal cord. The concomitant release of NE may reflect the activation of noradrenergic axons of passage or the ability of NRM neurons to modulate the activity of noradrenergic neurons which project to the spinal cord. Finally, the results of this study suggest that the elevation of nociceptive threshold produced by electrical stimulation of the NRM may be mediated by the release of both 5HT and NE in the spinal cord. (Supported by NS 16541 to TLY and the Mayo Foundation).

174.6 PROPERTIES OF A LONG LATENCY RESPONSE EVOKED IN DORSAL HORN NEURONS BY NOXIOUS THERMAL STIMULI IN DECEREBRATE CATS. K. C. Kajander*, D. C. Tam*, T. J. Ebner, and J. R. Bloedel. (SPON: G. King) Depts. of Neurosurgery and Physiology, Univ. of Minnesota, Minneapolis, 55455.

These experiments were designed to study the characteristics of the long latency response of dorsal horn neurons to noxious thermal stimuli. Specifically the extent to which this response can discriminate between different noxious temperatures was examined using signal detection theory. Also the effects of activating descending pathways on these measurements were determined. Experiments were performed in unanesthetized, decerebrate cats in which the lumbosacral region of the spinal cord was exposed by a laminectomy and stimulating electrodes were placed in the nucleus raphe magnus and the periaqueductal gray. Noxious thermal stimuli were applied to the ipsilateral hind footpad of the cat using a thermoelectric module capable of producing a 10°C rise in temperature in approximately 2 sec. Dorsal horn neurons selected for this study responded to maintained thermal stimuli at a temperature of 45°C and increased their discharge when the temperature was raised to noxious levels. The response of each neuron was assessed in the following manner. Transient thermal stimuli 10 and 12°C in magnitude were applied to the footpad from each of two baseline temperatures (42 and 45° C). The response of the neuron to each stimulus was analyzed individually. Based on plotted data from the individual trials, the time of onset (latency) and the duration of the average discharge rate of the neuron during a selected response period were then calculated. The mean frequency and standard deviation of the response evoked during several trials at each of the two transient temperature stimuli were used to calculate the parametric discriminability measure d'. The responses of most dorsal horn neurons had latencies greater than two seconds and durations which significantly outlasted the period of heating. The calculation of d' revealed that the responses to the two transient thermal stimuli were significantly different. Electrical stimulation to the periaqueductal gray and nucleus raphe magnus could attenuate the mean frequency of the response. However, its effect on d' was variable as d' was shown to increase or decrease. These data indicate that, based on an analysis of firing frequency, some dorsal horn neurons can discriminate between two transient noxious temperatures. In addition, the activation of descending pathways can alter the magnitude of the responses to each temperature pulse independent of their effect on discriminability. This research was supported by NIH Grant #5 RO1-NS13002.

174.7 COMPARISON OF RESPONSES OF ROSTRAL AND CAUDAL TRIGEMINAL BRAINSTEM NEURONES TO ELECTRICAL AND THERMAL STIMULATION OF TOOTH PULP IN CATS. J.W. Hu, G.J. Ball and B.J. Sessle. Fac. of Dentistry, Univ. of Toronto, Toronto, Canada M5G 1G6.

The subnucleus caudalis of the trigeminal brainstem sensory nuclear complex is generally implicated as the brainstem site vital for the relay of orofacial nociceptive information, yet paradoxically a large number of presumed nociceptive primary afferents from the tooth pulp has been shown in anatomical and electrophysiological investigations to project to more rostral parts of the trigeminal brainstem complex such as subnucleus oralis. However, since electrophysiological evidence for the latter projection is largely based on the activation of low-threshold mechanosensitive (LTM) neurones in oralis by electrical stimulation of the tooth pulp, we wished to test the response of oralis neurones to both electrical and natural (thermal) stimulation of the tooth pulp and compare it, in the same animals, with the response of caudalis neurones to both forms of pulp stimulation.

The activity of single neurones electrically driven from the ipsilateral canine tooth pulp was recorded in caudalis and oralis of anaesthetized cats. The neurones were functionally identified as LTM or cutaneous nociceptive neurones on the basis of criteria recently described (J. Neurophysiol., 45: 173, 1981). The effects on these neurones of controlled heating or cooling of the tooth crown were then examined.

In accordance with our earlier observations (e.g. Brain Res., 117: 211, 1976), we noted many LTM neurones in subnucleus oralis which had orofacial mechanoreceptive fields but which could also be driven at short latency by pulp electrical stimulation. However, none of 39 of these oralis neurones tested responded to heating (50-55° C) or cooling (0-5° C) of the tooth pulp. In contrast, 12 of 18 caudalis nociceptive neurones excited by facial noxious stimuli and pulp electrical stimulation responded also to tooth heating. The most vigorous and reproducible responses to heating were noted in those nociceptive neurones located in the superficial layers of caudalis. A weak response to thermal stimulation was seen in 4 of 28 LTM caudalis neurones that could be electrically driven from the pulp.

These findings raise uncertainties about the functional role, if any, played in tooth pulp nociception by those LTM neurones which can be excited by a synchronous barrage of impulses initiated by a pulpal electrical stimulus but not by a natural stimulus of the pulp. These neurones appear to predominate in subnucleus oralis.

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174.8 SYNAPTOLOGY OF CORTICOTRIGEMINAL PROJECTIONS. R.C. Dunn, Jr., L.E. Westrum and K.L. Chong*. Div. of Neurosurgery and Dept. of Anatomy, St. Louis Univ. Sch. of Med., St. Louis, Mo. 63104 and Dept. of Neurosurgery, Univ. of Wash. Sch. of Med., Seattle, Wash. 98195

Cortical input to the spinal trigeminal nucleus produces both presynaptic depolarization and hyperpolarization of trigeminal afferent fibers (Sessle, B.J., Dubner, R., Br. Res. 22:121, 1970). However, little is known about the synaptology of these cortical projections which are important in modification of facial sensation. To clarify these structural-functional relationships, an electron microscopic (EM) study of the corticotrigeminal projection is being made. Fourteen adult cats received either large sensorimotor region (6) or small coronal gyrus (8) cortical excisions. After survival periods from 30 hrs. - 21 days, animals were perfused and wafers of brainstem including p. interpolaris and p. caudalis were processed for EM. At 30 hrs., occasional small (1.6 μm) thinly myelinated axons are undergoing degeneration. Terminal degeneration is rarely seen at this time. By 72 hrs. widely separated electron dense terminals are encountered. Most are small (1 to 1.2 μm) and contain round vesicles (R-type terminals) but occasional small boutons with pleomorphic vesicles (NR-type) are also seen. Degenerating boutons frequently contact small (1.2 μm) dendritic shafts and spines. While the R terminals contact dendrites at asymmetrical membrane specializations, the NR-terminals may have symmetric contacts. Bouton degeneration is most pronounced at 4-5 days; even at this time, however, altered terminals are widely scattered. Although small degenerating axons are numerous at 7-8 days survival, dense terminals have become less frequent, essentially disappearing after 14 days. By 21 days the major remnants of the cortical injury are engulfed masses of electron dense axon fragments. Of particular interest at 3-5 days survival are occasional cortical boutons which appear to be post-synaptic to NR terminals. This arrangement may be the anatomic substrate for presynaptic depolarization of corticofugal fibers by trigeminal afferents (Dubner, R. et al: Nature 223:72, 1969). No cortical terminals have been found presynaptic to other terminals within interpolaris. From this study we conclude: 1) the corticotrigeminal boutons are small and sparse; 2) at least two categories of boutons are present, R and NR; 3) the NR boutons may be R boutons undergoing degeneration, but the symmetric contact of some argues for a separate category of corticotrigeminal afferent; 4) some corticofugal afferents appear to participate in axoaxonic arrangements.

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174.9 NON-SEROTONERGIC CELLS AT THE ORIGIN OF THE DORSOLATERAL FUNICULUS (DLF) IN RAT MEDULLA. J.N. Johannessen*, L.R. Watkins* & D.J. Mayer (SPON: M.C. Boadle-Biber). Dept. of Physiology, MCV/VCU, Richmond, VA 23298.

Descending pathways within the spinal cord DLF have proved important in the production of analgesia. The medullary nucleus raphe alatus, (NRA - n. raphe magnus & n. reticularis paragigantocellularis), which gives rise to fibers which descend in the DLF, is a sensitive site for the production of analgesia by electrical & pharmacological methods. Indirect evidence suggests that descending serotonergic fibers from area B3 may play a role in descending inhibition. The cells of B3 show a similar distribution to those of NRA, however, there is no direct evidence that serotonergic fibers from B3 descend in the DLF.

The cell bodies of NRA were retrogradely labeled with HRP by implanting a small piece of HRP-gel (Griffin et al., Brain Res., 168 (1979) 595-609) unilaterally into the cervical DLF. Two days later, the method of Bowker et al. (Neurosci. Abstr., 6 (1980) 101) was used to simultaneously visualize both retrogradely labeled cells & cells exhibiting serotonin-like immunoreactivity (SLI).

Extremely few double labelled cells were seen. Retrogradely labelled cells of NRA exhibited a different distribution from SLI cells. The SLI cells of B3 lie ventral to NRA. Some intermingling of retrogradely labelled cells and SLI cells was seen, especially near the midline. At the level of the facial nucleus, 25 retrogradely labelled cells & 55 SLI cells were seen in a typical hemisection, while no more than two double labelled cells could be identified.

Quantitative comparisons of SLI cells in the retrogradely labelled & non-labelled halves revealed no difference in the number of SLI cells, ruling out the possibility that the retrograde staining prevented or obscured SLI. Conversely, most cells retrogradely labelled from the DLF were found in an area devoid of SLI on the controlateral side, indicating a differential distribution of SLI cells & retrogradely labelled cells. Furthermore, sections from a single brain which were processed for retrograde labelling, but not SLI, showed similar patterns and numbers of labelled cells to those sections processed for both. Heavy double labelling of serotonin-like cells could be seen after a liquid injection of HRP (4 ul - 40%) into the fourth ventricle.

These results suggest that serotonin is not a major component of the DLF projection which originates in the NRA. This is consistent with evidence indicating that a descending serotonergic projection is unnecessary to elicit analgesia. (Johannessen et al. Neurosci. Abstr., 6 (1980) 247).

This research was supported by Grant DA-00576 to DJM.

174.10 RELATIVE EFFECTS OF DESCENDING INHIBITION ON A-FIBER AND C-FIBER INPUTS TO SPINAL CORD DORSAL HORN NEURONS IN THE CAT. Bruce G. Gray and Jonathan O. Dostrovsky, Dept. of Physiology, Univ. of Toronto, Toronto, Ont. M5S 1A8, Canada.

It is now generally accepted that stimulation of certain brainstem regions can activate a descending inhibitory system whose major effect is to reduce the transmission of nociceptive information. This concept is supported by studies showing that the C-fiber (primarily nociceptive) induced excitation of dorsal horn neurons is more powerfully inhibited than the A-beta (non-nociceptive) induced excitation. However since suprathreshold stimuli sufficient to excite C-fibers produce a very intense and synchronous activation of low threshold A-beta fibers it is difficult to obtain a meaningful comparison of the relative effects of the descending pathways on these two responses. In order to overcome this problem, the present study examined the relative degree of descending inhibition on the responses produced when each fiber group was excited to a comparable level.

Single unit recordings were obtained from the lumbar spinal cord of barbiturate anesthetized adult cats. Bipolar stimulating electrodes were stereotaxically implanted into the periaqueductal gray (PAG), lateral reticular formation (LRF), nucleus raphe magnus (NRM) and nucleus gigantocellularis (NGC). Stimulation sites were subsequently verified histologically. Only cells classified as wide dynamic range and which received both A-fiber and C-fiber inputs were studied. To test for inhibitory effects the brainstem conditioning stimuli were delivered 130ms. prior to the response of the particular fiber input under study and consisted of a 100ms., 500Hz train of 0.1ms. pulses. Cells were excited using electrical skin stimuli or an air-jet to just suprathreshold levels by A-fiber input and the intensity of the threshold current applied to PAG,LRF,NRM and NGC needed to inhibit this response recorded. Then after raising the stimulus intensity to levels just suprathreshold for obtaining the C-fiber input the intensity of the threshold current necessary to inhibit this response noted. Using this method it was found that the current thresholds necessary to inhibit these responses were similar in 4 cells but in the remaining 3 tested, the threshold current for inhibiting the C-fiber input was markedly lower. Another method was to use suprathreshold stimuli and construct PSTH's. Through inspection of the distribution of spike activity from C-fiber input one could create a similar distribution for the A-fiber input by using low intensity natural or electrical repetitive stimuli. When comparing the relative magnitude of inhibition following stimulation to a given brainstem structure only in 1 of 5 cells was there a more powerful inhibition of the C-fiber input. These experiments have shown that in many cases there is no preferential inhibition of C-fiber input and thus the apparent selectiveity previously reported is probably due to the unequal synaptic drive produced by the two fiber groups.

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174.11 EFFECT OF LESIONS OF THE N. RAPHE MAGNUS & SURROUNDING AREAS ON SYSTEMIC & MICROINJECTION MORPHINE ANALGESIA. E.G. Young*, L.R. Watkins* & D.J. Mayer (SPON: R. Costanzo). Dept. of Physiol., MCV/VCU, Richmond, VA 23298.

Previous studies have implicated several medullary nuclei in descending inhibition of pain. Foremost of these are n. raphe magnus (NRM) & n. reticularis paragigantocellularis (NRPgc). It has recently been suggested that the combined NRM/NRPgc area, referred to as n. raphe alatus (NRA), forms the actual functional unit for descending pain inhibition (Br Res, 181:1, 1980). The question arises as to the relative involvement of these areas in systemic & microinjection morphine analgesia (MA). In Exper. 1, the effect of NRA, NRM, NRPgc & control lesions were tested for systemic MA. Pain sensitivity was measured (tail flick test) prior to & 40 min after 6 mg/kg morphine (i.p.) at 2 days prior to surgery and 5, 12 & 19 days after surgery. Lesions were made by passing 0.15-1.0 mA (DC) for 10 sec through a stainless steel pin insulated to 0.5 mm of the tip. Significant reduction in systemic MA was observed for NRM, NRPgc & NRA groups, compared to controls. A progressive reduction of MA occurred, wherein systemic MA decreased from 100% (pre-lesion) to 50-70% by 12 days after NRM, NRPgc & NRA lesions. Our NRM results differ from Proudfit's (Neurosci, In press) in that no change in baseline pain sensitivity was seen. In Exper. 2, MA was produced by 5 μg/0.5 μl morphine injected into the periaqueductal gray. The effect of NRM, NRPgc & control lesions was assessed as in Exper. 1. In contrast to the effects for systemic MA, NRM lesions abolished microinjection MA by 5 days post-lesion. Preliminary results indicate a comparable reduction of MA for NRPgc lesions.

It is known that MA involves a descending pathway in the dorsolateral funiculus (DLF) of the spinal cord. Supraspinal areas which project via the DLF may potentially be involved in pain inhibition. If axons from such areas pass through NRA, they would be destroyed by lesions in this area. To determine if such an axon-of-passage problem existed, horseradish peroxidase (HRP) in slow-release gel was implanted bilaterally in the cervical DLF of NRM, NRPgc, NRA & control rats. Comparison of HRP labelled nuclei in these 4 groups showed that no such axon-of-passage problem occurred; all areas which normally project via the DLF are still present after NRM, NRPgc & NRA lesions.

This work demonstrates that analgesia elicited by morphine microinjected into the periaqueductal gray can be abolished by lesions within the NRA. In contrast, these same lesions have a much smaller effect on systemic MA, indicating that multiple sites are involved in this opiate analgesia. This research was supported by Grant DA-00576 to DJM.

174.12 THE ORIGIN OF BRAIN STEM SEROTONERGIC AND NEUROTENSIN PROJECTIONS TO THE RODENT NUCLEUS RAPHE MAGNUS. A.J. Beitz. Dept. Anat., School of Med., Univ. of South Carolina, Columbia, SC. 29208.

The distribution of serotonin (5HT) and neurotensin (NT) immunoreactive cell bodies which project to the nucleus raphe magnus (RM) was localized in the brain stem of the rat utilizing the combined retrograde transport - immunohistochemical technique of Bowker et. al., (Brain Research, 1981). Twelve adult Sprague-Dawley rats were stereotaxically injected with horseradish peroxidase by means of either iontophoresis or pressure injection. One to 3 days later the rats received an intraventricular injection of colchicine (60μg in 6μl NaCl). The animals were subsequently perfused with 3.8% paraformaldehyde followed by a 25% sucrose-PBS solution. The brains were removed, and 40μm sections were processed for HRP histochemistry using $CoCl_2$ in the reaction to yield a black reaction product. Alternate sections were then incubated in antisera to 5-HT or NT and further processed for PAP-immunohistochemistry using the method of Sternberger (1974). Neurons which both contain 5-HT or NT and project to the RM were identified by the presence of both brown and black reaction product in the same cell. 5-HT immunoreactive cells which project to the RM were localized in the nucleus (N) reticularis paragigantocellularis, the ventrolateral periaqueductal gray and the medial aspect of the n. cuneiformis. A few double labeled 5-HT cells were also present in the n. raphe dorsalis. Several brain stem nuclei were found to contain double-labeled NT cells. The majority of NT immunoreactive neurons projecting to the RM were observed in the N. solitarius, the midbrain periaqueductal gray, the nucleus cuneiformis and the locus coeruleus. A few double labeled neurons were also evident in the parabrachial nuclei and the ventral most portion of the trigeminal nucleus caudalis. These results demonstrate several sources of 5-HT and NT input to the raphe magnus. Of notable importance is the demonstration of both a 5-HT and NT projection from the periaqueductal gray to the RM. It is possible that these projections subserve the descending pain control system and thus deserve further study. (Funded by NSF BNS 7906486. I thank Dr. Robert Elde for the anti-5-HT serum).

174.13 ANTAGONISM OF INHIBITIONS OF NOCICEPTIVE NUCLEUS RETICULARIS VENTRALIS NEURONES (NRV) EVOKED BY PERIAQUEDUCTAL GREY STIMULATION (PAG) BY MICROIONTOPHORETIC NALOXONE, IN RATS SHOWING STIMULATION EVOKED ANALGESIA (SEA). R. Morris* and R.G. Hill* (Spon: D.W. Lincoln), Department of Pharmacology, University of Bristol, Bristol BS8 1TD, U.K.

Under sodium pentobarbital anaesthesia 47 male rats were chronically implanted with teflon coated stainless steel, twisted wire, bipolar electrodes targeted on the PAG. After a 4-8 day postoperative recovery period the current thresholds for the production of explosive escape behaviour (EEB) (observed in an open field) and SEA (measured using the reflex responses to noxious thermal or mechanical stimulation of the tail) were assessed. In 35 animals EEB was observed, which included all the animals in which SEA was present. Six animals showed reproducible analgesia, which was clearly antagonised by naloxone (10 mg/kg, i.p.), at thresholds lower than those which produced EEB in these animals, a further 8 animals showed variable analgesia at an identical threshold to that producing EEB.

All the animals (N=14) showing some degree of analgesia were used in subsequent acute experiments. The animals were anaesthetised with urethane and conventional six barrelled microiontophoresis electrodes were used to record action potentials and apply drugs within the NRV. The stimulation parameters producing the behavioural changes (50 Hz, trains of 0.5 ms duration, 50-300 μA, square wave positive pulses applied for 5-10 s) had a predominantly inhibitory effect on NRV neurones which responded to noxious peripheral stimulation (N tested = 56, 57% inhibited, 13% excited, 30% no influence). In a condition-test paradigm these PAG evoked inhibitions powerfully suppressed the nociceptive excitations for poststimulation periods up to 2 min. Microiontophoretically applied naloxone was tested against 21 of these inhibitions and partial antagonism was observed in 14 units and no influence in the remaining 7 units. These data suggest that an endogenous opioid system may be acting on caudal reticular neurones during stimulation evoked analgesia but, alternatively, these results may be related to the explosive escape behaviour which was much more consistently evoked from PAG stimulation in these experiments (supported by the Wellcome Trust).

174.14 ACTIVATION OF PRIMATE SPINOTHALAMIC TRACT CELLS FOLLOWING INTRACARDIAC BRADYKININ INJECTION AND DURING CORONARY ARTERY OCCLUSION. R.W. Blair, R.N. Weber*, H.R. Holmes*, and R.D. Foreman. Dept. of Physiology, Okla Univ Hlth Sci Ctr, Oklahoma City, OK 73190

Neural impulses mediating the pain of angina pectoris are conducted through sympathetic afferents. Since the spinothalamic tract (ST) is known to convey impulses signalling pain to the brain, we have examined whether sympathetic afferents from the heart can activate ST neurons. Previously we demonstrated that electrical stimulation of these afferents could excite ST cells. The goal of the present study was to determine if stimuli applied directly to the heart could activate ST neurons. Extracellular potentials of ST cells of primates anesthetized with alpha chloralose and Na pentobarbital were recorded from gray matter in the T_3 to T_5 segments of the spinal cord. The cells were antidromically activated by stimulating their axons in the contralateral ventral posterior lateral nucleus. Although visceral input onto ST cells is emphasized in this study, all the ST cells also received somatic input. Bradykinin (BK) was injected (1-10 μg/kg) into the heart via a catheter placed in the left atrial appendage. Coronary artery occlusion (CAO) was produced by tightening a snare around the main left coronary artery. Of 20 ST cells tested for a response to intracardiac BK, 15 increased their discharge rate from an average of 13.2 ± 3.4 to 29.6 ± 4.5 (SE) Hz. The latency to onset of cells' responses to BK ranged between 4 and 20 seconds after BK injection; peak responses occurred 11 to 56 seconds after injection. Five cells were unaffected by BK. Injections of similar doses of BK into the descending aorta or femoral vein produced little or no change in cell activity, indicating that BK's effect on ST cells originated from the heart. The responses of 8 cells to CAO were determined. Four cells were excited (from 17.8 ± 9.1 to 24.3 ± 9.7 (SE) Hz), but the other four were unaffected by CAO. The 4 cells excited by CAO and 3/4 of cells unresponsive to CAO were excited by BK. In the 4 cells responsive to both stimuli, BK caused the ST cells to fire at a higher rate than did CAO. These results demonstrate that presumably noxious stimuli applied to the heart can excite some ST cells. Thus, the pain of angina pectoris may be conveyed via the spinothalamic tract. (Supported by NIH grant #22732).

174.15 THE CORTICAL PROJECTION OF THE NUCLEUS SUBMEDIUS IN THE CAT. A.D. Craig, Jr., S.J Wiegand and J.L. Price. Dept. Anat. & Neurobiol., Wash. Univ. Sch. Med., St. Louis, MO 63110

Recent results have indicated that the dorsal portion of the nucleus submedius (Sm) in medial thalamus is a major spinothalamic terminus, receiving input virtually exclusively from the marginal zone (Craig and Burton, J. Neurophysiol., 45: 443, 1981); although the sources of afferent input to the remainder remain unknown, the preceding observations suggest that at least part of Sm may be involved in specific nociception. Conflicting reports have been published regarding the efferents of Sm in the cat and the rat; the present experiments have identified a discrete zone of orbitofrontal cortex in the medial wall of the presylvian sulcus and the adjacent posterolateral gyrus proreus as the Sm cortical field in the cat.

Anterogradely transported label was observed in cats which had received 50-100 nl injections of tritiated amino acids (50-100 uCi/ul). In three of these cases, bilateral injections were placed in caudal Sm and rostral Sm on each side, respectively. Retrogradely transported label was observed in cats which had received unilateral 100 nl injections of 1% HRP conjugated with wheat germ agglutinin. Analysis of these cases indicates that Sm projects topographically onto layer 3 of a discrete agranular orbitofrontal cortical field; the postero-dorsal portion of Sm projects to the postero-dorsal portion of the cortical field, and the antero-ventral portion of Sm to the antero-ventral portion of the Sm cortical field. A diffuse layer 1 projection to the Sm cortical field may also arise from Sm neurons; however, a dense projection to superficial layer 1 of Sm cortex appears to arise from VM neurons immediately subjacent to Sm. Sm is reciprocally connected with its cortical field; a moderate cortico-thalamic projection to contralateral Sm is also present. The Sm cortical field is cytoarchitectonically distinguished from adjacent areas by a diminished cell size in layer 5 and an expanded layer 3. It extends anteriorly almost to the frontal pole, attains its greatest dorso-ventral extent at the level of the junction of the olfactory peduncle, and extends caudally into the fundus of the rhinal sulcus to the level of the agranular insular cortex. The evidence indicates that the feline Sm cortical field may be homologous to the ventrolateral orbital field of the rat (Krettek and Price, JCN, 171: 157, [1977]), since both are adjoined by areas receiving input from MD and VM.

These results indicate that a distinct orbitofrontal field may be involved with nociception. (Supported by NS09809 [to H. Burton], NS09518 and T-32-NS07507.)

174.16 NEONATAL INTRASPINAL 6-HYDROXYDOPAMINE OR 5,7-DIHYDROXYTRYPTAMINE: SEXUALLY DIMORPHIC EFFECTS ON NOCICEPTION BUT NO EFFECTS UPON MORPHINE ANALGESIA. B. A. Pappas, R. Ings and D. C. S. Roberts. Carleton University, Ottawa, Canada, K1S 5B6.

Intrathecal administration of both alpha noradrenergic (NE) and serotonergic (5-HT) antagonists in the adult rat reportedly lower nociceptive thresholds. It is not clear, however, whether spinal NE or 5-HT systems mediate the antinociceptive effects of morphine (e.g., Yaksh & Wilson, J. Pharmacol. Exp. Ther., 1979; Reddy, Maderhut & Yaksh, J. Pharmacol. Exp. Ther., 1980; Proudfit & Hammond, Brain Res., 1981). To selectively deplete spinal NE and 5-HT, newborn rats were injected intraspinally on days one and two with either 6-hydroxydopamine (6-OHDA, 10 μg) or 5,7-dihydroxytryptamine (5,7-DHT, 8 μg) after desmethylimipramine pretreatment to deplete spinal NE or 5-HT, respectively. We tested these animals for basal nociception and morphine analgesia during adulthood. Intraspinal 6-OHDA depleted only spinal NE by 81, 78 and 87% in cervical, thoracic and lumbar cord, respectively. The 5-HT depletions after 5,7-DHT were 60, 55 and 66% in corresponding regions. There were no monoamine depletions in the brain — in fact, elevations of NE and 5-HT, respectively, were observed in the brainstems of the 6-OHDA and 5,7-DHT groups, presumably reflecting sprouting from the supraspinal origins of the damaged spinal terminals.

Nociceptive thresholds were determined immediately prior to and at 20 min. intervals after either s.c. saline, 1, 3 or 7.5 mg/kg morphine sulphate. Latency to tail flick after immersion of the distal tail in water (55°C) was recorded. The 6-OHDA and 5,7-DHT injections produced sex dependent effects on baseline nociceptive thresholds — tail flick latencies were significantly lowered in the spinal NE depleted females but normal in their male counterparts. Conversely, thresholds were significantly elevated in the spinal 5-HT depleted males but normal in their female counterparts. No sex differences were found, however, in the monoamine depletions after the 6-OHDA or 5,7-DHT treatments. While we do not as yet have an explanation for this sexual dimorphism, it may account for some conflicting reports of the role of spinal monoamines in nociception.

Neither spinal NE nor 5-HT depletion affected the antinociceptive effect of morphine at any of the three doses. Females were generally less affected by morphine, especially at the highest dose. Thus, while normal nociception requires intact spinal NE or 5-HT terminals in females and males, respectively, these terminals may not be necessary for the analgesic action of morphine in either sex.

175.1 NON-LINEAR HAIR CELL MODEL GENERATES SUMMATING POTENTIALS. T. McMullen*, D. C. Mountain* and E. Sanchez* (SPON: H. VOIGT). Depts. of Biomed. Engr. and Otolaryngology, Boston University, Boston, MA 02215.

Computer simulation was employed in an attempt to gain more understanding of the mechanisms responsible for the generation of the summating potential. Recent studies have shown that the operation of vertebrate hair cells is non-linear in at least two respects: (a) membrane conductance is voltage sensitive (Corey, D. and Hudspeth, A., Nature, 281: 625-627, 1979); and, (b) the relation between hair bundle displacement and the resulting conductance change is asymmetric and sigmoidal (Hudspeth, A. and Corey, D., Proc. Natl. Acad. Sci., 74: 2407-2411, 1977).

Prior to simulation of a model which included both of these elements, equivalent circuit models which simulated each of these phenomena separately were studied. Acoustic stimuli were modeled by time-varying modulations in the transducer resistance, and both intracellular and extracellular responses were examined. The variation in reponses to acoustic stimuli of various frequencies and amplitudes and, also, for different levels of endocochlear potential (EP) were compared to experimental data.

175.2 MUSCARINIC ACETYLCHOLINE RECEPTOR BINDING IN THE COCHLEA OF THE GUINEA PIG. William James, Mary Ann Cheatham* and William L. Klein. Dept. of Neurobiology and Physiology, Northwestern University, Evanston, IL 60201

Cell membranes from the guinea pig cochlea have saturable, specific binding of the potent muscarinic antagonist 3H-quinuclidinyl benzilate (QNB). Cochlear turns, dissected free of stria vascularis, were removed from Urethane-anesthetized animals, stored frozen until use, then sonicated in phosphate buffer. The homogenate was centrifuged at low speed to remove debris. Within a nanomolar range of 3H-QNB concentrations, half of the atropine-sensitive binding sites were filled at 0.7 nM and saturation was shown with 0.8 to 1.5 nM. Scatchard analysis revealed only one class of binding sites, with a maximum number at saturation of 41.7 fmol/mg protein. Muscarinic cholinergic compounds specifically blocked 3H-QNB binding sites at low concentrations. Binding of 3H-QNB was reduced by 50% (IC50) at these concentrations: acetylcholine (ACh) at 6 uM, atropine at 5 nM and oxotremorine at 0.4 uM. With a nicotinic antagonist, curare, the measured IC50 was 100 uM. Since curare is pharmacologically active at the micromolar range in the superfused system (Konishi, Acta Oto-laryng. 74, 1972), it is likely that nicotinic ACh receptors also are present in the cochlea.

Efferent innervation is maximal in the base of the cochlea, and receptor numbers show a similar distribution. In separate measurements on pooled first and second or third and fourth turns, we found 0.34 fmol/apical turns and 1.1 fmol/basal turns.

Our findings represent the first measurement of any neuroreceptor binding site in the cochlea, and are consistent with the use of ACh as a transmitter by the olivocochlear bundle.

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175.3 THE EFFECT OF NOISE EXPOSURE ON DEOXYGLUCOSE UPTAKE IN THE INNER EAR OF THE MOUSE. Jochen Schacht and Barbara Canlon*. Kresge Hearing Research Institute, The University of Michigan, Ann Arbor, MI 48109.

Deoxyglucose trapping in peripheral and central auditory structures was determined by microdissection of tissues and scintillation counting of the radioactive tracer. CBA mice received a pulse of ^{3}H-deoxyglucose into the tail vein (5 mCi/kg body weight) and blood samples and organs were taken after various time intervals. Prior to dissection, enzymatic activities were arrested by microwave irradiation. Radioactivity and protein concentrations were determined in homogenates of 1) organ of Corti, consisting of the auditory receptor cells as well as supporting cells; 2) lateral wall tissues, a combined preparation of stria vascularis and spiral ligament; 3) cochlear portion of the VIIIth nerve; 4) inferior colliculus. Radioactivity in all inner ear tissues was maximal at 45 to 60 min and the steady-state ratio of deoxyglucose-6-phosphate to deoxyglucose was 60:40. Animals were exposed to noise (white noise, free field) ranging from 25 dBA (anechoic chamber) to 115 dBA (acoustic overstimulation). There were no effects on serum glucose levels or serum kinetics of deoxyglucose. In all auditory structures examined deoxyglucose uptake increased 4 to 5-fold between 25 and 85 dBA and decreased by 25-50% between 85 and 115 dBA. Exposure and pulse times of 15 to 180 min were studied, and the effects were most clearly evident at 60 min. The increased uptake is consistent with analogous findings in the central nervous system and should represent increased metabolism and local blood flow. The finding that the lateral wall tissues which are devoid of neural innervation also respond to noise stimulation points to a close coupling of metabolism in the cochlea. It could indicate a regulation of cochlear blood flow at the modiolar level. The reason for the decreased uptake at high intensities remains speculative and may be related to vasoconstriction of cochlear vessels. It is interesting to note that electrophysiological responses of the ear follow a similar pattern as observed for deoxyglucose trapping: the cochlear microphonic potential rises with increased stimulus intensity and decreases with overstimulation. Likewise, firing rate of single auditory fibers in various species is maximal at moderate stimulation of 60 to 80 dB. Thus, deoxyglucose utilization in the auditory periphery is well correlated with the pattern of the electrophysiological response.
(Supported by research grant NS-12881, Program Project grant NS-05785 and Training grant NS-07106 from the National Institutes of Health)

175.4 EFFECTS OF SHORT-TERM ANOXIA ON THE INTRACELLULAR POTENTIALS FROM COCHLEAR INNER HAIR CELLS. M.C. Brown, A.L. Nuttall*, R.I. Masta* and M. Lawrence*. Kresge Hearing Research Institute, The University of Michigan Medical School, Ann Arbor, MI 48109.

Intracellular recordings were obtained from inner hair cells in the guinea pig cochlea. Short-term anoxia was induced in these paralyzed animals by turning off the respirator for brief periods. A 30 second anoxia induced a rapid and profound decrease in the DC receptor potential response to low intensity tone bursts at the characteristic frequency of the hair cell. Frequencies different from the characteristic frequency evoked smaller receptor potentials which showed proportionally less reduction during anoxia. The resting membrane potential was little affected during the anoxic period.

Other cochlear potentials were also affected by short-term anoxia. A large reduction was observed in the click-evoked compound action potential recorded from the round window. The percent reduction of the endocochlear potential was smaller in magnitude than the reduction of the DC receptor potential while the 4 kHz cochlear microphonics were least affected. These results suggest that the large effect of anoxia on the auditory neural potentials may be the result of oxygen depletion at elements peripheral to the nerve fibers.

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175.5 REFLEX INHIBITION AUDIOMETRY AS AN INDEX OF OTOTOXICITY. J.S. Young* and L.D. Fechter (SPON: W.R. Millington). The Johns Hopkins University, School of Hygiene and Public Health, Department of Environmental Health Sciences, Division of Toxicology, Baltimore, MD 21205

Attempts to provide a functional assessment of toxic damage to the auditory system face substantial constraints. The methods of traditional animal psychophysics are exquisitely sensitive, but require a substantial investment of time and effort in subject training. This limits the number of subjects which can be tested. Reflex procedures (e.g. Preyer's reflex, startle reflex) provide a much more rapid assay of hearing function, but are markedly less sensitive, with thresholds 50-60 dB above the detection threshold.

We have determined pure-tone audiometric functions in Long-Evans hooded rats, for stimuli between 2.5 and 40 kHz, in a procedure based on measuring the modulation of the startle reflex by weak stimuli which precede a startle-eliciting stimulus. No subject training is required for these determinations. Tone prestimuli of varying frequency and intensity are presented according to the psychophysical method of constant stimuli, and tone detection is assessed by comparing the amplitude of the whole-body startle response on trials containing a tone prestimulus to startle amplitude on interspersed trials on which only the startle stimulus is presented. We have further examined the effects of combined injections of an aminoglycoside antibiotic (neomycin) and aminooxyacetic acid, a treatment previously shown to produce rapid and severe hair cell damage (Cronin-Schreiber, R. et al., Soc. Neurosci. Abstr., 6:42, 1980).

We have found thresholds of startle inhibition to be quite similar to absolute detection thresholds as measured by operant techniques (e.g., Kelly, J.B. & Masterton, B., J. Compar. Phys. Psych., 91:930, 1977). Increases in the thresholds of startle inhibition of the treated subjects were noted within a few days of the combined injections.

Our results indicate that startle inhibition audiometry provides a rapid, sensitive index of hearing function, and of toxic insult to the auditory system. In addition, they provide a behavioral corroboration of the anatomical and electrocochleographic effects reported for combined administration of aminoglycosides and aminooxyacetic acid.

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175.6 STIFFNESS MEASUREMENTS OF STEREOCILIARY BUNDLES IN FROG CRISTA AMPULLARIS. S. Orman* and A. Flock* (SPON: J. Hind) Dept. of Neurophysiology, Univ. of Wisconson, Madison, WI, and Dept. of Physiology II, Karolinska Institute, Stockholm, Sweden.

Single stereociliary bundle displacements resulting from a small jet of frog's Ringer's solution of known velocity, have been measured in the sensory hair cells of the frog's crista ampullaris. The bundle stiffness -- the ratio of the fluid drag force to the angular displacement -- has been calculated to be approximately 4.6×10^{-11} N/rad .

The crista was dissected free from the ampulla of the posterior vertical canal and positioned in a fluid filled chamber where individual stereociliary bundles could be viewed with differential interference contrast optics. A 50 µm micropipette was brought into close approximation with an individual bundle allowing delivery of a fluid jet from a specially constructed microsyringe apparatus. The displacements were recorded on 16 mm film and both the angle of deflection and the time for the return to the stereocilia's resting position were measured on the film. The deflection curves reveal that the stereociliary bundles of the frog crista are highly overdamped and obey a Hooke's law type equation, i.e., the restoring torque returning the bundles to the resting position after the fluid jet was stopped was proportional to the angle of displacement. This supports the model that stereociliary stiffness restricts bending to a hinge region at the apical surface of the hair cell.

To determine if Ca^{2+} could influence the mechanical properties of the stereociliary bundles through action on their actin protein component, the calcium ionophore A 23187 was added to the Ringer's solution bathing the crista preparation. When the ionophore was added to a calcium free Ringer's bath no change was seen in the bundle's response to the fluid jet. When the ionophore was added to the normal Ringer's solution (2 mM Ca^{2+}) an immediate decrease in the deflection response to the fluid jet was observed. The resulting increase in bundle stiffness, due to the calcium, was calculated to be in the range of 20-25 %. The modulatory role of calcium in hair cell transduction through an active mechanical control mechanism will be discussed.

175.7 HAIR CELL ORIENTATIONS IN THE GRAVISTATIC ORGANS OF THE BLACK TETRA, A CHARACIN FISH. Christopher Platt. Dept. Biological Sciences, Univ. Southern California, Los Angeles, CA 90007.

The inner ear of the black tetra, Gymnocorymbus ternetzii, is interesting for two reasons. First, this is one of the few teleost fishes for which we know gravistatic functions of parts of its inner ear (von Holst, Z. vergl. Physiol. 32:60, 1950; Schoen, & von Holst, Z. vergl. Physiol. 32:552, 1950). Second, the black tetra represents the characins, a major family within the ostariophysines, a large group of teleosts with good hearing, from which only carps and catfish have had their ears examined. I have now mapped the tetra's inner ear sensory surfaces using scanning electron microscopy. Before removal from the head, the vestibular membraneous pouches were nicked for precise spatial orientation marks. Each sensory hair cell has an apical ciliary bundle, with an asymmetrically placed single kinocilium that gives each cell an observable orientation corresponding to its peak directional sensitivity.

Black tetras 30-35 mm long have roughly 10,000 hair cells in the utricle, their major gravistatic otolith organ. The cup-shaped sensory macula faces upward; its narrow posterolateral extension, the lacinia, is covered only by otolithic membrane. The striola of the main macula is a broad arc of cells with large prominent bundles; it courses almost 270° anteriorly around the macula, from the medial side to the base of the lacinia. Utricular hair cells form two oriented populations. From the central medial side, cell orientations radiate outward in a fanlike pattern. More marginal cells of the anterolateral side have orientations opposed to the central group, and so face radially inward. A line drawn between the two populations does not bisect the macula, but curves along the middle of the striola and lacinia. Thus a single utricle is asymmetrical, with a majority of cells oriented facing ipsilaterally.

The lagena is the tetra's other gravistatic otolith organ. Its macula is on its vertical medial wall, and contains a prominent anterior and posterior patch of large-bundled hair cells, both within a large oval area of small-bundled cells. Cells in the dorsal region face generally ventrally, while those ventral face dorsally. These two opposing populations form a boundary that runs near the middle of the macula from the rostral tip, then curves downward toward the caudal end.

These orientation patterns show that the unilaterally operated fish of von Holst and others indeed are left with asymmetric unequal populations of opposing cells in the remaining ear. The receptor patterns in all the tetra's inner ear surfaces, including also the saccule, macula neglecta and semicircular canal cristae, are extremely similar to those in other ostariophysines.

176.1 HYPERPLASIC GANGLIA AND THEIR INNERVATION PATTERNS IN FROGS WITH EARLY DORSAL ROOT GANGLION REMOVALS. M. Davis and M. Constantine-Paton. Dept. Biol., Princeton Univ., Princeton, N.J. 08544.

The problem of how sensory neurons project to appropriate CNS and peripheral regions can be studied by experimental alteration of the size of the target area. Increases in the periphery have been achieved by early removal of hindlimb dorsal root ganglia (DRG) in Rana pipiens tadpoles. The procedure has been reported to result in an increase in cell number (hyperplasia) in the adjacent ganglion relative to an equivalent ganglion on the opposite side (Bibb,H.,1977,J.Exp.Zool.200:265-275). We have further investigated the hyperplasic response to determine whether the size and position of the denervated region are consistently related to the increase in sensory cell number. We also asked whether the hyperplasic ganglia actually innervate the denervated central and peripheral areas.

Various combinations of tadpole hindlimb DRG were removed, and the animals were permitted to survive past metamorphosis. DRG cell counts were then used to assess the extent of hyperplasia. This information was combined with either physiological maps of hindlimb nerve receptive fields or with anatomical examinations of central and peripheral innervation. Examination of these areas was done by 1) ^{3}H-proline injections into the ganglion followed by autoradiography on the spinal cord, or 2)applications of horseradish peroxidase (HRP) to the cut sciatic nerves for determination of labeled cell numbers in ganglion 10(defined by Ecker,1889).

The location and degree of hyperplasia were highly variable. Although hyperplasias were found in the ganglia adjacent to those removed, some animals showed hyperplasia in non-adjacent ganglia. In these cases intervening ganglia were either normal in cell numbers or hyperplasic. Non-adjacent hyperplasias occurred most frequently in ganglion 10 which normally sends only a small branch to the hindlimb. Our HRP studies show that a hyperplasic #10 has many more labelled cells coursing through the sciatic nerve than contralateral #10. Receptive field mapping of hindlimb nerves shows that hyperplasic ganglia increase their peripheral fields.Autoradiography suggests no appreciable increase in the central terminations of hyperplasic ganglia compared to controls.

Thus, although the location of ganglionic hyperplasia cannot be reliably predicted from the type of tadpole ganglion removal, ganglia showing hyperplasia are often associated with increases in peripheral area. We conclude that the peripheral addresses of hindlimb DRG are not rigidly defined. The central processes of these same DRG do not show an equivalent capacity to expand their segmental spread within the spinal cord.

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176.2 TIME-COURSE FOR RECEPTIVE FIELD PLASTICITY OF DORSAL HORN NEURONS IN HEMISECTED CATS. Gene L. Brenowitz. Department of Anatomy, The Medical College of Pennsylvania, Philadelphia, PA 19129.

We have recently demonstrated a reorganization of cutaneous input to dorsal horn neurons following partial spinal cord hemisections (T 13, sparing the dorsal columns) in cats (Brenowitz and Pubols, Soc. Neurosci. Abstracts 6:847). The most striking change observed was a large increase in the size of light tactile, proximal hindlimb receptive fields in a discrete zone in the lateral dorsal horn ipsilateral to the lesion in animals with chronic (3-6 mo.) but not acute (6h-5d) survivals.

The present study was aimed at establishing when between 5d and 3 mo. the increase in receptive field size occurs. The sizes of light tactile, proximal hindlimb receptive fields (plotted with a 1gm vonFrey hair) and somatotopic organization, in general, of L7 dorsal horn neurons were studied in methoxyflurane anesthetized cats partially hemisected (T13, sparing the dorsal columns) 7-30d prior to recording.

Enlarged proximal hindlimb receptive fields were found in animals surviving 14d after a hemisection. The largest field observed extended from the dorsal midline, down the lateral leg, to a point midway down the lateral foot. The enlarged fields seen at this survival time were as large as those found in animals with 3-6 mo. survivals. These results indicate that by 14d the reorganization of cutaneous input responsible for this change is virtually complete and remains stable for at least 6 mo. Animals with 7-12d survivals did not have enlarged proximal hindlimb receptive fields, although it is difficult to detect subtle changes in field size.

The results of this study indicate that between 12 and 14d following a partial hemisection, increases in the size of light tactile, proximal hindlimb receptive fields become detectable and reach their maximum. These fields remain enlarged over the next 6 mo. The process or processes responsible for this reorganization may well begin earlier than 14d, going undetected until this time. In the future, this time-course for receptive field size changes should help differentiate between several alternative explanations for such plasticity.

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176.3 Ultrastructural Features of Dorsal Column Unmyelinated Fibers After Acute Injury of the Rat Spinal Cord and DMSO Treatment. P.K. Hill, J.C. de la Torre, S.M. Thompson,* S. Rosenfield-Wessells* and M.L. Beckett.* Department of Anatomy, Eastern Virginia Medical School, Norfolk, Virginia 23501 and Department of Neurosurgery, University of Miami School of Medicine, Miami, Florida 33101.

The project was conducted to elucidate the ultrastructural features of the adult rat dorsal funiculi unmyelinated fibers following acute injury and dimethyl sulfoxide (DMSO) treatment. Three groups of animals were included in the study: Group I (6 adult rats) received L-1 laminectomy, no contusion and 4 days of saline-filled Alza mini-pump implantation. Group II (6 adult rats) received L-1 laminectomy, contusion of L1-2 with a force of 0.17640 Newtons and 4 days of saline-filled Alza mini-pump implantation. Group III (6 adult rats) received L-1 laminectomy, contusion of L1-2 with a force of 0.17640 Newtons and 4 days of DMSO-filled Alza mini-pump implantation. Group II and III animals also received an intraperitoneal injection of saline and 1.5 gm/Kgm. body weight of DMSO, respectively, 3 times daily for 4 days in order to maintain systemic levels of the drugs. Qualitative ultrastructural examination of the right and left dorsal columns was performed at the lesion site (L1-2) as well as 1 centimeter proximal (T 11-12-13) and distal (L3-4-5).

The general trend of unmyelinated fiber response to DMSO treatment is that the usual clustering arrangement is better preserved at the lesion site as well as proximal and distal to that area when compared with control tissue. In both saline and DMSO treated tissues, clusters of small and medium-sized unmyelinated fibers remained intact to a greater extent than larger unmyelinated terminals. In Group III distal to the lesion, however, larger terminals consistently retained synaptic contacts and axoplasmic constituents to a greater extent than those in Group II.

The significance is that unmyelinated fibers of all sizes are better protected ultrastructurally from degenerative changes during acute injury in DMSO treated animals than are those of the saline treated group. Since functional return after experimental spinal cord injury as facilitated by DMSO treatment has been documented as a prognostic indicator of subsequent motor function return (J.C. de la Torre, et. al. Ann. N.Y. Acad. Sci. 243:362-389, 1975), structural preservation of unmyelinated dorsal column fibers may provide the initial step for potential recovery.

"Sponsored by the Eastern Paralyzed Veterans Association"

176.4 CENTRAL NEURAL TASTE RESPONSES IN PREPUBERTAL AND ADULT RATS. D.L. Hill, R.M. Bradley, and C.M. Mistretta. Dept. Oral Biol., Sch. Dent., U. Mich., Ann Arbor, MI 48109.

To determine whether there are functional changes in responses from central taste neurons after weaning in rats, electrophysiological recordings were made from chemosensitive units in the nucleus of the solitary tract (NST) in the medulla. Twenty-three single units were studied in rats aged 25-35 days (prepubertal) and 28 units in adults. Chemical stimuli applied to the anterior tongue were 0.1M and 0.5M NH4Cl, NaCl, LiCl, and KCl. Neural activity was measured for the first 5 sec after stimulation of the tongue; a comparable period of pre-stimulus spontaneous activity was subtracted to yield response frequencies.

For each stimulus, response frequencies in rats aged 25-35 days were lower than those in adults. However, only response frequencies to NaCl and LiCl were different at a statistically significant level ($p < 0.05$); response frequencies to these two salts in prepubertal rats were at least 50% lower than adult frequencies. Further differences were apparent when neurons were categorized on the basis of responding maximally to 0.1M NH4Cl, to 0.1M NaCl/LiCl, or equally to all 0.1M salts; no NST neuron responded maximally to 0.1M KCl. The percentage of neurons within each category are presented in the table.

	Maximally Effective Stimulus (% Neurons)		
Age	NH4	Na/Li	Equally
24-35 days	48	9	43
adult	14	22	64

Most younger neurons responded maximally to NH4Cl or equally to all salts; very few responded maximally to NaCl or LiCl. In adult rats, neurons generally responded equally to all salts, with about the same distributions in NH4 versus Na/Li categories.

We conclude that taste responses of rat NST neurons change after weaning. As in peripheral taste neurons, striking differences are found in responses of central neurons to NaCl and LiCl. Major response frequency changes to NaCl and LiCl in peripheral fibers, however, occur before weaning. Maturation of taste responses may be progressively delayed in more central neural structures, and adult rats would presumably differ from younger rats in behavioral responses to some taste stimuli. (Supported by NIH Postdoc. Fellowship NS06423 to D.L.H., NSF Grant BNS-8015737 to R.M.B. and C.M.M., and Res. Career Dev. Award, NIDR, DE-00066 to C.M.M.)

176.5 ANATOMICAL AND HISTOCHEMICAL STUDY OF THE ACCESSORY OLFACTORY BULB OF MOUSE. F. Miragall* and P.P.C. Graziadei. Biology, Florida State University, Tallahassee, Fla 32306

Recent studies have stressed the importance of the vomeronasal (VN)-accessory olfactory bulb (AOB) system in the sexual behavior of rodents. Anatomical observations, however, are mostly centered on the main olfactory bulb (MOB) while the AOB has received so far little attention.

The present investigation provides preliminary information on the morphological and chemical aspects of the AOB.

Light microscope observations with silver methods specific for the demonstration of the nervous tissue show that the glomeruli of the AOB stain in a characteristic blue-purple color as opposed to the pale red color of the MOB glomeruli. The same silver methods stain selectively the mitral and tufted cells of the MOB while they fail consistently to stain the same cells in the AOB. The differential stainability of the two centers is presently further investigated.

With immunohistochemical methods for the demonstration of the olfactory marker protein the AOB glomeruli show a faint yellow stain as opposed to the deep brown color of the MOB glomeruli.

The glomeruli of the AOB are arranged in a large, globose mass where the individual glomerular profiles are indistinct and the periglomerular cells are sparse. In the MOB the glomeruli are more discretely arranged and their profiles are separated by layers of periglomerular cells. In the AOB the external plexiform and mitral cell layers are not discretely arranged.

The above features will be illustrated together with the ultrastructural details of the organ.

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176.6 DEVELOPMENTAL DIFFERENCES IN THE BIRTH DATE OF OLFACTORY BULB PROJECTION NEURONS IN THE GOLDEN HAMSTER. Marjorie R. Grafe, Dept. of Neuroscience, Univ. of Florida, Gainesville, FL 32610

The golden hamster is born after an extremely short gestational period of 16 days. Although neonatal hamsters can respond to odors, their olfactory system undergoes many anatomical and functional changes in the first three weeks postnatally. We have previously reported a developmental change in the distribution of cells in the olfactory bulb (OB) which contribute axons to the lateral olfactory tract (LOT) (Grafe and Leonard, 1981). Cells which send efferents from the OB in the first week of life are concentrated in the medial quadrant of the bulb. The present study was undertaken to determine if there was a corresponding topographical gradient in the time of origin of these cells.

Pregnant hamsters were injected with ^{3}H-thymidine on gestational days 10 (E10), 11, 12, 13 and 14, and pups on postnatal days 1 (P1) and 2 (E16=P0). The pups were sacrificed at about P30, and heavily labeled mitral cells (MC) and tufted cells (TC) were plotted and counted from coronal sections of the OB. No MC or TC were heavily labeled after injections on E10. MC were formed only on days E11-12. TC were formed on E11-14. When classified according to their positions in the external plexiform layer (EPL), we found that inner tufted cells (ITC) were formed on E11-12, while middle and outer tufted cells (MTC and OTC) were formed on E12-14. By P1, the only heavily labeled neurons in the OB were granule cells. The projection neurons of the OB can be broken down into two groups on the basis of their birth dates: 1) the early-formed MC and ITC, and 2) the later-formed MTC and OTC. The time of origin of the MC and TC is much more compressed than in the mouse, which has a longer gestational period of 19 days. Hinds (J.Comp.Neurol.134:287,1968) found MC and TC originating on E10-18, with a fairly distinct progression from MC to ITC to OTC.

We found no consistent topographical gradients in the time of origin of MC and TC other than the depth distribution in the EPL. There was, however, a distinct tendency for labeled cells to cluster in small groups, sometimes widely separated from other labeled cells, suggesting a possible common origin of these cells. Studies are currently in progress combining thymidine labeling with retrograde transport of HRP to determine if there is a direct correlation between time of origin of OB cells and the arrival of their projections into olfactory cortex.

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176.7 BIOCHEMICAL CONTROL OF OLFACTORY NEURON REPLACEMENT. C. Camara and J.W. Harding. Department of Veterinary and Comparative Anatomy, Pharmacology and Physiology, Washington State University, Pullman, WA 99164.

Sectioning of the primary olfactory nerve is known to cause retrograde degeneration and death of the primary olfactory neurons in vertebrates. These neurons, unlike others in mature mammals, can be replenished from an undifferentiated stem cell population called basal cells. This replacement process, which is accelerated following injury, also occurs on a regular basis imparting to these neurons a finite lifespan. Using ^{3}H-thymidine incorporation into DNA to quantitatively assess mitotic activity and autoradiography to identify the actively dividing cell population, the mitotic activity of basal cells in olfactory epithelium was monitored at various times following olfactory nerve section. The mitotic rate increased dramatically after surgery, reaching a maximum at four days, at which time a 15-fold increase in mitotic rate was measured. Subsequently the rate declined, returning to control level by day 10. The rate of mitosis of basal cells appears to be controlled by the dipeptide carnosine, which is contained specifically within the mature neurons and its amino acid component, β-alanine. These compounds have antagonistic effects with carnosine acting as a powerful inhibitor and β-alanine working as a strong activator of ^{3}H-thymidine incorporation. The primary olfactory pathway makes an excellent model system to study the endogenous control of mitogenesis. The usefulness of this system is further amplified by the fact that the primary olfactory neurons represent the only neuronal population which is replaced on a regular basis in vertebrates.

176.8 OLFACTORY GRANULE CELL DEVELOPMENT IN NORMAL AND HYPERTHYROID RATS. P. C. Brunjes, W. T. Greenough and H. D. Schwark*. Dept. of Psychol. and Program in Behav. and Neural Biol., Univ. of Illinois, Champaign, IL 61820.

Neonatal hyperthyroidism results in accelerated anatomical maturation in many neural regions and in precocious functional capabilities, including early development of odor sensitivity (Brunjes & Alberts, Horm. & Behav., 14: 76, 1980). This study was designed to determine whether cellular maturation in the olfactory bulb parallels that of chemosensory abilities. The granule cell was investigated because its late formation and maturation suggested particular susceptibility to hormonal treatment. On the day after birth (Day 1), litters were trimmed to 8 rats (4♂, 4♀). Two males and 2 females received i.p. injections of L-thyroxine sodium (1 µg/gm body weight) on Days 1-4, while control pups received vehicle only. Eight littermate pairs (4♂, 4♀) were sacrificed at 7, 14, 21, and 60 days of age, their brains processed via Golgi-Cox techniques and sectioned at 120 µm. Fifteen internal granule cells were drawn (500x) per brain, using a camera lucida and criteria which maximized areas sampled. Tissue was coded to prevent experimenter bias during all stages of data collection. Measurements included: width and length of external plexiform layer portions of dendrites, number of 10 µm "Sholl" ring intersections, number and length of branches per order, and spines/dendritic length.

Development in both groups followed a similar course, with overproduction of dendritic material followed by subsequent regression. Nearly every measure (width of dendritic field, total dendritic length, total number of branches, ring intersections) exhibited peak values on Days 14-21, and decline thereafter. Similar "inverted U-shaped" curves of cellular, dendritic and synaptic development have been reported in several neural regions (Purves & Lichtman, Science, 210: 153, 1980) suggesting that the sequence may be a general feature of neural maturation.

Hyperthyroidism did not accelerate dendritic maturation in 7-day-old animals, the age at which precocious odor sensitivity was reported, and did not grossly affect development in older age groups. The results are striking because the bulb exhibits similarities with region in which major hormone-induced changes have been demonstrated (e.g. cerebellum, hippocampus). While our findings may be due to strain/dose differences, perhaps the bulb is relatively resistant to hormonal fluctuation. Nevertheless, the results are intriguing given that hyperthyroidism accelerates odor sensitivity but not the early anatomical maturation of the olfactory bulb.

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176.9 REGIONALLY SPECIFIC ALTERATIONS IN THE GROWTH OF OLFACTORY CORTEX FOLLOWING NEONATAL BULBECTOMY IN GOLDEN HAMSTERS. T. Schoenfeld, J.V. Corwin*, and C.M. Leonard. Department of Neuroscience, University of Florida College of Medicine, Gainesville, FL 32610

When the olfactory bulb is removed in the neonatal rat, intracortical (association) projections in the adult to the deafferented piriform cortex, normally restricted proximally, fill most of Layer I (Price et al., 1976; Westrum, 1975). However, the adult width of Layer I is reduced unless deafferentation occurs within 2 days of birth (Friedman & Price, 1978). We examined the generality of these findings for various parts of the olfactory cortex and for another rodent species, the golden hamster. The left olfactory bulb was aspirated in hamsters 5 (P5) or 19 (P19) days old, and the hamsters were sacrificed as adults 7-9 months later. The width of Layer I was measured bilaterally in the olfactory tubercle (OT), anterior piriform cortex (APC), and posterior piriform cortex (PPC).

Deafferentation at P5 produced a reduction in Layer I of the lateral OT of about 35% when compared to standard values from intact brains ($p<.05$). Deafferentation at P19 produced no change in the width of Layer I in the lateral OT. In the APC lateral to the lateral olfactory tract (LOT), a moderate 20% reduction relative to intact values (statistically ns.) was observed following bulbectomy at either age. Little or no alteration in the width of Layer I was seen in the medial OT, PPC, or APC deep to the LOT. Although terminals of the intracortical system (arising from APC, visualized with degeneration techniques) were found to extend to the pial surface in all three areas after bulbectomy at either age, regional differences in the growth of Layer I following deafferentation suggest that, in some cases of neonatal bulbectomy, the laminar distribution of intracortical innervation may represent some sprouting of terminals superficially while, in other cases, it may represent maintenance of a proximally restricted terminal field.

Of particular interest was the finding that bulbectomy at P19 but not P5 produced an expansion of Layer I in the contralateral lateral OT and the APC deep to the LOT (greater than intact, $p<.05$). This regional specificity in the expansion of Layer I suggests a role for the anterior olfactory nucleus (AON). Removal of AON terminals with a bulbectomy may result in an expanded arborization of AON terminals in other areas which the AON normally innervates, i.e., the lateral OT and the APC deep to the LOT. Expanded terminal arborization could then produce laminar expansion if synaptic density remained nearly normal. This expansion in olfactory cortex may represent another demonstration of the conservation of terminal arborization described by Schneider.

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176.10 NORMAL AND ABNORMAL AUDITORY CORTICOTECTAL PROJECTIONS IN RATS. L. L. Rose*, P. W. Land and A. R. Harvey* (SPON: N. S. Buckholtz). Department of Anatomy, Medical University of South Carolina, Charleston, South Carolina 29425

We examined the projections from the auditory cortex to the inferior colliculi (IC) of normal rats and rats which had received unilateral auditory cortex lesions at birth. Newborn rats were anesthetized with ether and the caudal and lateral portions of one cerebral hemisphere were removed by aspiration. Four months later, a lesion was made in the auditory cortex of the intact hemisphere. After a 4 day survival, animals were perfused with 4% paraformaldehyde and frozen sections through the brains were stained with the method of Fink and Heimer. The brains of several normal adult rats with acute unilateral auditory cortex lesions received similar treatment.

In normal adult rats, degeneration resulting from unilateral auditory cortex lesions was distributed in a pattern similar to that described for the cat. Coarse degenerating fibers interspersed with fine granules of degeneration spread over the dorsal, lateral and caudal aspects of the ipsilateral IC. This degeneration was largely confined to a narrow lamina of cells corresponding to the pericentral nucleus of the cat but also extended to some extent into the external nucleus. In addition, moderate to heavy degeneration extended into the dorsomedial portion of the central nucleus (dorsomedial nucleus). A light but consistent projection was traced through the commissure of the IC, and sparse degeneration extended into the dorsomedial portion of the contralateral central nucleus.

Lesions of the intact auditory cortex of rats with unilateral cortical ablation at birth produced patterns of degeneration in the ipsilateral IC which were similar to control animals. In contrast to normal rats, numerous fascicles of degenerating fibers occurred in the commissure of the IC of these animals and both coarse and fine granules of degeneration could be traced throughout most of the pericentral and external nuclei of the contralateral IC. Moreover, degeneration in the dorsomedial portion of the contralateral central nucleus was much denser than in control animals, and in some cases appeared denser than the degeneration in the corresponding portion of central nucleus ipsilateral to the lesion.

These results show that, as with other corticofugal pathways, the normal pattern of the auditory corticotectal projection is achieved, in part, through some cooperation between populations of axons from both hemispheres. However, since the projection from the auditory cortex is normally bilateral to a portion of the IC, while that from the visual cortex to the superior colliculus is exclusively ipsilateral in rat, there are likely to be other factors which influence the laterality of cortical projections to the mesencephalon. (Supported by USPHS grant EY03414 and Anatomy Department development funds to P.W.L.)

176.11 NEURAL TUNING MATURES FIRST AT HIGH CHARACTERISTIC FREQUENCIES IN COCHLEAR NUCLEUS NEURONS. Nigel K. Woolf and Allen F. Ryan*. Otolaryngology Research Laboratory, University of California at San Diego Medical School and San Diego VA Medical Center, San Diego, CA 92103.

Anatomical evidence indicates that the cochlea matures sequentially in a basal to apical direction. As a consequence of this, it would be expected that maturation of high frequency physiologic function would precede that for lower frequencies. The occurrence of neurones with high frequency CFs (characteristic frequencies) at the onset of hearing has not previously been reported and consequently this prediction has not been tested. In order to test the hypothesis, response properties of single neurons in the cochlear nuclei (CN) of Mongolian gerbils (Meriones unguiculatis) were examined by conventional extracellular recording techniques at postnatal ages 10,12, 14, 16, 18, 30, 60, 90 (young adult) days after birth (DAB),and adult. This range of chronological ages included the onset of neonatal hearing through achievment of mature auditory system characteristics.

Single neuron recordings in the neonatal gerbil CN revealed clear ontogenetic trends. At 10 DAB none of the neurons recorded from within the CN responded to acoustic stimulation. At 12 DAB only a small percentage of the neurons encountered were responsive. For subjects 14 DAB, or older, the vast majority of neurons isolated in CN were responsive to acoustic stimulation. Based upon data taken from neurons which were responsive, it was evident that with increasing chronological age threshold sensitivity rapidly and monotonically increased at all CFs. In contrast, the maturation of tuning in neurons with high-CF was quite different from that of neurons with low-CF. With increasing chronological age the frequency tuning curves for neurons with CF below 3 kHz exhibited a systematic increase in sharpness of tuning (Q10dB) and an increase in slope for both the high and low frequency boundaries for their tuning curves. This trend is consistent with previous reports. However, for neurons with high-CF ($CF \gg 3$ kHz) the sharpness of tuning (Q10dB), at all ages, can approximate normal adult values. That is, tuning at 12 DAB for high-CF neurons can be as sharp as that at maturity in spite of CF threshold disparities of 100 dBSPL or more. The existence at the onset of auditory function of neurons with high-CF and sharp neural tuning is a novel finding. This report thus supports the hypothesis that physiologic functional maturation in the central auditory nervous system is closely correlated with anatomic maturation in the cochlea.

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176.12 MORPHOLOGICAL DEVELOPMENT OF RAT COCHLEAR NUCLEI AND AGE-DEPENDENT EFFECTS OF ACOUSTICAL DEPRIVATION. J. Coleman, B.J. Blatchley, and J.E. Williams*. Departments of Psychology and Physiology, Univ. of South Carolina, Columbia, SC 29208.

The goal of this study was to examine changes in volume of the dorsal and ventral cochlear nuclei (DCN and VCN) during different stages of development and to determine the morphological effects of acoustically depriving groups of animals from various ages.

To determine the normal development of the cochlear nucleus, albino rats were sacrificed at 10, 16, 24, 36 or 70 days of age. Sections were drawn on a microprojector and the volumes of DCN and VCN were calculated from planimetric measurements. The volume of VCN increased as a function of age, with the greatest increase occurring between 10 and 16 days of age ($\alpha = .05$). Age-dependent volume differences of VCN were as follows: 71% between 10 and 70 days, 50% between 16 and 70 days, 33% between 24 and 70 days, and 17% between 36 and 70 days; the DCN also increased in size but the slope of expansion was less than for VCN.

The external auditory meatus was ligated in groups of rats at 10, 16, 24, or 36 days after birth and the animals allowed to survive to 70 days. The effects of monaural sound attenuation upon volume of the cochlear nuclei and upon the size of large spherical cells within the ACVN were then studied. The VCN of rats deprived from all ages were reduced in volume ($\alpha = .01$) with the largest difference in volume occurring between 10 and 16 days of age. VCN volume decreased for deprived groups relative to 70 day old normals by the following respective percentages: 43% for 10 day olds; 32% for 16 day olds; 24% for 24 day olds; and 20% for 36 day olds. Large spherical cells, which receive direct input from the auditory nerve, were smaller in the early deprived animals than those from normal cage-reared animals ($\alpha = .05$). Cell area differences between deprived animals and 70 day olds were: 36% for 10 day olds; 21% for 16 day olds; 17% for 24 day olds; and 15% for 36 day olds.

These results demonstrate that the most dramatic period of development for VCN and DCN occurs between 10 and 16 days in rat. The effects of acoustical deprivation on VCN and DCN volume and on large spherical cell area are most pronounced when deprivation is initiated within this same period of development. These findings concur with observations that there is increased auditory nerve activity during this stage of development in rat. (Supported by the Deafness Research Foundation.)

176.13 EARLY AUDITORY EXPERIENCE MODIFIES SOUND LOCALIZATION IN THE OWL. Phyllis F. Knudsen* and Eric I. Knudsen. Dept. of Neurobiology, Stanford University, Stanford, CA. 94305

Young barn owls were plugged in one ear and later tested to determine if they could accurately locate sounds. The owls compensated for the abnormal acoustic conditions created by the plugs and localized sounds without error.

Two barn owls (age 28 and 30 days) were monaurally occluded with foam rubber ear plugs. These owls and one unplugged owl were trained at approximately 8 weeks of age to turn their heads and fixate on sound and light stimuli delivered to them in an anechoic chamber. Owls naturally orient to sounds and lights but this behavior was positively reinforced to reduce habituation to the task. The sounds were repetitive noise bursts of various durations; the light was a light-emitting diode located at the center of the speaker cone. The speaker moved on a semicircular track that pivoted around a horizontal axis so that the speaker could be positioned nearly anywhere around the owl. Since an owl's eyes are stationary, the owl must turn its head to fixate an object visually. The experiments were performed in the dark and the owl's head movements were monitored with an infrared sensitive monitor.

For the behavioral experiments the speaker was moved to a random location, the sound or light stimulus was turned on, and the owl's orientation response was recorded. Forty to fifty responses to sound and light were collected from random locations around the owl. For each trial the owl's response, relative to the actual location of the speaker, was computed. Sound localization error was defined as the difference between the mean orientation to lights and the mean orientation to sound. In normal owls this error is less than 2° in azimuth and elevation.

The two experimental owls were tested after they had been plugged 110 days. Their respective localization errors were right 1.9°, down.8° and right 1.8°, down.5°. Thus, they localized sound with normal accuracy. We then removed their ear plugs and measured their sound localization error. The initial error of one owl, the day her plug was removed, was 6.7° left, 18.4° down. Over the next few weeks her error decreased and after 24 days her error was 1° left and 2° down. The second owl showed a similar pattern of compensation. His initial error was right 12.4° and up 7.6°. After 15 days his localization error was less than 2° in both azimuth and elevation.

We conclude that the auditory system of owls is capable of modifying its sound localization mechanism based on experience in early life.

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176.14 TOUCH DOMES AND THEIR MERKEL CELLS IN THE DEVELOPING AND MATURE RAT. J. Diamond, C.A. Nurse and B. Visheau. Dept. of Neurosci., McMaster Univ., Hamilton, Ontario L8N 3Z5.

In an accompanying Abstract (Nurse et al.) we show that epidermal mechanosensory target cells, the Merkel cells, can be labelled with the fluorescent dye quinacrine. Here we use this technique to follow the appearance of the quinacrine fluorescent cells (QFC) in the developing touch domes of rat skin. The density of dome "primordia" (DP) and the distribution of QFC per DP_2 were determined in rectangular strips of epidermis (10-40 mm^2). In animals < 2 weeks old, touch domes are not readily visualized structures, but in epidermis that is mechanically separated from the dermis following ½ hr incubation in 3 mg/ml dithiothreitol, the sites of the DP can be easily recognized by the flat elliptical annuli of QFC scattered across the basal epidermis. At day 1, the average density (σ) of DP was consistently higher on the dorsal (D) than on the ventral (V) trunk skin; the mean σ_D was about 1.3 DP/mm^2 and the mean σ_V was about 0.9 DP/mm^2. Both σ_D and σ_V subsequently decreased, and by two weeks they were usually <0.6 DP/mm^2. We are examining the likelihood that the DP are all established by birth or shortly thereafter, and then simply become further separated as the animal grows. The number of QFC per dome varied in both dorsal and ventral skin, from a low range of 3-50 QFC through an intermediate range of 51-100, to a high range of >100 QFC. At day 1, 50-75% of DP had QFC in the low range in both dorsal and ventral skin, although in the latter, about 10% had QFC in the high range as compared to 0-2% in the dorsal skin. Preliminary indications are that by day 7, the proportion of DP in the high range had increased in both the ventral (ca. 25%) and the dorsal (ca. 10%) skin. We are extending these studies to include embryos and older animals. Analysis of excised domes from adults indicates that often these have 50-170 QFC. This is surprising, since estimates based on nerve terminal counts have suggested that there are only about 25 Merkel cells per adult dome (Smith, J. Comp. Neurol. 131: 459, 1967). Electronmicroscopic (E.M.) examination of domes excised from animals even a few weeks old reveals, in addition to normal Merkel cells, abnormal ones, "transitional" cells (English et al., J. Comp. Neurol., 194: 475, 1980) and an occasional "dead" Merkel cell; any of these might have normal, abnormal or no nerve endings. We are attempting to combine nerve terminal staining with quinacrine fluorescence, together with E.M. examination of touch domes, to clarify the intriguing "modelling" that seems to be occurring within these sensory structures.

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176.15 DEVELOPMENT OF THE TRIGEMINAL PATHWAY IN THE MOUSE. D. Durham and T.A. Woolsey. Dept. Anat./Neurobiol., Washington U. Sch. Med., St. Louis, MO 63110.

The large mystacial vibrissae on the faces of rodents are represented within the sensory trigeminal pathway in discrete segregated loci, the pattern of which can be demonstrated with Nissl stains and/or the succinic dehydrogenase (SDH) histochemical technique. Damage to the vibrissae during the 1st postnatal week results in an anatomical alteration of the whisker representations, which decreases in severity with the age at the time of the lesion. In mice, a "critical period" for susceptibility to peripheral damage was determined from Nissl stained material to end on postnatal day (PND)-5 in the cortex and PND-3 in the thalamus (birth = PND-1). Using the SDH method, which apparently demonstrates the patterns of terminals, Killackey and Belford (1980) examined the vibrissae representations in rats damaged on various PND's and concluded that the cortical and thalamic "sensitive periods" were synchronous.

In the present study, various rows of vibrissae were damaged in timed litters of mice on PND 1,2,3,4, or 5. The animals were sacrificed on PND 15 60 and their brains prepared for SDH histochemistry. Paraffin sections of the muzzles were prepared to assess peripheral damage. We examined the 5 vibrissae representations for the pattern and density of the SDH stain.

In the brainstem trigeminal system, the appearance of representations in the principal sensory nucleus and spinal nuclei interpolaris and caudalis was similar regardless of the day of damage. Portions of the representations connected to damaged whiskers showed a relative paling of SDH stain density with no gross distortions of the overall pattern. We conclude that the critical period for the brainstem nuclei ends on or before birth and that the low SDH stain density is a result of the degeneration of 1° afferents from the damaged follicles. In the ventrobasal thalamus, we observed a graded response to damage, including "compensation" by the areas flanking the damaged zone, suggesting a critical period ending on PND-3. The results in the cortex parallel those seen in Nissl stained material with slightly more segmentation within the representations for a row; and the critical period ends on PND-5.

Taken together, our data suggest a sequential development along the whisker pathway, such that more peripheral stations could "instruct" the next central one.

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176.16 SHORT-TERM CHANGES IN THE FUNCTIONAL ORGANIZATION OF SOMATOSENSORY (SmI) CORTEX OF ADULT RACCOONS AFTER DIGIT AMPUTATION. A.M. Kelahan and G.S. Doetsch. Depts. of Physiology and Surgery (Neurosurgery), Med. Coll. Ga., Augusta, GA. 30912.

Amputation of a forepaw digit in neonate and adult raccoons has profound long-term effects on the functional organization of SmI cortex (Carson et al., 1981; Kelahan et al., in press). Electrophysiological study of barbiturate-anesthetized animals about one year after amputation revealed that the cortex was responsive, in a non-topographic fashion, to stimulation of large regions of the forepaw including the digit stump. Compared with normal cortex, the receptive fields (RFs) of neurons in chronically deafferented cortex (1) were larger and commonly included more than one digit, (2) often involved both glabrous and hairy skin, (3) were sometimes discontinuous and (4) were extremely variable in location as a function of recording distance across the cortical surface and of depth within cortical tissue; only minor changes were detected in neuronal submodality sensitivity and none in the distribution of tactile stimulus thresholds. The observed effects tended to be more pronounced in neonatal than in adult amputees. No such reorganization could be found immediately after amputation in barbiturate-anesthetized animals.

In the present study, the short-term effects of amputating forepaw digit 3 were studied in adult raccoons sedated with nitrous oxide, paralyzed and treated with local anesthetics. Extracellular recordings from single neurons and small clusters of neurons within the deafferented cortex revealed a shift in afferent drive from digit 3 to the adjacent digits and palmar pads within 30-60 minutes after amputation; the efficacy of this input gradually increased over time. In most respects, the short-term cortical changes were similar to the long-term changes: (1) novel RFs were larger than normal, comparable in size and location to those of long-term amputees; (2) no somatotopic organization was evident, i.e. RF location seemed to be unrelated to the cortical site of recording; (3) no major changes were found in neuronal submodality sensitivity. In contrast, following acute amputation (1) fewer neurons responsive to cutaneous stimulation could be found, (2) RF boundaries were more difficult to delineate and (3) neurons had somewhat higher stimulus thresholds than those of both normal animals and long-term amputees.

These results indicate that the local cortical reorganization following amputation does not require the development of new anatomical connections — unmasking and strengthening of pre-existing latent or weak off-focus inputs may suffice to account for the observed cerebral plasticity. These findings have important implications for the sensory phenomena associated with phantom limbs in human amputees.

176.17 POSTNATAL DEVELOPMENT OF THE ANTERIOR VENTRAL COCHLEAR NUCLEUS IN THE C57BL/6 MOUSE. Dept. of Anatomy, New York Med. Coll., Valhalla, N.Y. 10595.

The cytoarchitecture of the anterior ventral cochlear nucleus (AVCN) was analyzed with Nissl stain and Golgi-Kopsch or Rapid Golgi impregnations at 0,5,10,20 and 30 days in the standard planes. 30 and 20 day AVCN exhibit all the organization present in the mature mouse. Its rostral tip is capped by layers of granule cells which continue around dorsal and dorsolateral surfaces of AVCN. A characteristic cell type is the spherical (12-17 μm) cell with a dark staining nucleus and light staining cytoplasm. These cells are arranged in rostrocaudal and mediolateral rows among the ascending fibers. The dorsal area of AVCN lacks this arrangement. Occasional large multipolar (25-30 μm), spherical (25-30 μm), small (15 μm) and large fusiform (25-32 μm) are present. 10 day old AVCN has a homogenous cellular distribution while 5 and 0 day old AVCN have greater numbers of spherical but very few large neurons.

Golgi impregnations illustrate the following: 30 and 20 day old AVCN: Multipolar cells with 3 radiating primary dendrites, few 2° branches, distal varicosities and numerous short spines. Large spherical cells have 3 to 4 1° dendrites oriented rostrocaudally, few 2° branches, but many distal spines and varicosities. Ovoid neurons have thin and delicate dendrites caudorostrally oriented, few spines, some varicosities and little higher order branching. Larger and most numerous, spherical cells have 1 large 1° dendrite which branches profusely to give a "bushy" appearance. Spines and varicosities are present. 10 day old AVCN: Multipolar, spherical and fusiform cells have similar dendritic arrangements except the somata have appendages and dendritic processes. Some distal dendritic tips have expanded areas with projections. 5 and 0 day old AVCN: Have mostly spherical cells but various specific cell types are difficult to distinguish. The dendritic pattern for the spherical cells is more elaborate but there are no stereotype dendritic orientations. Supported by Grants No. 1-78-6 IN-A-P002R (NIA), 41-979-1 (NIH) and the Anatomy Department.

176.18 ABSOLUTE VISUAL THRESHOLD IS DETERMINED BY THE PROPORTION OF STIMULATED RODS IN THE GROWING GOLDFISH RETINA. Maureen K. Powers and Carl J. Bassi,* Department of Psychology, Vanderbilt University, Nashville, TN 37240.

New neurons are continually added to the retina of the adult goldfish in such a way that the density of rods remains constant while the density of all other cells decreases with age (Johns and Easter, J. Comp. Neurol. 176: 331, 1977). Thus, the receptive fields of ganglion cells in older fish contain more rods. We tested the effect of the relative increase in rods on the sensitivity of the rod system by measuring absolute threshold in three different sizes of goldfish. Small (3.3 ± 0.2 cm standard length), medium (9.5 ± 0.4 cm) and large (15.6 ± 1.4 cm) fish were classically conditioned to respond to a diffuse 532 nm stimulus that covered approximately the same number of ganglion cells in each fish. Threshold, determined by means of a staircase procedure, was defined as the intensity to which a given fish responded on 50% of the trials. We found that the retinal irradiance (q/sec per cm^2) required at threshold was the same in all three sizes of fish, and since double hits were unlikely (see Powers and Easter, Vision Res. 18: 1137, 1978), this result means that the proportion of rods stimulated at threshold was the same for all fish. However, because the stimulus occupied a larger retinal area in larger fish, a larger number of rods were stimulated at threshold in larger fish. The increase in the number of rods stimulated at threshold closely matches the increase in the convergence ratio of rods to ganglion cell fibers (computed from Easter, Rusoff, and Kish, J. Neurosci., in press). We infer that the steadily increasing convergence ratio in growing goldfish does not result in enhanced visual sensitivity with age; on the contrary, the goldfish maintains constant visual sensitivity despite its continually changing visual system.

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176.19 ACUITY AND PATTERN DISCRIMINATION IN YOUNG GERBILS. Frances E. Wilkinson and Leonard Gordon*. Department of Psychology, McGill University, Montreal, Quebec, Canada H3A 1B1.

The gerbil possesses a number of characteristics which should make it an excellent subject for behavioral studies of visual development. Locomotor competence, eye opening and weaning all occur within a period of a few days and the young animals display a high level of visual attentiveness and curiosity. The present study is an initial attempt to assess the pattern discrimination abilities and visual acuity of the developing gerbil.

Eight adolescent gerbils (48-58 days of age) from 2 litters were trained on a brightness discrimination followed by 1 of 3 pattern discrimination problems. A ninth animal from one of the same litters was tested for visual acuity in the same behavioral paradigm and the same conditions of illumination. Four adult gerbils (7-8 mo.) were also trained on the brightness and one pattern problem.

A Lashley jumping stand with food reinforcement was employed for behavioral testing. One or 2 20 trial sessions were given daily depending on motivation level. Acuity was assessed by having the animal discriminate vertical square-wave gratings from matched grey stimuli. The pattern problems used with the young gerbils were 3 pairs of Lie figures: (1) concentric circles vs. radiating line (N = 2); (2) vertical vs. horizontal gratings (N = 4); (3) oblique vs. upright sets of hyperbolae (N = 2). All 4 adults learned Problem 2.

A visual acuity measure of 1c/deg. was obtained under our test conditions (distance - 24 cm). Thus our pattern discrimination stimuli (single bar width: 2-8°) were well above threshold. Brightness discrimination was acquired in a mean of 106 trials. No difference was seen between BLACK + and WHITE + groups or between adolescents and adults. All 3 Lie pattern problems were solved very quickly. Average trials to criterion for adolescents: (1) circles vs. lines, 35; (2) vertical vs. horizontal, 48. (3) hyperbolae, 90; for adults: vertical vs. horizontal, 30. Transfer tests including contrast reversals rule out luminous flux and local brightness cues and suggest that gerbils in both groups rely on "pattern" information. The trend toward increasing difficulty from problems 1-3 is the same as that seen in kittens (Nature, 1980, 284: 258-9). The relation between our acuity measure and adult acuity, and the significance of the Lie pattern discriminations to models of visual system functioning will be discussed.

176.20 LATE EMBRYONIC DEVELOPMENT OF AMACRINE CELLS, DISPLACED AMACRINE CELLS, AND GANGLION CELLS IN THE MOUSE RETINA: AN ELECTRON MICROSCOPIC, SERIAL SECTION ANALYSIS. J. W. Hinds and P. L. Hinds*. Dept. of Anatomy, Boston Univ. Sch. of Med., Boston, MA 02118.

At embryonic day 15 (E15) amacrine cells (AC) appear to develop from cells in the ganglion cell layer (GCL) which lose their axon and migrate outwards to the future amacrine cell layer (ACL) - Hinds and Hinds '78 J. Comp. Neur. 179: 277. The present study examines AC and GC development at E17, the first day when an obvious inner plexiform layer (IPL) occurs between GCL and ACL. Many GC's resemble those at E15 in having an axon and dendrites in the optic nerve layer (ONL), but the ones with somata in the outer GCL now have well developed branching dendrites in the IPL. AC's with somata in the ACL also have dendrites arborizing in the IPL, but they are generally thinner and more tortuous. Unlike E15 a clear transitional series from ventricular cells (VC) to AC's occurs; intermediate elements are bipolar shaped cells with arborization in the IPL. An unequivocal daughter cell pair of immature AC's in the future ACL confirms the direct origin of some AC's from mitotic VC's at E17. In contrast, the development of displaced amacrine cells (DAC) closely resembles that described for AC's at E15: derivation from "ganglion cells" by loss of the axon and transformation of the cell. Three lines of evidence support this conclusion. (1) As at E15 cells resembling GC's but containing only an apparent axon remnant occur, but now their somata are restricted to the IPL and adjacent inner GCL; transitional cells that more closely resemble typical AC's at E17 are also found in the IPL. (2) One cell was found with an axon which morphologically appeared to be in the process of breaking up and degenerating. (3) The fraction of anaxonic cells that had somata in the GCL and IPL (2/79 (3%) in GCL; 12/90 (13%) in GCL plus IPL), even if all end up in the GCL, is too small to account for the large fraction (probably at least 35%) of DAC's found in the adult mouse (Dräger and Olsen '81 Inv. Ophth. 20: 285) ($P<0.001$). Autoradiographic studies show that few additional cells will join the GCL after E17, so the additional DAC's that occur in the adult must mostly be derived from cells that at E17 have axons that enter the ONL and resemble GC's. The present results suggest a natural explanation for the Golgi results of Perry and Walker ('80 Proc. Roy. Soc. 208: 415) that the 6 types of AC's with the widest field dendrites are found with somata on both sides of the IPL, but the 3 types with narrower fields have somata only in the inner nuclear layer: perhaps the former are derived by loss of an axon and become trapped on one side or the other of the forming IPL, while the latter generally are later arising cells that form directly from VC's. (Supported by NIH Grant EY 01398).

176.21 CORTICAL ACTIVATION OF CELLS IN TECTAL TRANSPLANTS IN RATS. G. T. Golden, A. R. Harvey* and R. D. Lund, Department of Anatomy, Medical University of South Carolina, Charleston, South Carolina 29425

In previous studies we found that tectum taken from fetal rats and transplanted adjacent to the superior colliculus of newborns continues to mature and forms connections with the host brain. Anatomical studies indicate a substantial projection from the host occipital cortex to the transplants. Frequently, both cerebral cortices contribute to this pathway, however the projection from the cortex ipsilateral to the side with which the transplant connects is the heaviest and most consistent. In this study we have investigated whether the cortical projection is capable of physiologically activating cells in the transplant.

Three to nine months after transplantation, rats were anesthetized with urethane and ketamine. An array of 6 stimulating electrodes was placed in the left occipital cortex spanning areas 17 and 18a. Single units were recorded in transplants with varnished or glass-coated tungsten microelectrodes. Small electrolytic lesions were made during the recording penetrations to allow subsequent reconstruction of electrode tracks. Brains were fixed after recording for histology.

A total of 20 electrode penetrations were made in 7 transplants. Four of these transplants connected with the left and three transplants connected with the right superior colliculus. Of the 124 transplant units tested with electrical stimulation, 20 (16%) were orthodromically activated from host cortex. A higher proportion of cells was driven in the transplants connected ipsilaterally to the stimulated cortex. Minimum latencies after cortical stimulation ranged from 3.5 to 300 msec., however all but three transplant units had latencies less than 15 msec. The mean latency for these 17 cells was 7.2 msec. For any individual unit the latencies fluctuated over a range from 3 to 5 msec. Most units responded to about 50 to 70% of the shocks applied to the host cortex.

The results indicate that cells in transplants are indeed driven by host cortical afferents, however the activation seems less secure than that found in the corticotectal pathway in normal animals. (Supported by NEI grant EY 03327 and MUSC postdoctoral fellowship)

176.22 CORTICAL EVOKED POTENTIAL AND SINGLE UNIT ACTIVITY TO VISUAL AND AUDITORY STIMULI IN MATURE AND IMMATURE FERRETS (MUSTELA PUTORIUS). G. H. Rose*. (SPON: R. J. Ellingson). Department of Psychology, Bowdoin College, Brunswick, ME 04011.

The ferret is a comparatively inexpensive, easily obtained, relatively tame carnivore with a cat-like gyrencephalic brain. Used fairly extensively in endocrinology, reproductive physiology and virology studies, its use in brain research is limited to animal models of CNS birth defects induced by neurotoxins administered during pregnancy. With the exception of a study on the cerebeller center (Llinas et al., J. Neurobiol., 4, 69-94, 1973), virtually no information is available on the structure and functions of the mature or immature CNS in spite of the above mentioned advantages. It is of particular interest from a developmental viewpoint since it is extremely immature at birth. Eyes in ferrets open at 30 days of age in contrast to 4-7 days of age in kittens. The neurophysiological results obtained appear to be the first reported regarding the sensory cortex of this species.

The cortical topography and other parameters of evoked potential and single unit activity in response to visual and auditory stimuli were delineated in 10 adult and 15 immature ferrets (3-10 weeks of age). Records were obtained from anesthetized and unanesthetized subjects. Unique species typical data, in terms of VEP waveform, unusually short latency regions (visual), and late developmental onset (25-30 days after birth) of electrocortical responses, were obtained. The data are contrasted with developmental neurophysiological findings prevalent in other species.

177.1 DEVELOPMENT OF THORACIC MUSCLES IN MUSCLE-SPECIFIC MUTANT AND NORMAL DROSOPHILA MELANOGASTER. Walter J. Costello and John B. Thomas*. Dept. of Biol., Yale Univ., New Haven, CT. 06511

Using anatomical, physiological, and genetic techniques, we have been studying the interaction between nerve and muscle in developing motor systems of D. melanogaster. The specific systems examined are those involved in escape-the jump response and subsequent flight. The jump is mediated by the tergo-trochanteral muscle (TTM),a tubular muscle; flight is accomplished by two opposing sets of fibrillar type muscles, the dorsal longitudinal (DLM) and the dorso-ventral (DVM).

Several mutations affecting these motor systems are expressed by highly specific phenotypes. One set of mutations disrupts only the jump system, while another set affects solely the flight motor system.

Jump Response Mutants: two mutants, nj 42 and 45T (latter isolated by L. Salkoff), disrupt only the TTM. In the adult, the TTM is almost wholly missing, though the indirect flight muscles, DLM and DVM, are normal.

Flight Response Mutants: the mutant allele stripe (sr) affects only the DLM (Costello,1979,Neurosci.Abstr.5:243). With only one copy of the mutant allele (genotype sr/Df(3)sr; one chromosome carries sr, the other has a deletion at that locus), no DLMs are present in the adult; The DVMs and TTM are unaffected. In nj 307, only the DVMs are affected. Wild-type flies have 6 sets of DVMs (3 per side). In nj 307, one set of DVMs is missing; the adjacent DVMs, DLMs, and TTM are normal.

The development of these muscles during pupation was studied. In every mutant, the affected muscle was at first present, and only subsequently degenerated in mid- to late pupal stages. In normal and mutant flies, we found that in late prepupal and early pupal stages, the myocytes giving rise to DLM,DVM, and TTM appear to migrate from the everted 2nd leg disc, complementing earlier findings (Shatoury,1956,J.embryol.exp.morphol. 4:228). Also, the nerves innervating the larval muscles which serve as substrates for DLM/DVM apparently persist, becoming the adult motoneurons supplying DLM/DVM. The adult myocytes migrate along these nerves in early pupae to reach the larval muscles where they commence differentiation into adult muscles. Thus these nerves might provide orientation for the migrating myocytes to reach their proper destination.

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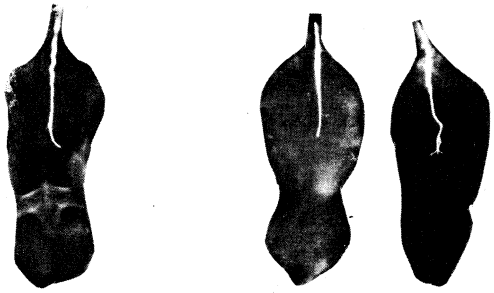
177.2 A SINGLE GENE MUTATION ALTERS THE MORPHOLOGY OF THE GIANT FIBER IN DROSOPHILA. John B. Thomas* (SPON: Robert J. Wyman) Dept. of Biology, Yale Univ., New Haven, CT 06511.

An X-linked recessive mutation, nj262, has been isolated in D. melanogaster which alters the terminal bend of the giant fiber (GF). In mutant flies the GF cell body position and dendritic morphology is indistinguishable from wild-type and each of the two GFs descends unbranched to the thoracic ganglion. In wild-type flies the GF always bends laterally before ending abruptly (Koto et. al., Brain Research , in press). However, the GF in nj262 flies fails to make this characteristic bend and either terminates before making the bend or sends out numerous abnormal branches from the tip (see fig.). At the terminus of the bend the GF normally contacts the TTM motorneuron. This synapse is disrupted physiologically in the mutant.

However, the GF does drive the TTM in the mutant, perhaps by a different pathway. The TTM can be driven by the GF only at extremely low frequencies (0.5 Hz vs. the normal 200 Hz) and its response latency is abnormally long (3.0 msec vs. the normal 1.0 msec). In contrast, the DLM is driven normally by the GF in nj262. This is a disynaptic pathway in wild-type mediated by the PSI interneuron which the GF contacts before making its characteristic bend (King and Wyman, J. Neurocytol., in press).

Supported by USPHS #NS-07314 to R.J. Wyman.



WILD-TYPE NJ262

Lucifer Yellow fills of wild-type and mutant GFs.

177.3 ANALYSIS OF MUTANTS WITH INCREASED MEMBRANE EXCITABILITY IN DROSOPHILA MELANOGASTER. B. Ganetzky,* A.-X. Liu,* and C.-F. Wu. (SPON: D. Murphy). Lab. of Genetics, Univ. of Wisconsin, Madison, WI 53706, and Dept. of Zoology, Univ. of Iowa, Iowa City, IA 52242.

Three genes (Sh, bas^{MW1}, and Eag) involved in the regulation of nerve membrane excitability in Drosophila melanogaster were studied using the larval neuromuscular preparation. Mutations in any of these genes results in enhanced nerve excitability and neuromuscular transmission. Double mutants exhibit striking synergistic interactions suggesting close functional relationships among these three genes.

In Sh, single nerve stimuli evoke excitatory junctional potentials (ejps) of increased amplitude and duration even at 0.1 mM $[Ca^{2+}]_o$ which are associated with repetitive spikes in motor axons (Jan et al., Proc. Roy. Soc. Lond. B. 198:87, 1977; Ganetzky and Wu, Soc. Neurosci. Abstr. 6: 569, 1980). In bas^{MW1}, several stimuli at 10Hz trigger a train of nerve spikes accompanied by a prolonged ejp. Nerve evoked ejps are normal in Eag(0-2mV at 0.1 mM$[Ca^{2+}]_o$). However, Eag differs from normal and the other two mutants in causing the ocurrence of spontaneous ejps ($\sim$ 5Hz). These ejps have normal time course and amplitude.

Double mutants display additional defects not found in any of the single mutants:

1. Duration of evoked ejps is increased at least 10 fold.
2. Spontaneous ejps of this duration occur even at 0.1 mM$[Ca^{2+}]_o$.
3. Both evoked and spontaneous ejps correlate with repetive firing of the motor axon.
4. Nerve terminals sustain very prolonged synaptic transmission upon electrotonic stimulation even when axonal conduction is blocked by TTX.

Mutations such as these can provide the material to analyze the molecular mechanisms of membrane excitability.

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177.4 THE TRANSPLANTATION OF EYES TO GENETICALLY EYELESS SALAMANDERS. W.A. Harris*. (Spon: A.I. Selverston). Dept. of Biology, UCSD, La Jolla, CA. 92093.

The eyeless axolotl was discovered as an autosomal recessive mutant (Humphrey, R., Anat. Rec. 163: 306, 1969). Hibbard and Ornberg (Exp. Neurol. 50: 113, 1976) showed that vision could be restored to such animals by embryonic transplantation of eye primordia. The experiments presented here describe several new findings on eyeless animals to which eyes have been transplanted. These are: 1) The central projections of such eyes, as traced with autoradiography or HRP, may be bilateral or ipsilateral to the optic centers of the midbrain and thalamus, or down the spinal cord. The probability of each of the different classes of projection is correlated with the site of nerve entry as in eye transplants to normal animals (Harris, W.A., J. Comp. Neurol. 194: 303, 1980) and is not therefore influenced by the host's eyes (contrary to Giorgi, P.P. and Van der Loos, H., Nature 275: 746, 1978; and Schwenk, C. and Hibbard, E., Exp. Neurol. 55: 498, 1977). 2) Somatosensory serotonergic input to the tectum which is restricted to the deep neuropil in normal animals, and is superficial in eyeless mutants, becomes normally deep in genetically eyeless tecta innervated by transplants. 3) Acetylcholinesterase staining in the tectum, which is limited to the superficial optic neuropil in normal animals, and is largely absent in eyeless mutants, is restored by transplantation. 4) The transplanted retinas make topographic maps on the host tecta, either normal or rotated depending on the orientation of the transplant, even though these transplants were performed before stage 34, the supposed time of "retinal specification" in these animals (Stone, L.S., J. Exp. Zool. 145: 85, 1960), and even though the optic fibers take abnormal routes to the tectum. 5) Whereas in normal animals the axes of the visual and somatosensory projections to the tectum are in topographic register, in eyeless animals to which a rotated eye has been transplanted, these axes are not in register. Thus, the visual map does not orient the somatosensory one.

177.5 K^+-RESISTANT MUTANTS OF PARAMECIUM TETRAURELIA FAIL TO "ADAPT". C. L. Shusterman and C. Kung* Lab. Molecular Biology and Dept. of Genetics, University of Wisconsin, Madison, WI 53706.

A class of behavioral mutants of Paramecium tetraurelia was obtained by a nonbehavioral selection, and used to study the membrane events involved in "adaptation". 35 mM K^+ added to the culture medium kills wild-type P. tetraurelia. The mutants were obtained by adding 35 mM K^+ to mutagenized, autogamized cultures, and isolating the cells that grew. At least 27 independent K^+-resistant mutants resulted, and some survive at up to 80 mM K^+. Paramecia grown in non-lethal levels of K^+ (15 mM) show a behavioral deficit called adaptation. Wild-type P. tetraurelia grown overnight in 15 mM K^+-supplemented medium fails to respond when challenged with Ba^{++} or a thermal gradient. However, the K^+-resistant mutants show little or no such adaptation. The K^+ resistance and lack of adaptation occur together in all of the mutants and cosegregate in genetic crosses. Genetic complementation tests define at least four complementation groups, and the degrees of non-adaptation and K^+ resistance are correlated within the groups.

The mutants have been studied electrophysiologically in an investigation of the membrane phenomena involved in adaptation. Mutants from three different complementation groups and a double mutant of two of these were studied with current clamp and voltage clamp in comparison with the wild type. The mutants are not significantly altered in their resting, depolarization-sensitive or hyperpolarization-sensitive g_K's. One of the mutants has a reduced calcium activation and inward current, indicating a reduction in g_{Ca}. The double mutant, which shows the "pawn" behavioral phenotype (lack of avoiding reactions to any stimulus) has a complete loss of calcium activation and zero inward current. These results indicate that adaptation in Paramecium is a membrane phenomenon involving a change in the membrane conductance to Ca^{++}. Supported by P. H. S. grant GM22714 and N. S. F. grant BNS79-18554 to C. K.

177.6 GRANULE CELL DEATH DURING POSTNATAL CEREBELLAR DEVELOPMENT OF STAGGERER ↔ WILD-TYPE CHIMERAS. Karl Herrup, Dept. of Human Genetics, Yale Medical School, New Haven, CT 06510.

Staggerer is a mutation of mice that causes the improper development of the cerebellar cortex. Virtually all of the granule cells die after migration to the internal granule cell layer (IGL) and the Purkinje cells (PCs) are morphologically abnormal, ectopic, and reduced in number. In staggerer ↔ wild-type chimeric adults all of the phenotypes of the staggerer PCs are retained and thus are intrinsic properties of cells with that genotype. Based on preliminary observations of staggerer ↔ ichthyosis chimeras the death of staggerer granule cells is an extrinsic event, caused almost certainly by the absence of their postsynaptic target, the PC dendritic spine. In normal development granule cells are produced in approximately the correct numbers as very little cell death is observed in the IGL. In adult staggerer chimeras, the volume of the IGL is intermediate to mutant and wild-type. The question thus arises, are the granule cells in the chimera overproduced during development followed by cell death or is their production adjusted to the intermediate number of other wildtype cells?

Using β-glucuronidase activity as an independent cell marker genotypically staggerer PCs were identified in four 16 to 17 day old chimeras. This identifies these animals as staggerer chimeras in the absence of the ability to progeny test. The volume of the chimeras' IGL varied inversely with the percent of staggerer in the chimera. Further, significant granule cell death was observed in the IGL. The extent of this necrosis increased with the percentage of staggerer in the chimera. The latter finding demonstrates that granule cells in the staggerer chimera are overproduced and then "pruned" back to the number found in the adult. The reason for the overproduction, could be either i) a certain number of granule cells are always generated regardless of the number of other cells present or ii) the staggerer PCs are capable of stimulating granule cell multiplication but, due to the absence of PC dendritic spines, are incapable of supporting them. The results also indicate that there is a limited plasticity to the developmental interaction between the Purkinje and granule cell. If the granule cells could be sustained by many fewer PCs or if the wild-type PCs could sustain many more granule cells, little or no granule cell death would be expected in the young staggerer chimeras.

Supported by March of Dimes Birth Defects Fndn; NIH #HD12213

177.7 CEREBELLAR PURKINJE CELLS ORIGINATE FROM A SMALL NUMBER OF PROGENITORS COMMITTED EARLY IN DEVELOPMENT. Richard Wetts and Karl Herrup, Dept. of Human Genetics, Yale Medical School, New Haven, CT 06510.

During the development of a neuron most cytological differentiation (i.e. the growth processes by which a cell elaborates its characteristic morphology) follows the cycles of cell division that generate the adult number of cells. However, the time at which a neuron (or any other mammalian cell type) undergoes commitment to its adult phenotype is not known. This time of genetic and/or spatial commitment can be any time during or after the cycles of division. Results from our laboratory provide evidence that cerebellar Purkinje cells (PCs) are descended from a small number of progenitor cells which become committed very early in development.

Chimeric mice were produced by the aggregation of lurcher (+/Lc) and wild-type 8-cell embryos. The nervous systems of these chimeras are fine-grained mosaics of mutant and wild-type cells. As previously reported, the lurcher PCs degenerate because of some intrinsic mechanism. Thus all of the PCs remaining in the adult are descended from the wild-type embryo. In one chimera (χ11), the number of remaining PCs (approximately 18,000) is the lowest number observed in a mosaic animal. These cells may represent a single clone; that is all of the PCs of χ11 may have descended from a single progenitor. This argument is supported by the fact that in the other lurcher chimeras the numbers of PCs are roughly integer multiples of the number of PCs in χ11. The number of PCs in wild-type mice is approximately 10 times the number in χ11, suggesting that they are descended from 10 progenitors. If a greater number of progenitors gives rise to PCs, smaller clone sizes would have been seen. We cannot know, however, whether these progenitors also give rise to other cell types.

Because cells increase in number by doubling, 14-15 cell divisions are needed for each progenitor to produce the number of PCs in χ11. Assuming an average doubling time of 7-9 hrs, commitment occurs 4.1-5.6 days before the last cell division. In the mouse, the birthdates of the PCs are E11-E14, and so commitment occurs between E5.4 and E9.9 and most likely between E6.9 and E8.4. At this time in development, the primitive streak has appeared, followed by the formation of the neural plate and, later, the formation of the neural tube. Therefore, approximately 10 progenitors are committed to becoming PCs at the earliest stages of nervous system development.

Supported by March of Dimes Birth Defects Fndn; NIH #HD12213.

177.8 ALBINO C57BL/6J-c^{2J} MOUSE OPTIC NERVE: ELECTRON MICROSCOPIC AXON COUNT. J. M. Bolam* and I. S. Westenberg. Institute for the Study of Developmental Disabilities, Chicago, IL 60608.

The mouse is valuable in the neurosciences because of many neurological mutants with appropriate genetic controls. The effects of the albino mutation on retinal ganglion cells can be assessed at the retina or more centrally. Previous estimates placed the number of retinal ganglion cells in the albino C57BL/6J-c^{2J} mouse eye between 27,000 and 39,000. However, it has been hypothesized that these estimates are low. This hypothesis can be tested by counting the number of axons in the optic nerve. The small diameter of the mouse optic nerve allows viewing of a complete cross-section electron microscopically, as required to resolve the smallest axons.

The left optic nerve of an 111-day old albino male C57BL/6J-c^{2J} mouse was processed (aldehyde - osmium - ethanol) and embedded in Epon 812. Serial electron micrographs (scope mag.= 2080X) of an unstained whole nerve cross-section on a filmed slot grid were assembled into a montage. Axons were counted in 27 10 μm X 10 μm sample areas (total mag.= 6650X) evenly distributed across the section. The full cross-sectional area of the nerve was calculated from the montage, and the total axon number was estimated.

The section was ovoid in shape with a long axis of 382 μm, a short axis of 253 μm, and an area of 71,700 μm^2. The total sample area of 2700 μm^2 represented about 4% of the total area. 1800 axons were counted in this small sample. Thus the estimated number of axons for this nerve was 47,800. This estimate is close to other electron microscopic estimates of mouse optic nerve axon number. This confirms the hypothesis that the previous estimates of the number of retinal ganglion cells based on light microscopic studies of the retinae of albino C57BL/6J-c^{2J} mice are low.

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177.9 ALTERED THYMIDINE KINASE IN DEVELOPING STAGGERER CEREBELLUM. Anne Messer, Michael Savage*, and Thomas P. Carter*. Div of Laboratories and Research, N.Y. State Dept. of Health, Albany, N.Y. 12201.

The mouse cerebellar mutant staggerer (sg/sg) exhibits a reduced rate of proliferation of cerebellar granule cells and degeneration of the remaining granule cells, as well as a 70-80% loss of Purkinje cells (with the remainder abnormal) prior to the time of granule cell generation. Several lines of evidence, including monolayer cell culture studies, quantitative agglutination, and tests utilizing antibodies, indicate that staggerer cerebellar cells retain embryonic cell surface characteristics. This may be secondary to a lack of interaction with normal Purkinje cells.

The enzyme thymidine kinase (TK) is associated with initiation of DNA synthesis, and shows a sharp increase in activity in the mouse cerebellum at postnatal days 6-8. The present experiments demonstrate that levels do not increase developmentally in the mutant cerebellum at ages when the wild-type level is high. Mixing experiments show that this effect is not due to an endogenous inhibitor of the enzyme. Both the K_m and the susceptibility of the TK to nucleotide inhibitors are unaltered in the mutant animals. It is therefore hypothesized that the enzyme is not induced normally in the mutant.

This finding could offer a mechanistic link between the observations of reduced granule cell proliferation and the mutant cell surface changes. Further experiments to examine the relationship between granule cell surface changes and proliferation, in vivo and in vitro, are in progress.

177.10 MITRAL CELL DEGENERATION IN THE MOUSE MUTANT PCD. Charles A. Greer and Gordon M. Shepherd. Section of Neuroanatomy, Yale Univ. Sch. Med., New Haven, CT 06510

Purkinje Cell Degeneration (pcd) is a mutant gene in mice which, when present in the homozygous recessive condition, has several postnatal effects on the nervous system. These include the rapid loss of cerebellar purkinje cells and slower degenerative losses of photoreceptors, some thalamic nuclei, and mitral cells of the olfactory bulb. Mitral cell degeneration, assessed with Fink-Heimer methods on the lateral olfactory tract, was not pronounced until 10 mo. postnatal. We have studied olfactory bulbs of pcd mice to establish more precisely the timecourse of mitral cell degeneration and the consequent effect upon the functional organization within the bulb.

C57BL/6J mice carrying the pcd gene at one or both loci were obtained from Jackson Laboratories and at either 4 or 8 months postnatal were either sacrificed for routine histological examination or tested for functional organization with the ^{14}C-2-deoxy-D-glucose (2DG) technique. The heterozygous (pcd/++) littermates of affected (pcd/pcd) mice were employed as controls since our early studies demonstrated normal olfactory systems in the heterozygous genotype.

At 4 months postnatal pcd/pcd had only 19% of the number of mitral cells found in pcd/++. By 8 months, only 4% remained. In parallel with the loss of mitral cells the thickness of the external plexiform layer (EPL) decreased. This may reflect the decline in number of mitral cell primary and secondary dendrites. Also, orderly arrangement of the granule cell population into islets appeared in disarray. The olfactory nerve and glomerular layers in pcd/pcd did not exhibit any gross morphological changes. Interestingly, on gross inspection, the population of tufted cells in the EPL does not appear to be subject to the influence of the pcd gene. Examination of the functional organization in the olfactory bulb with the 2DG technique revealed essentially normal spatial density patterns within the glomerular layer of the pcd/pcd mice after stimulation with odor.

These results demonstrate that the degenerative loss of mitral cells in the olfactory bulbs of pcd/pcd mice is occurring earlier than previously suggested. Also the 2DG analyses raise the possibility that the olfactory receptor input is functionally normal despite loss of mitral cells. Consequently this murine mutant would seem to be a promising model for studying the role of the mitral cells in olfactory bulb function, the effect of mitral cell loss on synaptic interactions of remaining elements, and plasticity and reorganization in the olfactory system.

Supported by NINCDS F32-NS06159 and NSF BNS 78-16545.

177.11 MORPHOLOGICAL DEFECTS IN MICE HOMOZYGOUS FOR t^{w1}: EFFECTS ON EARLY NEURAL TUBE DEVELOPMENT. Linda C. Chaney. Dept. of Zoology & Neuroscience Program, Michigan State Univ., E. Lansing, MI 48824.

The t mutants (t-haplotypes) identified in mice have been genetically divided into seven complementation groups. In the homozygous state, alleles from each complementation group affect different stages of early embryonic development. The late (from day 14 of gestation to birth) embryonic effects of mutants t^{w1}, t^{w3}, t^{w12}, and t^{w20} from the t^{w1} complementation group have been previously described (D. Bennett, S. Badenhausen & L.C. Dunn, J. Morph., 105: 105, 1959). This study re-examines in greater detail the effects of one mutant, t^{w1}, upon developing embryos at mid-gestation.

Mice heterozygous for t^{w1} were mated and embryos were collected from 9 to 13 days post-coitus (p.c.). Embryos were fixed in Bouins solution, embedded in paraffin, serially sectioned at 8 µm, stained with hematoxylin and eosin, and examined by light microscopy. Embryos homozygous for t^{w1} (t^{w1}/t^{w1}) could not be distinguished from normal littermates ($+/+$, $+/t^{w1}$) on the basis of gross morphology prior to 11 days p.c. From 11 to 13 days p.c., t^{w1}/t^{w1} embryos could be identified by their decreased size, enlarged hearts, and degenerating neural tissues. At the light microscope level, t^{w1}/t^{w1} embryos could be detected as early as day 9 p.c. by the presence of pycnotic cells within the mantle layer of the rostral portion of the rhombencephalon. As mutant embryos continued to develop, the pycnotic cells appeared along the neural axis in a specific pattern. Pycnotic cells were always found first in the ventral region of the rostral portion of the rhombencephalon. With increasing age of the embryos, a wave of pycnosis subsequently appeared along the ventral neural tube in rostral and caudal directions, extended into regions of the dorsal neural tube in the area of the caudal rhombencephalon, and spread along the dorsal neural tube towards the tail. Other neural ectoderm structures did not appear to be affected. In general non-neural structures continued to develop in the presence of the abnormally developing neural tube. By days 12 to 13 p.c. mutant embryos were in the process of being resorbed, the heart was not beating, and neural as well as non-neural structures were pycnotic.

Tail or heart explants taken from t^{w1}/t^{w1} embryos at 9 to 12 days p.c. were cultured in vitro for 2 to 3 months. These studies support the hypothesis that, unlike earlier acting t-haplotypes, the action of the gene t^{w1} is specific for cells within the neural tube and does not affect all cells of the embryo.

177.12 THE USE OF CHIMERAS FOR THE STUDY OF THE MYELIN DEFICIT IN THE CNS OF QUAKING MICE. A. Berarducci*, A. Peterson, A. Aguayo, I. Tretjakoff*. Neurosciences Unit, Montreal General Hospital, Montreal, Quebec, Canada.

The understanding of the mechanisms involved in the pathogenesis of inherited disorders of myelination is hampered by the difficulty in separating the relative role played by neurons and glial cells. To explore this question several in vivo and in vitro techniques have been used in recent years (Bray et al, Ann. Rev. Neurosci. 4:127-162, 1981). The present study utilizes experimental chimeras (Peterson et al, Soc. Neurosci. Symp. 4:258-273, 1979) to investigate the pathogenesis of the myelin disorder in the quaking mouse - an animal counterpart of the human leucodystrophies (Sidman et al, Science 144:309-311, 1964).

Quaking and normal 8-cell embryos were aggregated and allowed to develop into chimeras in which the mutant and normal cell lines coexisted throughout primary development. In mature chimeras highly variable areas of normal and mutant-like myelin are distributed in a highly variegated pattern throughout the central nervous system. Normal and quaking-like myelin were found to share the same axons, suggesting that axons are not responsible for the myelin deficit in the quaking mouse.

If, as indicated, quaking oligodendroglia are intrinsically defective, then identification of quaking and normal myelin in chimeras will permit the study of clonal deployment of these cells in the central nervous system.

177.13 INHERITANCE OF NIGROSTRIATAL TYROSINE HYDROXYLASE ACTIVITY IN THE CXB RECOMBINANT INBRED MOUSE STRAINS. H. Baker, C. Vadasz*, and D. J. Reis. Lab. of Neurobiology, Cornell Univ. Med. Coll. New York, NY 10021, and Res. Inst. for Neurochem., Wards Island, NY 10035.

The genetic control of tyrosine hydroxylase (TH) activity was studied in the nigrostriatal system in male mice of seven recombinant inbred (RI) strains, their progenitor strains (BALB/cByJ and C57BL/6ByJ), their reciprocal F_1 hybrids and a $CB6F_2$ segregating generation. TH was measured in cell bodies, in substantia nigra (SN) and terminals in corpus striatum (CS). TH activity in the SN fell into 3 subsets: highest in the BALB/cByJ strain; lowest in the CXBI; with intermediate values in the others (3.21 $\pm$ .06, 2.23 $\pm$ .04 and 2.48 $\pm$ .09 to 2.86 $\pm$ .01 nmols DOPA/SN/hr respectively; Duncans multiple range test). The distribution of TH activity in the $CB6F_2$ generation was unimodal with significant genetic as compared to environmental variance. Heritability estimates from both the RI lines (0.48) and the $CB6F_2$ (0.89) generation were high indicating that only a few genes control TH activity in the SN. In the CS a significant source of variance of TH activity ($p<0.01$) was attributable to strain differences but no non overlapping subsets of strains were observed indicating a polygenic mode of inheritance. TH activity was highest in the C57BL/6By strain and lowest in the CXBI/J strain and intermediate in the others (C57BL/6By, 9.37 $\pm$ .09 and CXBI, 6.82 $\pm$ .78 nmols DOPA/mg prot./hr). However, the distribution of striatal TH activities in the $CB6F_2$ generation was bimodal indicating that few genes control TH activity in this brain region. Heritability estimates for striatal TH activity differed for the RI and $CB6F_2$ generations (0.25 and .79 respectivtely). A high and significant ($P < .001$) between-strain correlation ($R = 0.82$) of TH activity in SN and CS suggested the presence of pleitropy or linked genes. The number of midbrain midbrain neurons immunocytochemically labeled with antibodies to TH were counted: in the two strains with the greatest difference in TH activity BALB/cBy mice had more TH-containing neurons than the CXBI strain (9686 $\pm$ 561 vs 8059 $\pm$ 579, respectively). We conclude that: (a) TH activity varies in the cell bodies and terminals in the CXB Recombinant Inbred strains; (b) TH activity in both SN and CS is under the control of relatively few genes; (c) some of the controlling genes for TH activity in CS and SN are shared; (d) the branching of SN nigral neurons may be regulated by additional factor(s); (e) the variations in nigrostriatal TH activity between RI strains may be attributable to differences in the number of midbrain DA neurons.

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177.14 ACHE AND BUCHE ACTIVITY IN INBRED RATS. J. A. Edwards and S. Brimijoin. Dept. Pharmacology, Mayo Foundation, Rochester, MN 55905.

We have previously reported wide individual variation in acetylcholinesterase (AChE, EC 3.1.1.7) and butyrylcholinesterase (BuChE, EC 3.1.1.8) activities in adult male Sprague Dawley rats. The regulation of AChE in this outbred strain appeared to be tissue-specific, while BuChE activity was relatively similar in all tissues of a given rat. In order to determine whether the variation of the cholinesterases is genetically influenced, we have examined AChE and BuChE in 15 normal male rats of the inbred Fischer 344 strain and 12 normal male rats of the Wistar-Furth strain, age 5 months (300-350g). Serum, liver, diaphragm, brain, and superior cervical ganglia (SCG) were assayed radiometrically in detergent-containing extracts. AChE activities exhibited at most a 3-fold range (in serum), and varied by about 2-fold in other tissues. BuChE activities also varied about 2-fold in most tissues, although a 5-fold range was seen in serum. These ranges are much smaller than previously noted in the outbred Sprague Dawley rats. In general the coefficients of variation of AChE and BuChE activities in the inbred strains were 40-60% as great as those in corresponding tissues of the outbred rats.

No pair of tissues in the inbred Fischer and Wistar rats showed a significant correlation of AChE activities. This result implies that, as in the Sprague Dawley rats, the regulation of AChE in the inbred strains is tissue-specific. BuChE activities in the diaphragm and liver of the Fischer rats and in the serum and liver of the Wistar rats were correlated at a significant level ($r=0.692$ and 0.643, respectively). However, this result contrasts with the universal correlation of BuChE activities among the tissues of the Sprague Dawley rats. We conclude that body-wide regulation of BuChE is possible, but is not necessarily the rule. To determine if AChE and BuChE were regulated coordinately in the same tissues, correlations between the two enzymes were examined. Most tissues showed little or no correlation between AChE and BuChE activities, as was also found in the Sprague Dawley rats. In contrast, however, a significant correlation between the two enzymes was discovered in SCG in both inbred strains. Further work is required to determine the biochemical mechanisms by which these presumably genetically mediated strain differences in the regulation of cholinesterases arise.

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178.1 INTERACTIONS BETWEEN CO-CULTURED AMPHIBIAN SENSORY NEURONS AND MECHANOSENSORY TARGET TISSUES. K.M. Mearow, C.A. Nurse*, B. Visheau and J. Diamond. Dept. of Neuroscience, McMaster Univ., Hamilton, Ontario L8N 3Z5.

Previous studies in this laboratory have established that in salamanders the epidermal Merkel cell is the target for sprouting and for regenerating mechanosensory axons. We are now attempting to study these neuron-target interactions under controlled conditions in vitro. We began by co-culturing dissociated salamander DRG neurons and skin tissue. The isolated neurons would survive for some weeks, and were electrically excitable. In general however the (bipolar) neurite outgrowth was sluggish, and electronmicroscopic (E.M.) examination of the skin for the relatively sparse Merkel cells was tedious. We therefore turned to the more embryonic Xenopus tadpole, whose two tentacles appear to be mechanosensory structures containing numerous Merkel cells (Ovalle, Cell Tiss. Res. 204: 233-241, 1979). Explants of tentacle pieces and of trigeminal ganglia (the normal source of innervation) or DRGs from stage 52-57 tadpoles were grown on pre-formed collagen-gel discs either alone or in co-culture, in 70% L-15 medium plus or minus exogenous protein (e.g. serum, insulin and NGF). The fluorescent dye quinacrine (see accompanying Abstract by Nurse et al.) was used as a marker for Merkel cells; positive cultures were further processed for E.M. examination to confirm the presence of these cells.

Little neuritic outgrowth was observed in cultures of sensory ganglia by day 4-5. However when a tentacle piece was present there was a marked enhancement of neurite outgrowth by this time even in the absence of exogenous protein (other than the collagen-matrix). Further, a significant proportion of these neurites were oriented towards the tentacle explant or its epithelial-like outgrowth. In longer term cultures (> 10 days) phase microscopy revealed that many neurites not clearly associated with the target-tissue cells appeared to be degenerating; in contrast, those judged to be most strongly associated with the target tissue looked healthier and persisted longer. Areas of tentacle outgrowth in intimate contact with such neurites often contained quinacrine-fluorescent cells. In the E.M. we have found Merkel cells in the original explant and in the cellular outgrowth; nerve bundles were prominent near basement membranous material. We are now investigating whether morphological neurite-Merkel cell contacts develop in vitro, whether mechanosensory function is established, the characteristics of the target stimulus for neurite outgrowth, and the nature of the recognition of the target by nerve endings.

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178.2 OPTIC LOBE EXTRACT STIMULATING NEURITE EXTENSION IN EMBRYONIC RETINAL EXPLANTS *IN VITRO*. N. G. Carri and T. Ebendal*. Department of Zoology, Uppsala University, Box 561, S-751 22 Uppsala, Sweden.

Normal visual function depends on the ordered projection of retinal ganglion cell axons onto the optic centers. During development, the extending optic fibers seem to follow selective orientational cues to establish contact with their appropriate targets. At present, however, little is known about the exact nature of these cues whether acting en route or in the terminal field for the visual projection. We designed a tissue culture procedure to study factors controlling the outgrowth of neurites from embryonic chick retina. Using glass capillary tubes, organotypic explants were punched out from the retina in chick embryos (white Leghorn) of Hamburger-Hamilton stage 28-29 (6 days of incubation). The circular explants (0.5-1.5 mm in diameter) were cultured, vitreal side down, for 4 days on top of medium-containing collagen gels with serum added at 20% (10% fetal calf serum and 10% donor horse serum). At intervals, the length of extending retinal neurites was measured in the dark field and phase contrast microscope. Under the described conditions neurites growing out from the retina were sparse and less than 0.2 mm in length. Retinas were then tested for their response to extracts of the optic lobe, telencephalon, and thigh muscles from 18-day-old chick embryos. These additives increased the neuritic outgrowth considerable. Best results were obtained with optic lobe extract, closely followed by the telencephalon extract; the mean neuritic lengths were 1.2 and 1.0 mm, respectively. Moreover, optic lobe extract distinctly improved the survival of retinal cells in the explants. The stimulative effects of the optic lobe extract were also evident although reduced under serum free conditions. Neuritic outgrowth was not enhanced by either mouse nerve growth factor (NGF), or embryonic chick heart extract, both being stimulators of neuritic extension in peripheral ganglionic neurons. Our data suggest that extractable materials from appropriate target areas may support the growth of retinal axons and possibly present orientational cues needed for setting up the visual projection. It is not yet known whether this control of fiber extension is exerted by "pathway" molecules coating the substratum (e.g. acting to increase growth cone adherence) or by "neuronotrophic" factor(s) active in solution.

(N. G. Carri is a Fellow of CONICET, Argentina).

178.3 TROPHIC INFLUENCE OF THE TECTAL TISSUE ON THE OUTGROWTH OF NEURITES FROM THE CO-CULTURED RETINAL TISSUE, EXPLANTED FROM ADULT GOLDFISH. Myong G. Yoon and Frank A. Baker* Department of Psychology, Dalhousie University, Halifax, Nova Scotia, Canada, B3H 4J1.

Trophic interactions between the regenerating neural processes from the retinal tissue and the tectal tissue were studied in vitro under various experimental conditions by co-culturing the retinal and the tectal tissues, explanted from the adult goldfish. The optic nerve was pre-crushed about two weeks before retinal explantation, and 5-15μ Ci of H^3-proline was injected into the eye 24-72 hrs prior to retinal explantation. This procedure allowed us to label the entire length of newly grown neurites from the retinal explant, maintained in culture for 3-4 weeks. If a rectangular piece of the retina was dissected free along the nasotemporal axis near the equator, and implanted on a culture dish, the neurites sprouting out from the retinal explant usually grew in the radial direction towards the phantom optic disc. As they grew out further, these retinal neurites curled up to form clockwise spirals, in the absence of their target tissue. When a piece of the tectal tissue (dissected from the topographically matching area) was introduced, however, the curling retinal neurites tended to straighten, and grew preferentially towards the tectal tissue, and eventually invaded it. The trajectories of the ingrowing retinal neurites within the tectal explant were traced by autoradiographic methods through serial sections. Preliminary result suggests that these retinal neurites tend to grow towards the appropriate layers of their usual passages and terminations within the co-cultured tectal tissue.

(Supported by grants from NSERC and MRC of Canada)

178.4 NERVE GROWTH FACTOR ACTION AT THE GROWTH CONE. P.J. Seeley* and L.A. Greene* (SPON: A. Chalazonitis) Dept. Pharmacol., New York Univ. Med. Ctr., New York, NY 10016

The shape and motility of growth cones of PC12 cells and sympathetic neurons were observed to alter rapidly on withdrawal or readdition of nerve growth factor (NGF) to the culture medium. These effects have been documented by time-lapse video recording of light microscope images of living cells. The clonal pheochromocytoma line PC12 has been used for these experiments since it expresses sympathetic neuronal characteristics without being dependent on the factor for its survival. The growth cones of PC12 neurites exposed to NGF are broad, lamellopodial structures which are commonly fringed by filopodia adherent to the substrate. The geometries of both filopodia and lamellopodia are constantly changing on a time scale of minutes. In addition, tentacle-like structures made up of lamellae and microspikes, appearing to originate at the upper surface of the cone, probe the medium above the terminal with rapid gyrations.

Removal of NGF from neurite-bearing cultures of PC12 cells provokes a sequence of changes in the growth cone. Over 1-2h a large proportion of lamellopodia and filopodia are withdrawn, resulting in a neurite tip which is butt-ended. Subsequently a 'bead' forms close to the terminus which is followed by thinning of the neurite and collection of other bead-like structures along its length. Motion of components of the cone gradually decreases and by 4h after initiating removal of NGF, the terminal is almost static. This time-course is partly contemporary with likely slow dissociation of NGF from cell surface receptors. Readdition of NGF to deprived cultures quickly induces (< 5min) rapid motion of filopodia and tentacle-like structures, but spreading of lamellopodia and re-initiation of neurite elongation occur after about 30min. These events induced on readdition of NGF are not blocked by interventions which retard influx of extracellular calcium, nor can they be mimicked by addition of dibutyryl-cAMP. The main features of the growth cone response to NGF withdrawal or readdition occur also for cultures of neurons from rat superior cervical ganglia.

The rapidity of effects observed in the growth cone on re-adding NGF implies a local mechanism for trophic interaction of a growing axon with NGF which is distinct from longer-term, transcription-dependent events elicited by NGF at the cell body (~d). Supported by grants from the March of Dimes and NIH (NS 16036). P.J.S. is a N.A.T.O. Fellow.

178.5 REGENERATION OF NEURITES IN LONG-TERM CULTURES OF SYMPATHETIC NEURONS WITHOUT NERVE GROWTH FACTOR. R.B. Campenot* (SPON: R. Hoy). Sect. of Neurobiology & Behavior, Cornell Univ., Ithaca, NY 14850.

Sympathetic neurons from newborn rats were plated into center compartments of three-compartment dishes. Neurites elongated along a series of parallel collagen channels which served to orient them to the left and right. Neurites readily penetrated silicone grease barriers and entered the separate fluid environments of the left and right side compartments where their progress was monitored by means of a stage micrometer, and their density was evaluated visually (see Campenot, R.B., Methods in Enzymology, 28: 302, 1979). 7S nerve growth factor (NGF) at 1 µg/ml was used. Formulations and general culturing procedures were as described in Hawrot, E. & Patterson, P.H., Methods in Enzymology, 28: 574, 1979), except that side compartments were given medium free of serum and ascorbate.

Neurites readily penetrated barriers when NGF was present in the side compartments, and NGF was removed from the center compartments 4 days after plating (see Campenot, R.B., Proc. Nat'l. Acad. Sci., 74: 4516, 1977). Cultures matured for 20-55 days when neurites were removed from the side compartments by vigorous washing with distilled water. Within 24 hrs, luxurient, regenerating neurites were observed in side compartments given NGF subsequent to washing. They advanced along the channels at about 1 mm/day during at least 4 days of observation. Surprisingly, regeneration also occurred in cultures where neither side compartment was given NGF subsequent to washing. Without NGF the neurites were less dense (but substantial), advanced somewhat more slowly, stopped in about 2 days at distances of 1-2 mm, and held this position for as long as 20 days of observation. Neurite elongation resumed when NGF was subsequently added to previously deprived side compartments, but addition of NGF to center compartments did not augment regeneration into NGF-deprived, side compartments. Also, regeneration was repeatedly observed in cultures subjected to successive washings over periods as long as 20 days without NGF given anywhere, making it very unlikely that residual NGF could have supported the regeneration.

It has been shown that cultured sympathetic neurons from newborn rats die if deprived of NGF during days 10-20 in culture, but that some will survive NGF withdrawal at later times (Chun, L.L.Y. & Patterson, P.H., J. Cell Biol., 75: 705, 1977). The present work strongly suggests that in addition to survival capability, at least some sympathetic neurons develop the capability to regenerate neurites without the trophic support of NGF.

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178.6 LECTIN AFFINITIES OF PNPF, A POLYORNITHINE-BINDING NEURITE-PROMOTING FACTOR. Ruben Adler, Marston Manthorpe and Silvio Varon. Dept. of Biology, Univ. of Calif., San Diego, La Jolla CA 92093.

PNPF is a neurite-promoting factor present in many conditioned media, and particularly concentrated in serum-free medium exposed to the Schwannoma cell line RN22. The RN22 PNPF does not by itself have survival-promoting properties, and its neurite-promoting activities are expressed only after it is bound to polyornithine (PORN) substrata. Dissociated 8-day chick embryo ciliary ganglionic (CG) neurons cultured on PORN with adequate support from the eye-derived trophic factor CNTF survive but fail to grow neurites in the absence of PNPF, and generate a lavish neuritic network in its presence. Neurons from dorsal root and sympathetic ganglia, as well as some neurons from the spinal cord also respond to PNPF. PNPF activity is associated with a heat- and trypsin-sensitive, acidic macromolecule.

We report here current studies on the lectin affinity of PNPF. PNPF binds to agarose-coupled concanavalin A (Con A), wheat germ agglutinin (WGA) and Ricinus commonis agglutinin (RCA), but not to Dolichos Biflorus agglutinin (DBA) or Ulex Europaeus agglutinin (UEA). The activity remains bound to Con A and WGA columns during 1M NaCl elution, but can be eluted from Con A with α-methyl mannoside (αMM), and from WGA with N-acetylglucosamine. Investigations with RCA are impaired by the appearance of toxicity in the eluted fractions. Pretreatment of PORN-bound PNPF with con A prevents neuritic growth from CG neurons, and this inhibition can be removed with αMM. Attempts to block PNPF activity in the CG assay using WGA have so far been unsuccessful.

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178.7 FURTHER STUDIES ON THE MECHANISMS OF AXONAL GUIDANCE DURING DEVELOPMENT OF THE CORPUS CALLOSUM. Jerry Silver. Dept. of Anatomy, Case Western Reserve Univ., Sch. of Med., Cleve, OH 44106

During normal ontogeny of the mouse forebrain, and before the arrival of the pioneer fibers of the corpus callosum at the midline (E15), a population of primitive glial cells migrates medially (through the dorsal septum and rostral to the lamina terminalis) from the ependymal zones of each hemisphere. At the midline these cells unite to form a sling-like structure suspended below the interhemispheric fissure (E 16). The first callosal axons grow along the surface of this glial scaffold as they travel towards the contralateral side of the brain (E17). The critical question remains. Does this transient glial tissue actually provide guidance cues to the callosal axons?

I have adopted two courses of study in order to answer this question. Firstly, the developmental stages of a congenitally acallosal mouse mutant (strain BALB/c, supplied by D. Wahlsten) have been analysed to see if malformations occurred in the regular development of the glial "sling" in advance of the first morphological signs of the callosal abnormality. Secondly, the "sling" has been lesioned surgically through the uterine wall in the normal embryo (strain C57Bl/6J at stages E15-16).

In the acallosal mouse mutant the "sling" does not form. Although fusion of the septal midline does occur forward of the lamina terminalis, the glial elements which normally comprise the "sling" do not migrate far enough nor in numbers sufficient enough to form a cohesive structure. Although the would-be callosal axons approach the midline on schedule, they do not cross. Instead, most whorl into a pair of large neuromas adjacent to the interhemispheric fissure. Apparently, some fibers, whose destinations are yet unknown, eventually enter the septum (Ivy and Killackey,'81). Surgical disunion of the glial "sling" in normal embryos (without touching the lamina terminalis) also resulted in acallosal individuals. The anterior and hippocampal commissures were unaltered. It is noteworthy and rather remarkable that the callosal pathology in these affected animals mimicked exactly that of the genetically lesioned mutant.

There are several especially significant aspects of these investigations. Firstly, my observations suggest that the primitive glial "sling" over which the callosal fibers grow is just as important to the guidance of axons as the targets with which they must eventually synapse. Secondly, the highly oriented geometry of this glial structure is apparently organized under genetic control. Thirdly, it appears that the proper development of the corpus callosum has little to do directly with the lamina terminalis, at least in the mouse. Supported by NIH (NS-15731).

178.8 GROWTH CONES EXPAND IN-VITRO BY DEPOLARIZATION UNDER CONDITIONS WHICH PERMIT Ca^{2+} ENTRY. L. Anglister*, A. Shahar*, I.C. Farber* and A. Grinvald. Dept. of Neurobiology, Weizmann Inst. of Sci. and Institute for Biol. Res., Ness-Ziona, Israel.

Calcium action potentials were recently recorded at or near growth cones of regenerating neurites (Meiri et al., Science 211, 709, 1981) and developing neurites (Grinvald & Farber, Science 1981, in press). In order to investigate if Ca^{2+} entry plays a role in neurite growth, we have studied the morphological changes induced by experimental conditions which permit inward calcium currents. Differentiated N1E-115 neuroblastoma cells (4-10 days) were depolarized either by 30mM potassium (for 10-20 min) or by stimulating the soma (for 20-120 min) with an intracellular electrode. Morphological changes in individual cells were followed by time-lapse video recordings and subsequently by scanning electron microscopy. Steady state potassium depolarization or long trains of 0.5 Hz action potentials induced in more than 60% of the experiments pronounced flattening and an increase of 20-120% in the apparent contact area of the growth cone. Statistical comparison of control and potassium depolarized cells (in the presence of 5mM Ca^{2+}), by scanning electron microscopy, indicated that depolarized growth cones had larger contact area with the substrate at least by 25% relative to the control cells. The growth cone membranes were flattened, had a smaller number of micro-spikes, and contained markedly more vesicular protuberances. Similar morphological changes were also observed in restricted zones of expanded membrane area occurring along the neurites of these cells. The same results were obtained when the cells were stimulated in a medium containing 10^{-6}M TTX and 15mM TEA. However such morphological changes could not be observed in the presence of 0.2mM Ca and 1mM Cd^{2+} which block the inward Ca^{2+} currents. Removal of the Cd^{2+}, or elevation of the Ca^{2+} concentration to 5mM, even in the presence of 1mM Cd^{2+}, again led to the changes in these cells. These results indirectly suggest that Ca^{2+} inward current might be the primary trigger of the observed morphological changes. The above changes may play a role in neurite growth.

In some cases we have observed that growth cones of different processes of a single cell responded differently to depolarization e.g., while some growth cones were expanded by 110%, others were not changed at all. One possible explanation might be the regional variations in the distribution of functioning Ca^{2+} channels. This possibility is being examined by local microperfusion combined with focal extracellular recordings and optical recordings from different processes and growth cones of the same cell.

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178.9 **CHEMOTAXIS AND REGENERATION OF MAMMALIAN NERVES.**
M.J. Politis, K. Ederle*, and P.S. Spencer. Inst. of Neurotoxicology, Albert Einstein College of Medicine, Bronx, N.Y. 10461.

We have continued to reexamine the Weiss/Taylor 1944 rebuttal (1) of Cajal's hypothesis (2) that Schwann cells in distal nerve stumps exert an "attractant" (chemotactic) effect on regenerating axons. Proximal stumps of transected feline peroneal nerves were individually inserted into the single "input" end of hollow, Y-shaped Silastic implants so that regenerating axons grew over a 4-mm distance toward either a tibial (T_{out}) or peroneal (P_{out}) distal stump inserted into the paired output ends of the implant. At 4.5 weeks postoperatively the number of unmyelinated and myelinated axons greater than one micron in diameter in implant forks leading to distal peroneal and tibial nerves were assessed morphologically, and at 6 weeks the number of myelinated axons. At both time points 2-3 times more peroneal axons were found in implant forks leading to "native" P_{out} than to "foreign" T_{out}. Treatment of P_{out} with dry ice and RNA/DNA-synthesis inhibitors to compromise Schwann cells resulted in approximately 10 times more regenerating peroneal axons to T_{out} than to P_{out}. Preliminary data utilizing axonally transported radioactive proteins indicate a preferential growth of both motor and sensory peroneal axons to untreated tibial rather than treated peroneal stumps. Morphological assessment of implant forks revealed a similar though less marked effect when 8-week denervated tibial distal nerve stumps(containing "undifferentiated" Schwann cells, an enhanced density of fibroblasts and little or no myelin debris) were individually inserted adjacent to freshly transected treated distal P_{out}. When Nuclepore filters (0.2-um pore size) were placed between the implant and either treated P_{out} or untreated T_{out}, marked preferential growth to the latter was observed. These results suggest that peripheral nerve regeneration is influenced by diffusible chemotactic factors, possibly of Schwann cell origin.

Supported by NIH grant NS 13106

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178.10 AN ELECTRON MICROSCOPIC STUDY OF THE HAMSTER PYRAMIDAL TRACT.
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A previous light microscopic study using anterograde tracing techniques demonstrated that the growth of the pyramidal tract of the hamster is primarily postnatal. Thus, the pyramidal tract provides a convenient model system to study the development of fiber pathways in the mammalian central nervous system. We therefore undertook a qualitative and quantitative electron microscopic (EM) study of the development of the pyramidal tract to investigate the mode of growth of the axons, the possibility of fiber degeneration during development, and the process of myelination.

Golden hamsters ranging in age from three to 200 days were perfused intracardially and blocks containing the medullary pyramids were prepared for EM analysis. The total number of fibers in the tract at the level of the inferior olivary nucleus was determined for several postnatal ages by taking the product of axon density and tract area. We found that the pyramidal tract grows through the medulla as a compact bundle containing nearly twice the number of fibers as the mature tract. During the second postnatal week there is a substantial decline in the number of fibers in the tract coincident with the appearance of dark degenerating profiles in the tract. The decline in axon number continues in the third and fourth postnatal weeks at a reduced rate and by 34 days after birth the number of axons reaches the adult value. Myelination in the hamster pyramidal tract begins at seven days and continues at a very slow rate until the third postnatal week, when a dramatic increase in myelin formation occurs. By 34 days after birth the number of myelinated axons is approximately 80% that of the adult. As has been reported for other CNS tracts, there does not seem to be a "critical diameter" of an axon that absolutely determines the presence or absence of myelin. However, all axons above 0.5 in diameter are myelinated at approximately the same rate, while those under this diameter are myelinated much more slowly and even in the adult make up only a small percentage of the total myelinated fibers.

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178.11 AXONAL PROJECTIONS OF SUBCORTICAL NEURONS IN CEREBELLAR TISSUE CULTURES.
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Cerebellar explants derived from newborn mice can be prepared to include or exclude portions of underlying pons. When portions of dorsal pons are included, a clear separation usually becomes evident after maturation in vitro between two subcortical groups of neurons, namely those derived from intracerebellar nuclei and those originating from nuclei of the tegmentum pontis. Large intracerebellar nucleus neurons fall into two major categories when stained with silver and Golgi impregnation methods, including multipolar and spindle-shaped cells. Both forms have aspinous as well as spiney dendritic branches. A variety of neuronal types is evident in dorsal pontine portions of cerebellar explants, ranging from medium size to giant multipolar neurons with predominantly spiney dendritic branches to occasional unipolar mesencephalic V nucleus neurons. Catecholamine positive neurons are infrequent in those portions of tegmentum pontis routinely incorporated with cerebellar explants. The axons of intracerebellar nucleus neurons invariably turn toward cerebellar cortex after a brief ventrad excursion, and multiple branches terminate in dispersed cortical regions. The axons of most pontine tegmental neurons loop back to the explant after initially growing toward or into the ventral outgrowth zone, and their branches terminate in widely separated portions of cortex. A constant exception to this pattern are the peripheral axons of mesencephalic V nucleus neurons, which terminate in far reaches of the outgrowth zone. Mossy fiber terminals are readily evident on ultrastructural examination of cortical regions of cerebellar explants with incorporated segments of dorsal pons, while such terminals are rare in cultures without pontine tegmental neurons. It would thus appear that dorsal pontine neurons, many of which project mossy fibers to the cerebellar cortex in situ, are the source of most mossy fiber terminals in cerebellar explants. Axons of intracerebellar nucleus neurons, deprived of their usual target cells, may universally turn toward cortex in a generally unsuccessful search for central targets, and not because they mimic an in situ nucleocortical projection. (Supported by the Veterans Administration).

178.12 GROWTH OF CORTICOSPINAL AXONS THROUGH LESIONED NEONATAL SPINAL CORD.
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The anterograde transport of horseradish perioxidase was used to label the growing axons of corticospinal neurons in neonatal rats that had suffered spinal cord lesions. The appearance of these axons, their length, and the path taken by them were compared to those in unlesioned animals on the ninth day after birth at which time corticospinal axons reach the lumbosacral enlargement. Spinal cord lesions were of two general types: a complete surgical transection, usually involving a limited length of spinal cord, or a thermal lesion produced by brief contact of a heated rod on the dorsal aspect of the vertebral column. The latter type of lesion had a greater rostrocaudal extent, but with varying dorsoventral involvement. All lesions were made at low thoracic levels at different times before the arrival of the first corticospinal axons. The overall growth rate of corticospinal axons was not significantly altered, except in cases where complete transection or massive damage constituted an insurmountable barrier. Corticospinal axons grew toward a lesioned area at the same rate as controls and if they grew through and beyond a lesion, their growth rate was unaffected. The tips of the growing axons appeared normal. The paths taken by growing axons in the vicinity of a lesion were often markedly aberrant. Virtually any remaining neural tissue could provide a substrate for growth through a lesion, but axons never extended outside the CNS. Displaced axons that were successful in passing a lesion sometimes, but not always, returned to their normal position in the dorsal columns distal to the lesion.

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178.13 IDENTIFICATION OF CULTURED MOTONEURONS BY RETROGRADE TRANSPORT OF HRP. K. W. Tosney* and L. T. Landmesser. Biology Department, Yale University, New Haven, CT. 06511.

Recent work suggests that motoneurons innervating the chick hindlimb are specified early in development, and that their precise and specific responses to environmental cues play a large role in generating specific patterns of innervation. For instance, motoneuron axons sort out into discrete tracts before they reach the limb (Lance-Jones and Landmesser, 1981, Proc. Roy. Soc. Lond. In press). Furthermore, when experimentally displaced from their normal point of entry into the limb, motoneuron axons can still specifically innervate their appropriate targets (Lance-Jones and Landmesser, 1980, J. Physiol. 302:581). In order to analyze further the abilities of motoneurons to fasciculate specifically and to locate their targets, we have developed an in vitro system in which specific populations of motoneurons are identifiable.

Motoneuron pools are labelled in vivo by injecting 5% HRP into specific sites of stage 24-31 chick hindlimbs and allowing retrograde transport for 3 hours (h) in an oxygenated Tyrode bath at 31° C. The ventral quadrant of the lumbosacral cord is then plated either as segment-sized explants or as dissociated cell cultures on tissue culture substrata that have been coated with polyornithine and rinsed with acetic acid-solubilized collagen. The culture medium, containing 10% horse serum and 5% embryo extract, is previously conditioned by confluent monolayers of chick heart ventricle cells. Neurons begin to extend neurites 3 h after plating under these conditions. After fixation, the cultures are processed with diaminobenzidine to allow visualization of label.

One population of neurons is heavily labelled with granular reaction product in dissociated cultures derived from embryos in which all the limb muscles have been injected with HRP. The labelled neurons are 12-15μ in diameter and 80-90% have extended neurites by the time of fixation, 18-24 h after plating. Only a background level of label is present in glial cells or in neurons of other size classes or with dramatically different morphologies. In explants, labelled neurons occur only within the lateral motor column, and are restricted to the appropriate segments when only a single muscle is injected with HRP.

We conclude that specifically labelled populations of motoneurons retain their label in our cultures and actively extend neurites. We are currently using this culture system to examine neurite-neurite and neurite-target interactions which may be important in generating specific patterns of innervation during development.

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178.14 AN IN VITRO MDOEL OF CEREBELLAR GRANULE CELL MIGRATION. E. Trenkner, N. Segil* and R. Liem* (SPON: U. Drager). Dept. of Pharmacology, New York Univ. Med. Ctr., New York 10016.

It has been suggested that astro glia cell types play a decisive role in the histogenesis of the CNS by serving as guidance for neuron migration (1). We have developed a micro-tissue culture system which allows us to characterize and to manipulate cell migration during cerebellar development (2). We have previously described the process of pattern formation in these cultures. After dissociation, cells reaggregrate and form cables which connect the reaggregates. A population of presumptive granule cells then migrates out of the reaggregates onto the cables. This study was undertaken to confirm the identity of these cells and to further characterize their interaction with glial elements.

At postnatal day 3 (P3), the majority of cells dividing in the cerebellum are external granule cells, a subpopulation of which are destined to begin migrating within 24 hrs. (3). Therefore, we injected ^{3}H thymidine into P3 animals and at various times thereafter prepared cerebellar cultures in order to determine whether this labeled, migrating population of cells appeared as migrating cells in our cultures. 3 days after culture of P4 animals (injected 24 hrs earlier) 30%-40% of the migrating cells were labeled. This % was much lower in cultures prepared 96 hrs (P7) after ^{3}H thymidine injection, indicating that there might be a predetermined length of time during which these cells can migrate. To check this hypothesis, P3 cerebellar cells were labeled and then cultured for various times up to 7 days. The largest % of labeled cells on cables was found after 3 days in vitro. Subsequently, the % of labeled cells on cables declined to near 0, most probably as a result of migration into reaggregrates (2). This study confirms the electron microscopic evidence that the cells migrating on cables are granule cells and suggests that there may be a predetermined length of time for migration to take place.

In an attempt to characterize the cellular composition of the cables, anti-glial filament antibodies were employed immunohistochemically. It was observed that granule cells only migrated onto cables which contained glial elements in addition to the parallel fiber elements previously described (2). However, no direct contact between glial elements and migrating granule cells seemed to be necessary. These observations further substantiate the possible role of glia during granule cell migration.

(1) Rakic, P. (1971) J. Comp. Neur. 141:283.
(2) Trenkner, E. and Sidman, R.L. (1977) J. Cell Biol. 75:915.
(3) Fugita et al. (1966) J. Comp. Neur. 128:191.

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178.15 GROWTH CONES OF GOLDFISH RETINAL NEURITES GENERATE DC CURRENTS, AND ORIENT IN AN ELECTRIC FIELD. J.A.Freeman, J.M.Weiss, G.J.Snipes, B.Mayes, J.J.Norden. Vanderbilt Medical School, Nashville, TN 37232.

The optic nerves of fish and amphibia are remarkable for their ability to regenerate after injury. Little is known about the mechanisms that control the rate or direction of axonal growth. Some of these mechanisms can be studied to advantage in tissue culture. Goldfish retinal explants were cultured on poly-L-lysine coated petri dishes 10-12 days after optic nerve crush, using a modification of the method of Landreth & Agranoff (Brain Res., 1979). Prolific neuritic outgrowth occurred within 3-5 days. Individual neurites grow at a rate of 15-25μm/hr; the majority of long neurites appear to arise from ganglion cells, and tend to organize themselves into fascicles. The fascicles appear under SEM to be held together by glial cell processes, not by direct contact between neighboring neurites. By contrast, growth cones appear to adhere directly to the substrate. Filopodial processes are often seen extending towards small particles, some of which are negatively charged as shown by their direction of migration in an applied electric field. To further explore the influence of an electric field on neurite growth, a chamber was constructed through which a linear field could be applied at different angles. Fields of 2-3V/cm caused a dramatic orientation of neurites towards the cathode, and increased the rate of growth, as also shown by Jaffe & Poo (J.Expl.Zool., 1979). Applied fields also caused new processes to sprout.

Growth cones themselves generate DC currents of 15-30nA/cm^{2}. These were measured by a platinized microelectrode caused to oscillate in a circle of 15-20μm diameter at 300Hz by a specially constructed driver. A computer was used to average the voltage gradient across the diameter of the electrode's traverse at different angles. Signal averaging and the rapidity of the electrode motion greatly decreased electrode noise, permitting currents of <5nA/cm^{2} to be measured and displayed in polar coordinates. A current appears to flow inwards across the plasma membrane at the tip of the growth cone, and back outwards at the base. Calculations based on these measurements are consistent with the hypothesis that these endogenous currents are produced by the strategic location of a class of ionophores or of pump sites on the tips of the growth cones. These currents might play a role in the molecular assembly and organization of the growth cone, and possibly in controlling the direction of growth, which seems to be quite sensitive to and possibly determined by short-range electric field interactions.

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179.1 ABSENCE OF MORPHOLOGICAL RESPONSES TO INTRACELLULAR INJECTION OF NERVE GROWTH FACTOR INTO CELLS OF THE NEURON-LIKE CLONE PC-12. S. Huttner* and P. O'Lague. Dept. Biology, Univ. of California, Los Angeles, CA 90024.

Nerve growth factor (NGF) triggers the extension of neurite-like processes by clonal pheochromocytoma cells (PC-12). The mechanism (s) of NGF action is not understood but the presence of high affinity binding sites for NGF on both the plasma and nuclear membranes suggests that NGF may have both intracellular and extracellular actions.

The role of cytoplasmic NGF on neurite extension was studied by comparing the effects on PC-12 cells of NGF applied either exogenously or microinjected from a pipette into the cytoplasm or nucleus. Giant multinucleate PC-12 cells, used to facilitate microinjection were produced by chemically induced cell fusion and were grown in cell culture (O'Lague and Huttner, 1980, PNAS 77:1701). Final intracellular NGF concentrations produced by injection were roughly estimated to range between 0.1-5 µg/ml for injection volumes up to 5% of total cell volume. Some control cells were injected with phosphate buffer. Approximately 90% of injected cells (experimental n=150, control n=100) survived the injection for at least 1 week.

Within 2 min many cells exposed to exogenous NGF (1 µg/ml) showed dramatic morphological changes. Individual cells observed with phase contrast optics exhibited an increase in the number of fine microspikes on the cell surface, then by 20 min the lengths of some filopodia increased, and by 24 hrs in many cases neurite-like processes had extended. Cells were also examined in the scanning electron microscope.

NGF-injected cells showed no detectable differences from control cells at any time observed (30 min to 1 week after injection). However the injected cells retained the ability to respond to external NGF, applied in the growth media or ejected from the pipette immediately outside the cell, with the morphological changes seen in uninjected cells exposed to exogenous NGF.

179.2 NGF-DEPENDENT RECOVERY OF FETAL MOUSE DRG AND DORSAL CORD NEURONS AFTER CHRONIC TAXOL EXPOSURE IN ORGANOTYPIC CULTURES. E.R. Peterson and S.M. Crain (SPON: E. Masurovsky). Dept. of Neurosci. Albert Einstein College of Medicine, Bronx, N.Y. 10461.

Taxol induces a striking extensive assembly of "stabilized" microtubules in cultured fibroblasts and Hela cells (Schiff and Horwitz, PNAS, '80) and in neurons and supporting cells in explants of spinal cord and dorsal root ganglia (DRGs) (Peterson et al, & Masurovsky et al, J. Cell Biol.'80). Cross-sections of 14-day fetal mouse spinal cord with attached DRGs were explanted on collagen-substrate coverslips and exposed to 1 µM taxol in a nutrient medium supplemented with NGF (300 B.U./ml). In cultures exposed to 1 µM taxol for 4-6 days without NGF, almost all neurons degenerated, whereas in the presence of NGF survival of DRG neurons was comparable to controls without taxol. The DRGs showed, however, some cytoplasmic aberrations, nuclear distortions, and neuritic outgrowth was essentially blocked. The spinal cord showed rapid necrosis of glial cells and variable patterns of neuronal degeneration that were more clearly evaluated after withdrawal of taxol. In cultures exposed for 4-6 days to taxol + NGF and returned to control media without NGF, only ~ 60 neurons survived per DRG and the spinal cord was almost totally destroyed. (Comparable numbers of DRG neurons survive in control cultures which have never received NGF.) On the other hand, when after 4-day taxol + NGF, the recovery medium was supplemented with NGF (300 B.U./ml), there was not only an almost total survival of DRG neurons (> 90%), but also an unexpected and remarkable survival of dorsal cord tissue -- whereas ventral cord was often reduced to a monolayer. Dorsal cord tissue in the 4-day taxol-treated DRG-cord explants not only showed good morphologic integrity after return to high NGF, but characteristic dorsal-horn network responses could be evoked with DRG stimuli (Crain & Peterson,Br.Res.'74) at 3 wks in vitro. Even after 6 days in taxol + NGF, 30-70% of the DRG neurons recovered in high NGF and small islands of dorsal cord occasionally survived. Abundant swirling bundles of naked neurites were maintained in the outgrowth of these cultures for several weeks, while Schwann cell replication and migration was blocked. Thus, although the presence of high NGF fully prevented degeneration of most DRG neurons during exposure to taxol, additional NGF was required for their recovery after return to normal medium. Furthermore, chronic exposure of cord-DRG explants to taxol appears to alter dorsal cord neurons so that they become unexpectedly dependent for their survival either on the presence of NGF or on trophic influences from NGF-stimulated DRG neurons.

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179.3 LOCAL SPREAD OF NERVE GROWTH FACTOR IN THE SPINAL CORD B.A. Green, T. Khan, R.J. Perez Polo,(SPON: J. Trimble). Dept. of Neurological Surgery, University of Miami Medical School, Miami, Fla. RER&D Center, VA Hospital, Hines, IL. Dept. of Biochemistry UTMB, Galveston, Texas.

This study evaluates the local spread of nerve growth factor (NGF) from the site of infusion in the spinal cord I^{125} labelled β NGF was infused into the experimentally contused spinal cord of rabbits. One hour post injury a dorsal myelotomy was performed and intramedullary infusion of I^{125} NGF was accomplished by inserting a small catheter into the spinal cord through the myelotomy. The other end of the catheter was attached to a small osmotic pump (Alzet). The pump contained 0.2 ml of I^{125}β NGF and a continuous infusion of I^{125} at the rate of 0.3 uc/hour of NGF was accomplished. The duration of infusion was one day and one week post injury. The animals were then anesthetized and perfused through the left ventricle with 4% formaldehyde and 0.5% glutaraldehyde in a phosphate buffer. The spinal cord was removed, dehydrated, embedded in parafin and sectioned. Serial longitudinal frontal sections, 10 microns thick were mounted on gelatinized slides, deparaffinized, dipped in Kodak NTB 2 photographic emulsion and stored for 3 weeks or 6 weeks. At the end of this period slides were developed in DK170 and counterstained with cresyl violet.

Examination of slides infused with I^{125} NGF for one day showed most of the labelled area to be at the site of infusion. Labelling extended circumferentially to 1-2 mm from the edge of the wound. Some cells of dorsal root ganglion (DRG) next to the infusion site were labelled. The sections of spinal cord infused for one week showed some labelling at the site of infusion within the scar tissue. The dorsal root ganglion next to the site and a segment away showed labelling of cells. The labelling of spinal cord, dorsal as well as ventral aspect, away from the lesion area was noted though this labelling seems to be nonspecific.

179.4 POSSIBILITY OF A STABILIZING EFFECT BY NERVE GROWTH FACTOR ON INJURED NEURONS. T. Khan, B.A. Green, J.R. Perez Polo. RER&D Center, VA Hospital, Hines, IL. Dept. of Neurological Surgery, University of Miami Medical School, Miami, Fla. Dept. of Biochemistry, UTMB, Galveston, Texas.

Nerve growth factor (NGF) has been shown to have a positive stabilizing effect on the injured spinal neurons. The effect of NGF was studied on the experimentally contused spinal cord. Since, it was suggested that NGF may not penetrate the blood brain barrier (Freed, W.J., Brain Res. Bull. 1:393-412, 1976), NGF was infused locally in the spinal cord. The lesion was produced by dropping a known weight from known height on surgically exposed spinal cord, sufficient to produce permanent paraplegia. One hour post injury, a myelotomy was performed at the site of the lesion. An implantable silastic osmotic pump reservoir (Alzet) was used for continuous infusion of NGF into the grey matter of spinal cord at myelotomy site. The pump was changed every week and NGF was delivered for 2 weeks, 6 weeks and 12 weeks post trauma. The results were evaluated by histological serial sections stained with the Bodian Protergol method. Our preliminary results showed a beneficial effect of NGF on the central fibers. The dorsal and ventral funiculus showed the same effect. The extent of scar tissue formation and fiber regrowth depends on the duration of infusion of nerve growth factor.

179.5 LOW LEVELS OF IMMUNOLOGICAL CROSSREACTIVITY BETWEEN AFFINITY PREPARED ANTI-MURINE β-NGF AND HUMAN PLACENTAL β-NGF. C. E. Beck*, M. Blum*, K. Turpin*, Claude Jacque* and J. R. Perez-Polo (SPON: R. W. Stach). Dept. of Human Biol. Chem. & Genet., Univ. Tex. Med. Br., Galveston, Texas 77550; Dept. of Med., Univ. of Reims, Reims, France and INSERM, Hopital de la Salpêtrière, Paris, France.

Nerve Growth Factor (NGF) is a multimeric protein that is required for the development and maintenance of the vertebrate sympathetic nervous system. It has been reported that alterations in the levels of NGF, as determined by radioimmunoassays with antisera directed against murine NGF, can be correlated with a number of neuropathies. These attempts to quantitate NGF levels in human serum, body fluids, and tissues using non-species specific antisera have yielded conflicting results. We have used a number of radioimmunological assays in order to determine if there is significant crossreaction between antibodies directed to murine NGF when challenged with human NGF in human biological fluids of interest. The use of a two-site radioimmunoassay (RIA) procedure reduces problems associated with NGF binding proteins found in sera, but results in false values when the total protein concentrations are not comparable in all samples tested. At detectable levels of 500 pg β-NGF per ml of serum, using this RIA technique, we have been unable to reproducibly demonstrate human β-NGF in either normal adult sera or in amniotic fluid samples collected at varying times during pregnancy. We feel that this failure to detect human NGF could be due to the low crossreactivity we have observed between monospecific murine antibodies and human β-NGF isolated from human placenta at term. More recently we have found a high level of crossreactivity between murine α-subunit antisera and an acidic protein associated with NGF activity in human placenta. Contamination of antisera with small amounts of anti-α-subunit may be responsible for the discrepancies in previously reported levels of NGF in human sera and tissues.

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179.6 RADIOAUTOGRAPHIC LOCALIZATION OF ^{125}I-NGF IN PC12 CELLS. P. Bernd* and L.A. Greene* (SPON: C.R. Noback). Dept. of Pharmacology, New York Univ. Med. Ctr., New York, NY 10016.

The localization of bound nerve growth factor (NGF) was studied by light and electron microscopic radioautography on a cell line derived from a rat pheochromocytoma (PC12). In response to NGF, PC12 cells cease mitosis and develop neuron-like processes. In all experiments, cells that had not been previously exposed to NGF (naive) were examined, as well as those that were grown with 50 ng/ml of NGF for one week (primed).

Cells were grown on collagen-coated coverslips or directly on collagen-coated plastic dishes at a density of 200,000 cells per 35mm dish in RPMI medium containing 5% fetal bovine serum and 10% heat-inactivated horse serum. ^{125}I-NGF was prepared by the lactoperoxidase method (3.8 x 10^4 cpm/ng). Cells were incubated with 5 ng/ml of ^{125}I-NGF for 15 min, 1 hr, 6 hrs or 24 hrs (control included a 1000-fold excess of nonradioactive NGF). Primed cultures were extensively washed with NGF-free medium prior to exposure to ^{125}I-NGF. The cultures were then washed with iced PBS, fixed (3% glutaraldehyde in 0.1M phosphate buffer containing 3% sucrose, pH 7.5; 1 hr, 25°C), and washed with the above buffer. Coverslips were mounted on slides and allowed to dry; cells in dishes were postfixed in osmium (1% in the above buffer; 1 hr, 4°C), dehydrated and embedded in epon 812. Whole mounts and sections were coated with Ilford L4 emulsion and exposed at 4°C for periods ranging from 1 week to 2 months.

Examination of whole mount radioautographs revealed that all cells were labelled and that the distribution of grains was homogeneous. Primed cells were labelled on neurites and growth cones, as well as cell bodies, and also had a greater density of labelling than naive cells. These patterns were identical for all time points studied. The binding of ^{125}I-NGF was specific, since control cultures showed only background levels of grains. Thick sections (1 μm) were examined to determine if cytosol and/or nucleus were labelled by internalized ^{125}I-NGF. In all cells, the cytosol was heavily labelled while the nucleus was relatively unlabelled; however, in a small percentage of cells (~15%) the nucleus was labelled and the grains often appeared to be concentrated over nucleoli. This apparent nucleolar labelling was observed at all time points studied and was present in both naive and primed cells. Preliminary statistical analysis of electron microscopic radioautographs showed a specific association of grains with lysosomes and a possible association with both heterochromatin (assumed to be nucleolus), the nuclear membrane and the plasma membrane. This study supported by NIH grant # NS 16036, March of Dimes grant # 1-704 and a Pharmaceutical Manufacturers Association Foundation fellowship.

179.7 MOUSE NERVE GROWTH FACTOR-LIKE ACTIVITY (mNGF) IN THE BRAIN OF ADULT MICE: DETECTION BY PC-12 CELL BIOASSAY. S.M. Scott*, R. Tarris*, D. Eveleth*, M.E. Weichsel, Jr., D.A. Fisher*, Perinatal Research Laboratory, Harbor-UCLA Medical Center, Torrance, CA

Previously we reported the detection of NGF in mouse brain by RIA. However, bioassay data from several laboratories have not been in agreement and the presence of NGF in brain remains controversial. Thus we conducted studies of NGF bioactivity in brain tissue using a sensitized PC-12 cell bioassay system (SCBS). PC-12 cells are derived from a rat pheochromocytoma and respond to NGF with neurite outgrowth. The SCBS is sensitive to 100 pg/ml NGF.

Three pools of seven brains each from adult Swiss-Webster mice were homogenized 1:1 wt to volume with PBS and the supernatants collected for bioassay. Prior to bioassay, the supernatants were dialyzed against media used for maintenance of the PC-12 cells except that serum was omitted. The cells were placed in a bioassay plate at time zero at a concentration of 10^4 cells per well in a medium containing 50 ng/ml of mNGF (total volume of 500λ per well); at 48 hrs, the cells were changed to mNGF-free media. At 72 hrs six conditions were established prior to reading the bioassay at 96 hrs. Each condition was duplicated and each brain pool studied on a different day. The pre-immune serum and specific anti-NGF antibody were from the same rabbit: (% outgrowth = % of cells with neurite growth)

Condition	Media	Pre-immune	Brain pool	Antibody	% Outgrowth
1.	500	--	--	--	5%
2.	400	100	--	--	5%
3.	400	--	--	100	5%
4.	400	--	100	--	80-100%
5.	300	100	100	--	80%
6.	300	--	100	100	5%

Results: 3-8% normal PC-12 cells will have neurite extension under control conditions (condition #1). The addition of pre-immune serum and antibody (#2 and #3) show lack of toxicity by these factors. #4 shows the positive response of brain supernatant; #5 shows failure of normal rabbit serum to block this effect. #6 shows the ability of specific anti-mNGF antibody to block the neurite growth promoting effect of brain supernatant. We conclude that the brains of Swiss-Webster adult mice contain an NGF-like substance, probably NGF itself, which promotes neurite outgrowth in PC-12 cells and that this effect is blocked by specific NGF antiserum.

179.8 NERVE GROWTH ACTIVITIES IN RAT PERIPHERAL NERVE. T. Ebendal* and P. M. Richardson, Inst. of Zoology, Uppsala University & Dept. of Neurosurgery, McGill University.

Nerve growth activities in the rat sciatic nerve were assessed by recording the neuritic outgrowth from chick embryo ganglia cultured for two days in collagen gels in close proximity to nerve fragments. Sometimes the normal sciatic nerve was used; sometimes the nerve had been cut at the sciatic notch 2-15 days previously or had been prepared as an autologous graft in the leg two days earlier. Frozen and thawed specimens from normal nerves, like those from several other rat tissues, induced, from both sympathetic and ciliary ganglia, neuritic outgrowth which was not significantly diminished by anti-NGF. Living nerve fragments, on the other hand, released activity resembling NGF in its effect on sympathetic ganglia and almost totally blocked by anti-NGF. Thus, from observations on normal nerves, two agents promoting neuritic extension were deduced to exist although the presence of additional factors has not been excluded. NGF-like activity was not detected in frozen and thawed segments from normal nerves but its level increased markedly in nerve grafts and, to a lesser extent in the distal stump of a nerve transected two days previously. Nerve fragments with strong NGF-like effect on sympathetic ganglia also evoked neuritic outgrowth, partly suppressible with anti-NGF from ciliary ganglia. This phenomenon has not been fully explained but may reflect the actions of a high concentration of one agent similar but not identical to NGF or the combined effect on ciliary neurons of two factors, one recognized by anti-NGF. Two weeks after nerve transection, the distal nerve stump contained little or no nerve growth activity of either kind. However, when then taken to culture, nerve fragments were seen to have retained the capacity to produce NGF-like activity although the course of maximal activity had shifted from the endoneurial to the perineurial region. In summary, cells in the rat peripheral nerve can release soluble agents promoting neurite outgrowth. One factor resembles NGF and its concentration in tissues and site of synthesis are altered by manipulation of the nerve in vivo.

179.9 FURTHER CHARACTERIZATION OF A NEW NEURONAL GROWTH FACTOR AND ITS ANTISERUM. M.D. Coughlin, J.A .Kessler and I.B. Black. Div. of Developmental Neurology, Cornell Univ. Med. Coll., New York, NY 10021.

We recently reported that a new neuronal growth factor (CMF), isolated from mouse heart cell conditioned medium and distinct from nerve growth factor (NGF), stimulates morphological and biochemical development of the embryonic mouse superior cervical ganglion (SCG; Coughlin et al., Develop. Biol. 82:56, 1981). This study further characterizes CMF and describes the effects of an antiserum to the factor which specifically inhibits neurite outgrowth in culture.

All biological activity of the CMF fraction eluted in the void volume of a Biogel P300 column. Use of a larger pore size agarose gel (Biogel A-5M) with or without the dissociating agent sodium lauryl sulfate (SDS) produced similar results. Electrophoresis on standard and SDS polyacrylamide gels gave similar findings: no activity and very little protein entered 3.75% gel. However, both protein and activity migrated through a 0.6% agarose-1.2% acrylamide combination gel. These results suggest that CMF activity is associated with very high molecular weight (>500,000 daltons) material.

Rabbits immunized against CMF developed high titres of antiserum (anti-CMF) with activity specifically directed against CMF. The antiserum inhibited neurite outgrowth in a dose dependent fashion, and the effects were reversed by addition of excess CMF.

Although anti-CMF inhibited NGF-stimulated neurite outgrowth from the neonatal SCG, it did not inhibit the NGF-stimulated increase in tyrosine hydroxylase activity. Moreover, addition of excess NGF did not reverse the effects of anti-CMF. Finally, ganglia incubated for 2 days in the presence of anti-CMF were subsequently capable of producing neurites when washed and cultured in medium free of anti-serum. Thus, in contrast to the effects of anti-NGF in this system, anti-CMF did not cause cell death or irreversible damage.

These findings suggest that CMF activity is associated with very high molecular weight material, and that this factor or antigenically similar material is required for neurite extension in culture.

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179.10 THE EFFECT OF A GLIAL CELL RELEASED FACTOR ON SPINAL CORD NEURON GROWTH AND ITS MODULATION BY STEROIDS. B.G.W. Arnason, R.C. Yu, L. Amico* A. Arenander*, J. DeVellis. Dept. of Neurology, University of Chicago, Chicago, Illinois 60637 and Dept. of Anatomy, U.C.L.A., Los Angeles, Calif. 90024

Dissociated embryonic spinal cord neurons (SCNs) survive for only a short period of time in vitro if glial cells are eliminated from the culture, yet SCNs can be maintained in organotypic cultures for several months. This suggests a glial influence on the growth and survival of SCNs in vitro.

We used explant cultures from 14-day-old rat embryonic spinal cord sections to assess the effect of factors released from glial cells on neuronal growth. Fresh serum free F-10 medium was conditioned with rat C_6 Glioma by incubating the medium with semi-confluent C_6 monolayers previously grown in medium supplemented with 10% fetal calf serum. Spinal cord explants were maintained in conditioned medium or in non-conditioned medium as a control. Neuritic outgrowth from explants grown in conditioned medium consistently exceeded that of cultures grown in control medium both in the number as well as in the length of neurites seen. This growth promoting effect was not observed when spinal sensory ganglion cultures were used. Conditioned medium collected from C_6 cultures pre-exposed to medium containing cortisol (1μm) showed neuritic outgrowth which was reduced significantly below control levels. In contrast C_6 conditioned medium from cultures pre-exposed to estrogen (1μm) increased outgrowth beyond that seen with C_6 conditioned medium alone. Extracts from C_6 glioma grown as a solid tumor in young rat brain did not show any growth promoting effect when added to culture medium.

Dissociated SCN from 4-day chick embryos were grown in C_6 conditioned or control medium. SCN in control medium died; in conditioned medium they survived and extended numerous long neuritic processes. Thus the C_6 conditioned medium effect is not species restricted and supports survival and growth of dissociated SCN.

These results lead us to conclude that a glial cell released factor(s) specific to SCNs favors their survival in tissue culture and their capacity to extend processes. The production of this factor by glial cells can be modulated by certain steroids. Growth factors specific for SCN could be relevant to diseases of man, such as amyotrophic lateral sclerosis, in which SCN die.

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179.11 EFFECTS OF BRAIN EXTRACTS ON THE NUMBER OF ACETYLCHOLINE RECEPTORS IN PRIMARY CULTURES OF RAT MYOTUBES. J. Robert Bostwick* and Stanley H. Appel (SPON: J. Eichberg). Dept. of Neurology, Program in Neuroscience, Baylor College of Medicine, Houston, Texas 77030.

Several laboratories have reported the enhanced effect of peripheral and central nervous system extracts on the synthesis of acetylcholine receptors (AChR) in cultured myotubes. We report here the partial characterization of a soluble factor from rat brain which induced an increase in AChR number in primary cultures of rat myotubes. This effect was not species specific since extracts of embryonic chick (12 days old) or rat (16 days old) brains induced the same response. Daily additions of extract to post-fusion cultures resulted in an optimal 65% increment in AChR number of 8 and 9 day old cultures as compared to non-treated controls. Similar increases were observed in 9 day old cultures which received only a single dose 24 hours previous to assaying for AChR. This response was concentration dependent and saturable. Brains from 16 day old rat embryos appeared to be more enriched in activity on a per milligram protein basis than were brains from new born rats.

The inclusion of protease inhibitors in the culture medium did not affect activity, indicating that the putative humoral factor itself was not susceptible to proteolysis during the incubation period. The trophic substance is probably a protein since its activity was not stable to tryptic digestion or boiling but was stable to lyophilization. When the extract was dialyzed against a 0.1 M phosphate buffer, pH 7.4, containing 0.5mM DTT and 1mM EGTA activity remained in the retentate.

The activity precipitated a 30-55% ammonium sulfate fraction and could be back-extracted in 42% salt for a 3-fold enrichment relative to protein. Gel filtration chromatography of this material on a column of Sephadex G-50 fine yielded two peaks of activity. The first peak eluted in the void volumn with little enrichment; the second peak contained 75% of the activity loaded onto the column and was recovered in an elution volume similar to ribonuclease A (M.W.=13,700). An overall purification of 60-fold from the original extract was obtained. SDS polyacrylamide gel-electrophoresis of the combined fractions indicated a band at 17,000-16,000 daltons which was significantly enriched compared to unfractionated extract.

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179.12 NEUROTROPHIC FACTOR INITIATES MYOGENESIS. Heinz Popiela, Daniel L. Taylor*, and Stanley Ellis*. Biomedical Research Division, NASA, Ames Research Center, Moffet Field, California 94035.

NTF was recently purified about 90% from peripheral nerves of adult chickens using a quantitative assay in which the amount of thymidine incorporated into chick muscle cells in vitro was measured (Popiela and Ellis. 1981. Dev. Biol. 83: 266-277).

Primary chick muscle cells are plated in the absence of NTF but presence of horse serum to allow cell attachment to the gelatin substratum; a maximum number of cells attaches 5-8 h after plating but cells do not proliferate in the absence of NTF or do so extremely slowly. Subsequent to a routine attachment period of 6 h, the medium is aspirated and replaced with medium containing 2-20 ug/ml of NTF and 5% horse serum. Upon exposure to NTF, in the obligatory presence of serum but absence of embryo extract, an 8 h lag ensues; then, thymidine is incorporated at NTF dose-dependent rates by growing cell populations but also by single cells. The rates of incorporation into cell populations depends on the initial number of cells plated and the amount of NTF supplied. Thus, populations of growing cells incorporate more thymidine at increasing doses of NTF. Cell counting indicates that dose-dependent proliferation of muscle cells has occurred during the first 48 h of exposure to NTF. Division of incorporated thymidine per dish by the number of cells present per dish reveals NTF dose-dependent increases in the amount of thymidine incorporated per cell. The specific activity of NTF is repeatedly shown to be at least 10-fold greater than unfractionated nerve extract. Shortly after 48 h exposure to NTF, myoblasts characteristically begin to fuse with one another and myogenesis commences. At 72 h of exposure to NTF, dose-dependent myogenesis is visible. Presently, we have no evidence that myogenesis takes place in response to NTF, or that myogenesis occurs automatically due to greater NTF-induced cell densities.

H.P. is a National Research Council Resident Research Associate.

179.13 EMBRYONIC BRAIN EXTRACT STIMULATES COLLAGEN SYNTHESIS BY CULTURED MYOTUBES: POSSIBLE ROLE OF COLLAGEN IN ACETYLCHOLINE RECEPTOR AGGREGATION. C. Kalcheim*, Z. Vogel*, and D. Duksin*. (SPON: M. Schneider). Departments of Neurobiology and Biophysics, The Weizmann Institute of Science, Rehovot, Israel.

Recent studies (1-4) have shown that components of the extracellular matrix are involved in nerve-muscle interaction and in the induction of acetylcholine receptor (AChR) aggregates on the muscle cell surface.

We report here that incubation of cultured rat myotubes with an extract of embryonic rat brain increased the number of receptor aggregates on the myotubes and in addition increased the synthesis and secretion of types I, III and V collagens into the culture medium. A 16 h incubation of myotubes with 0.5 mg/ml of extract of 18 day-old embryonic rat brain produced a seven fold increase in the incorporation of [^{3}H]Proline into collagen in the medium and a three fold increase in the number of AChR aggregates. Extract of adult brain was much less effective in the stimulation of both processes. Ascorbic acid (50 ug/ml) stimulated collagen secretion (2.5 fold) and also promoted a 1.6 fold increase in the number of receptor aggregates formed.

Evidence for the involvement of collagen in receptor aggregation comes from the observation that concentrations of purified bacterial collagenase sufficient to prevent accumulation of extracellular collagen also prevented the brain-extract induced receptor aggregation.

It is an attractive hypothesis that during neuromuscular synapse formation the nerve induces the muscle to synthesize and scerete one or more types of collagen. These collagen molecules either directly or via the interaction with other extracellular matrix components have a role in aggregating the AChR to the junctional areas on the muscle surface.

1. Sanes, J.R. et al. J. Cell Biol. 78: 176 (1978).
2. Burden, S.J. et al. J. Cell Biol. 82: 412 (1979).
3. Rubin, L.L. et al. Neurosci. Abstr. 6: 330 (1980).
4. Vogel, Z. et al. These Abstracts.

Supported by US-Israel Binational Foundation and by the Muscle Dystrophy Association.

179.14 MOLECULAR BEHAVIORS OF THE CILIARY NEURONOTROPHIC FACTOR(S). Gilles Barbin, Marston Manthorpe and Silvio Varon. Dept. Biol., Sch. Med., Univ. Calif. San Diego, La Jolla, CA 92093.

Ciliary neuronotrophic factor(s) (CNTF) supports the in vitro survival and growth of dissociated chick embryo ciliary ganglionic (CG) neurons. Intraocular tissues which contain the muscle cells innervated by CG neurons are highly enriched in CNTF activity. During eye development CNTF activity increases sharply during the exact period when CG neurons are forming synapses with their target cells. We have previously reported that eye CNTF could be partially purified (to about 6 x 10^4 Trophic units/mg protein) using microdissection, ion-exchange chromatography and ultrafiltration. In order to evaluate potential steps for further purification we have subjected extracts from selected intraocular tissues, CIPE (choroid, iris-ciliary body and pigment epithelium), to various fractionation protocols.

Fractional precipitation of CIPE extract using ammonium sulfate resulted in recovery of two species of CNTF activity which together contained all of the starting activity. Approximately one-half of the recovered activity and protein precipitated using 50% saturation and could be reconstituted in a small volume. The remaining half of the recovered activity was still soluble above 80% saturation.

When CIPE extract was passed through an immobilized heparin column, only 15-30% of the loaded activity could be recovered using a batchwise elution with 0.35 M NaCl. This same behavior was seen when the recovered salt eluate was diluted and passed over a second heparin column, except that 50% of the loaded activity was recovered.

CNTF activity within CIPE extract did not bind to various immobilized lectin columns (Con A, WGA, DBA, RCA and UEA) indicating that the activity is not associated with bindable carbohydrate moieties. However, when CIPE extract was passed through a Con A column, 70% of the loaded protein (but no CNTF activity) was retained.

Molecular sieving of CNTF activity within the CIPE extract was performed by gel filtration and comparisions were made using separately run molecular weight standards. CNTF activity did not migrate as a single molecular species. At low ionic strength it eluted in regions corresponding to 30-40,000 molecular weight; by increasing the ionic strength (elution in 3 M NaCl) there was a considerable shift of CNTF activity toward the lower molecular weight region, suggesting a dissociation of CNTF from aggregates or complexes.

Further data to be presented include actual protein and activity recoveries, specific activities and gel electrophoretic profiles of selected fractions. Supported by NINCDS grant NS-16349.

179.15 TESTOSTERONE ENHANCES [^{14}C] 2-DEOXYGLUCOSE UPTAKE BY RAT LEVATOR ANI MUSCLES IN VIVO. J. Toop* and S. R. Max (SPON: R. F. Mayer). Dept. of Neurology, Univ. of Maryland Sch. of Med., Baltimore, MD 21201.

Androgens influence skeletal muscle mass and function. However, mechanisms underlying the anabolic effects of these hormones remain inchoate. To address this problem, we used the 2-deoxy-D [^{14}C] glucose ([^{14}C] 2DG) autoradiographic technique of Sokoloff (J. Neurochem., 28:897, 1977) to detect early metabolic responses of muscle to hormonal stimulation. We chose the levator ani muscle (LAM) of the rat because it has a higher concentration of androgen receptors than ordinary skeletal muscle. This large number of receptors may be linked to the rapid growth of the LAM that occurs when rats are treated with androgens. Growth of the LAM is accompanied by enhanced protein synthesis, which may depend upon increased uptake of glucose as an energy source. In the present study, we used immature male rats, which have low circulatory levels of androgens. We administered testosterone propionate (TP) in DMSO, i.p., at a dose of 2.5 mg/100 g body weight; controls received DMSO. At 1, 3, 10 and 20 hr after hormone injection, paired experimental and control rats were each given 12.5 μCi of [^{14}C] 2DG in 0.2 ml 90% ethanol, i.p. Forty-five minutes later the rats were decapitated, and the LAM was dissected out and rapidly frozen in isopentane cooled in liquid N_2. Frozen sections of LAM were cut in a cryostat and picked up onto pre-cooled slides that had been coated with emulsion. Sections were thawed and dried before exposure at -80°C. Exposed slides were developed, rinsed, fixed, washed and dried. Sections overlying the emulsion were stained with Ehrlich's hematoxylin and eosin. No difference in grain numbers was discernible in muscle from TP-treated rats at 1 hr after injection. A slight increase was seen at 3 hr. By 10 and 20 hr, however, [^{14}C] 2DG uptake was markedly enhanced in muscle fibers in LAM's from TP-treated rats. The LAM is histochemically rather uniform on the basis of myosin ATPase staining, but more variable with NADH-diaphorase. However, [^{14}C] 2DG uptake appeared to be the same in all fibers. The latent period suggests an indirect (genomic) effect of the sex hormones rather than a direct effect. This influence of TP on [^{14}C] 2DG uptake presumably involves androgen receptors. Enhanced glucose utilization, as shown by increased [^{14}C] 2DG uptake, may be a metabolic precursor to the events leading to androgen-induced hypertrophy. These data show the [^{14}C] 2DG technique to be a valuable tool for studying the effects of sex hormones on muscle. [Supported in part by grants from NIH (NS 15766) and NASA (NAG 2-100)].

179.16 ANDROGEN RECEPTOR IN REGENERATING RAT LEVATOR ANI MUSCLE. S. R. Max, S. Mufti* and B. M. Carlson*. Depts. of Neurology and Biochemistry, Univ. of Md. Sch. of Med., Baltimore, MD 21201 and Dept. of Anatomy, Univ. of Michigan, Ann Arbor, MI 48109.

The development of the cytosolic androgen receptor was studied following degeneration and regeneration of the rat levator ani muscle (LAM) after a crush lesion (Gutmann and Carlson, Exp. Neurol. 58:535, 1978). An important feature of muscle regeneration is that it appears to recapitulate myogenesis in many respects. It therefore provides a model tissue available in sufficiently large quantity for investigating the ontogenesis of the androgen receptor. The receptor in the cytosol of the normal levator ani muscle has characteristics similar to those of the cytosolic receptor in other androgen sensitive tissues. Binding isotherms showed that the LAM contains a single class of cytosolic androgen receptor molecules, of limited capacity and high affinity (K_D 0.74 nM; Bmax fmoles/mg protein). Competition studies with a number of steroid hormones (androgens, estrogens, progestins, glucocorticoids) showed the ligand-specificity to be similar to that exhibited by the receptor in "classical" androgen target organs, such as kidney and prostate gland. Also, the steroid-receptor complex binds selectively to DNA-cellulose, as expected for a cytosolic androgen receptor. After crushing the LAM, the receptor number decreased by day 2 to about 50% control. By the 3rd day after crushing the muscle, androgen receptor number further declined to 25% of control values. This decrease was followed by a 4-5 fold increase in receptor number, which attained control values by the 7th day after crush. Receptor number remained stable at the control level through day 30 after crushing. By day 60, however, the number of receptors decreased to 75% control. These results correlated with the morphological development of the regenerating muscle following crushing. It is concluded that there is little, if any, cytosolic androgen receptor present in the early myoblastic stages of regeneration; rather, synthesis of the receptor occurs after the fusion of myoblasts into myotubes and during the differentiation of myotubes into cross-striated muscle fibers. [Supported in part by grants from NIH (NS-15766 and NS-14538) and from NASA (NAG 2-100)].

179.17 PROTEINS OF THE EXTRACELLULAR FLUID IN THE MOUSE BRAIN: CHARACTERIZATION AND MODE OF SECRETION. R. Hofstein* and V.E. Shashoua. Harvard Medical School and Mailman Research Center, McLean Hospital, Belmont, MA 02178.

Recent experiments in our laboratory have demonstrated that increased metabolism of cytoplasmic (CYTO) proteins and their release into the extracellular fluid (ECF) are major steps in a chain of transduction events leading to the processing and storage of newly acquired information. We therefore developed a method for the isolation and characterization of proteins released into the ECF. In brief, balb c mice were ether anesthetized, perfused via the heart with isotonic saline and the brains then dissected out and incubated at 0° in isotonic sucrose solution containing various proteinase inhibitors.

The maximum protein yield obtained was 0.05% of the tissue wet wt. One- and two-dimensional SDS gel electrophoretic patterns of the ECF proteins were obtained and compared to those of the cytoplasmic and serum proteins. Distinct features of the gel patterns characterized ECF fractions as a separate population of brain proteins. That the ECF proteins were not simply due to a nonspecific leakage of cytoplasmic proteins during the period of incubation was established by assays of tyrosine hydroxylase (TH) activity using high pressure liquid chromatography. No TH activity was detected in ECF (<0.01 ng dopa/mg protein/30 min), whereas in CYTO the activity was 100 ng dopa/mg protein/30 min.

The volume of extracellular space as measured by diffusion rate of ^{14}C-inulin into brain tissue at 0° was found to be 20% of tissue wet wt. The protein concentration in ECF was 0.3%, which is considerably higher than the previous values for CSF (0.03%). The extent of ^{14}C-inulin diffusion into mouse brain was confirmed by autoradiographic studies which revealed that the most extensive uptake was into the frontal cerebral cortex posterior to the rhinal fissure.

The pattern of radioactively labeled ECF proteins was determined following i.p. injections of ^{14}C and ^{3}H-valine. Specific protein bands separated by gel electrophoresis were found to be heavily labeled. One of these migrating at 33,000 daltons, P33, was found to increase after the mice were trained in a T-maze. Concurrent studies of cytoplasmic proteins indicated that the observed changes at P33 might be preceded by the labeling of a higher molecular weight cytoplasmic protein which is released into ECF and converted by a series of selective proteolytic steps into P33 protein. These observations suggest that rapidly labeled proteins can be influenced by training and that proteins in ECF might have a functional role as trophic factors in neural plasticity.

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179.18 SCIATIN: IMMUNOCYTOCHEMICAL LOCALIZATION OF A MYOTROPHIC PROTEIN IN CHICKEN NEURAL TISSUES. T. H. Oh, C. A. Sofia*, Y. C. Kim*, C. Carroll*, H. H. Kim*, G. J. Markelonis and P. J. Reier. Dept. of Anatomy, Univ. of Maryland Sch. of Medicine, Baltimore, MD 21201.

A myotrophic protein (sciatin) purified from chicken sciatic nerves has "trophic" or "maintenance" effects on cultured muscle. We have elicited a specific antiserum against sciatin in rabbits. Using this antiserum, we investigated the distribution of sciatin in embryonic and adult chicken tissues by an unlabeled peroxidase-antiperoxidase method at the light microscopic level. Adult chicken tissues were fixed in cold 95% ethanol or 4% paraformaldehyde-0.1% glutaraldehyde in 0.1M cacodylate buffer (pH 7.2) and embedded in paraffin. Dissociated embryonic cells were prepared from 12-day old chick embryos and cultured on collagen-coated plastic dishes. The antiserum stained adult chicken neural tissues in situ and cultured embryonic neurons. Staining was intense in the cell bodies of spinal cord neurons and the axoplasm of sciatic nerves. Cerebral cortical neurons were weakly stained by the antiserum. No staining was apparent in Schwann cells, oligodendrocytes or astrocytes. Non-neural tissues such as skeletal, smooth and cardiac muscle, kidney and liver were also unstained by the antiserum. Cultured spinal cord neurons, cerebral cortical neurons and sensory neurons were stained immunocytochemically by the antiserum. There was no reaction product seen in the glial cells which are usually present in neuronal cultures or cultured cells from liver, kidney, skeletal muscle, smooth muscle and cardiac muscle. Our results thus show that the myotrophic protein is localized in neuronal perikarya and their processes.

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179.19 SCIATIN INCREASES ACETYLCHOLINE RECEPTORS AND MAINTAINS RECEPTOR CLUSTERS IN CULTURED SKELETAL MUSCLE. G.J. Markelonis, M.E. Eldefrawi*, L. Guth and T.H. Oh. Depts. of Anatomy and Pharmacology & Experimental Therapeutics, Univ. Maryland School of Medicine, Baltimore, Maryland, 21201.

Factors present in neural extracts or in media conditioned by neurons have been shown by others to increase both the number of acetylcholine receptors (AChRs) and the number of receptor clusters in cultures of embryonic skeletal muscle. We have shown that the neuronal glycoprotein, sciatin, has pronounced myotrophic effects on developing muscle in vitro, and we therefore added sciatin to aneural cultures of chick skeletal muscle to ascertain sciatin's effects on AChRs. The effects can be summarized as follows: (1) Sciatin caused significant increases in the number of AChRs/culture dish as measured by binding of ^{125}I-α-bungarotoxin (α-Btx) and in acetylcholinesterase (AChE) activity/culture dish in differentiating muscle cells. The increase in AChRs elicited by sciatin was due solely to increased receptor synthesis and incorporation. The rate of AChR synthesis in sciatin-treated cultures was as much as five times the control rate and was significantly reduced by cycloheximide (10 μM). AChR degradation was unaffected by the trophic protein. (2) Although the number of AChRs/culture dish was increased by sciatin during myogenesis, AChR specific activity, expressed as fmoles ^{125}I-α-Btx bound/mg cell protein was only transiently increased by the trophic protein. This contrasted with AChE specific activity in sciatin-treated cultures which remained elevated throughout differentiation. (3) Autoradiographs of ^{125}I-α-Btx-labeled cultures showed that sciatin caused an increase in the number and size of AChR "hot spots" and maintained the integrity of these AChR clusters in aneural muscle cultures for up to 5 weeks. At this time control cultures had completely degenerated. (4) The mechanism by which sciatin enhanced the synthesis of AChRs appeared to be distinct from that of tetrodotoxin (TTX), an agent which abolishes muscle activity, since the effects of sciatin and TTX on AChR synthesis were additive. The results of this study suggest that sciatin may be identical to the diffusible factor(s) from motor neurons described by others which has trophic effects on AChRs. Furthermore, since sciatin produces only a transient increase in AChR specific activity but promotes the maintenance of AChR "hot spots" for prolonged periods in the absence of innervation, this trophic protein may function in vivo as the initial neuronal regulatory signal for directing the metabolic machinery of the target muscle cell.

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179.20 EFFECTS OF NEONATAL 6-HYDROXYDOPAMINE ADMINISTRATION ON CEREBELLAR DEVELOPMENT. KATHRYN L. LOVELL. DEPT. OF PATHOLOGY, MICHIGAN STATE UNIV., E. LANSING, MI 48824.

NOREPINEPHRINE (NE) HAS BEEN PROPOSED TO INFLUENCE CENTRAL NERVOUS SYSTEM DEVELOPMENTAL PROCESSES PRIOR TO AND SEPARATE FROM ITS ACTION AS A NEUROTRANSMITTER. SUCH EFFECTS OF NE ARE LIKELY TO BE EXERTED PRIOR TO DEVELOPMENTAL EVENTS AND POSTNATAL DEPLETION OF NE DOES NOT GENERALLY CAUSE MAJOR DEVELOPMENTAL CHANGES IN THE CEREBRAL HEMISPHERES, WHERE MOST CELL DIFFERENTIATION IS COMPLETED BY THE TIME OF BIRTH. POSTNATALLY DIFFERENTIATING CELL POPULATIONS IN THE CEREBELLUM PROVIDE AN OPPORTUNITY TO EXAMINE EFFECTS OF NEONATAL NE DEPLETION ON NEURONAL PROLIFERATION, DIFFERENTIATION AND MIGRATION. ALTHOUGH THE CEREBELLAR EXTERNAL GERMINAL LAYER (EGL), THE SOURCE OF BASKET, STELLATE AND GRANULE CELLS, IS PRESENT AT BIRTH, MOST OF ITS PROLIFERATIVE ACTIVITY OCCURS DURING THE FIRST 2 POSTNATAL WEEKS. IN ADDITION, A SYSTEM IN WHICH ALL EGL DEVELOPMENT OCCURS POSTNATALLY CAN BE PRODUCED BY METHYLAZOXYMETHANOL (MAM)-INDUCED NEONATAL EGL DESTRUCTION FOLLOWED BY PARTIAL EGL RECONSTITUTION BEGINNING SEVERAL DAYS AFTER BIRTH. THE OBJECTIVE OF THIS STUDY WAS TO CHARACTERIZE ALTERATIONS IN POSTNATAL EGL DEVELOPMENT PRODUCED BY NEONATAL NE DEPLETION IN THE NORMAL MOUSE CEREBELLUM AND THE IN VIVO MODEL SYSTEM PRODUCED BY MAM ADMINISTRATION. REDUCTION IN NE CONTENT WAS ACHIEVED BY INJECTION OF 6-HYDROXYDOPAMINE (6-OHDA; 50 MG/KG, S.C.) ON POSTNATAL DAYS 1, 3 AND 5. THIS DOSE PRODUCED DEPLETION OF CEREBELLAR NE TO 12% OF CONTROL VALUES 8 HR AFTER INJECTION ON DAY 1 AND DEPLETION TO 20-30% OF CONTROL VALUES 24 HR AFTER EACH INJECTION. IN THE FOUR EXPERIMENTAL GROUPS (MAM + 6-OHDA, MAM + VEHICLE, SALINE + 6-OHDA, SALINE + VEHICLE), MORPHOLOGICAL STUDIES WERE CONDUCTED AT 5, 8, 11, 14 AND 21 DAYS OF AGE TO ASSESS CHANGES IN CEREBELLAR SIZE AND FOLIATION PATTERN, IN THE SCHEDULE AND EXTENT OF GERMINAL CELL PROLIFERATION AND IN THE MIGRATION OF BASKET, STELLATE AND GRANULE CELLS. THE CROSS-SECTIONAL AREA OF THE CEREBELLAR VERMIS WAS REDUCED IN THE 6-OHDA + SALINE GROUP COMPARED TO CONTROL ANIMALS, BUT THE REDUCTION IN VERMIS AREA PRODUCED BY MAM WAS NOT ALTERED BY 6-OHDA ADMINISTRATION. IN THE 6-OHDA + SALINE GROUP, NE DEPLETION PRODUCED CHANGES IN THE FOLIATION PATTERN AND ALTERED THE TIME COURSE OF EGL DEVELOPMENT AS MEASURED BY EGL WIDTH IN THE CULMEN AND NODULUS. THE NUMBER OF GRANULE CELL PROFILES IN THE CULMEN WAS REDUCED DUE TO THE 6-OHDA INDUCED DECREASE IN INTERNAL GRANULAR LAYER AREA. THESE STUDIES PROVIDE EVIDENCE TO SUPPORT A ROLE OF NE IN REGULATION OF NEURONAL DEVELOPMENT IN THE CEREBELLUM. (THIS RESEARCH WAS SUPPORTED BY BRSG FUNDING FROM THE COLLEGE OF OSTEOPATHIC MEDICINE, MICH. STATE UNIV.)

179.21 NERVE GROWTH FACTOR (NGF) AND EPIDERMAL GROWTH FACTOR (EGF) ARE BOUND BY THE SAME PC12 PHEOCHROMOCYTOMA CELL: VISUALIZATION BY DOUBLE-ANTIGEN IMMUNOFLUORESCENCE. Leonid Pevzner*, David End*, Stanley Vinores*, and Gordon Guroff. Section on Intermediary Metabolism, Laboratory of Developmental Neurobiology, National Institute of Child Health and Human Development, NIH, Bethesda, MD 20205.

A rat pheochromocytoma cell line, PC12, which has been shown in this laboratory to contain both NGF and EGF receptors was further investigated to determine if both NGF and EGF receptors could be found on the same cell. PC12 cells, growing in tissue culture flasks or on collagen-coated cover slips, were incubated with saturating concentrations (350-500 ng/ml) of NGF and EGF for 45 min at 0°C. After fixation, dilutions of sheep anti-NGF sera and rabbit anti-EGF sera were added and after a 30-40 minute incubation at room temperature, fluorescein-conjugated rabbit anti-sheep $F(ab')_2$ and rhodamine-conjugated sheep anti-rabbit $F(ab')_2$ were added.

Fluorescence microscopy revealed a green fluorescence of PC12 cells due to the NGF binding and a red fluorescence due to the EGF binding. By changing fluorescein and rhodamine filters while observing the same field it was evident that each PC12 cell possessed both kinds of the fluorescence. No background fluorescence was seen with high dilutions of the antisera when the growth factors or specific antisera were omitted.

The double fluorescence observed was similar whether the growth factors and their corresponding antisera were added successively or simultaneously. There was no cross-reaction fluorescence between NGF and anti-EGF sera or between EGF and anti-NGF sera.

The NR11 subclone of PC12 cells, isolated by Bothwell et al., (Cell, 21, 857, 1980), which does not have NGF receptors, was characterized under the same conditions of double-antigen binding experiment by a red EGF-binding fluorescence alone. No green fluorescence was observed. Absence of the NGF binding to these cells was confirmed by the more sensitive peroxidase-antiperoxidase method of Sternberger (L.A. Steinberger, Immunocytochemistry, 2nd edition, John Wiley, New York, 1979).

Red fluorescence due to the EGF binding to the PC12 cells was substantially reduced when an agent blocking the EGF receptors, TPA (200 ng/ml), was added simultaneously with EGF. Red fluorescence was also markedly reduced when cells were grown for 72 hours in a medium containing NGF (50 ng/ml), a condition shown in this laboratory to result in a profound decrease in EGF receptors.

180.1 INTRAMUSCULAR "COMPARTMENTALIZATION" OF THE CAT BICEPS FEMORIS AND SEMITENDINOSUS MUSCLES: ANATOMY AND EMG PATTERNS. A.W.English and W.D. Letbetter, Emory University, Atlanta, GA.

The anatomy and innervation patterns of cat biceps femoris (BF) and semitendinosus (ST) muscles were examined to see if they were organized about the same principles of compartmentalization found in the calf muscles. On the basis of gross fiber architecture and nerve supply, a distinct posterior head of BF (BFp) can be identified while the remainder of the muscle appears to be divided into an anterior (BFa) and a middle component (BFm). Glycogen depletion, through stimulation of primary muscle nerve branches, confirmed this compartmentalization scheme for BF, although the organization of BFm may be somewhat more complex than that of BFa and BFp. BFa and BFm insert into an aponeurosis attached at the femur. BFp inserts into an aponeurosis attaching laterally to the leg and forming a crural fascial hood which extends as far distad as the ankle. ST consists of two heads arranged in-series about a tendonous inscription. Each head is supplied by a separate branch of the ST muscle nerve. By glycogen depletion techniques, each of the proximal (STp) and distal (STd) heads was shown to contain distinct populations of muscle fibers. STd's aponeurosis of insertion is attached to the tibia, but mainly forms the medial part of the crural fascial hood. To examine the possible functional roles of these compartments, EMG recordings were made from surgically implanted electrodes and synchronized with stepping movements using high speed (200fps) cinematography. Although all EMG records showed substantial variability in onset, duration and intensity of activity (i.e., relative to individual step cycles), some patterns can be recognized. BFa tended to be active during the stance phase, sometimes early (during E^2 or the E^1-E^2 boundary) but most often late (E^3). BFp activity occurred mainly during the mid to late swing phase (F-E^1 boundary) but activity was also recorded during the early stance phase (E^2), or at both times. BFm was generally active in phase with BFa, although late swing phase activity was sometimes also present. STd's activity was similar to BFp's, but more strongly biphasic. STp showed only weak activity, in phase with STd, except at high speeds of stepping. It is concluded that the differences in patterns of EMG activity in the in-parallel components of BF may reflect mechanical differences in their roles as hip extensors, knee flexors or crural fascial tensors. The intramuscular localization of monosynaptic group I inputs to motor neurons supplying these compartments (Botterman et al., Neurosci. Lett. in press) may thus serve to reinforce these separate mechanical linkages. The differences in EMG patterns for STp and STd may reflect its peculiar, in-series linkage and its role (along with BFp) in tensing the crural fascia. (Supported by AM19916 and NS15452 from the USPHS).

180.2 THE RELATIONSHIP BETWEEN PERIPHERAL INTRAMUSCULAR "COMPARTMENTS" AND SPATIAL ARRANGEMENT OF BICEPS FEMORIS AND SEMITENDINOSUS MOTOR NUCLEI IN THE CAT LUMBAR SPINAL CORD. William D. Letbetter and Arthur W. English, Department of Anatomy, Emory University, Atlanta, GA 30322.

Retrograde transport of horseradish peroxidase (HRP) was used to label motoneurons belonging to the biceps femoris (BF) and the semitendinosus (ST) motor nuclei in cats. One of the whole muscle nerves was soaked in a 10% HRP-1.6 mg% hyaluronidase solution in the ipsilateral hindlimb while one of the distinct intramuscular nerve branches supplying either the anterior (BFa), middle (BFm), or posterior (BFp) head of BF muscle, or the proximal (STp) or distal (STd) head of ST muscle, was soaked in the contralateral limb. After a 3-day survival period, spinal cord segments L6-S1 were serially sectioned at 20 μm so that a detailed analysis of the spatial organization of labeled motor nuclei could be made from 3-dimensional serial reconstructions.

BF motoneurons were distributed over longitudinal distances of 12-14 mm within spinal segments S1, L7, and L6. Those supplying BFp were located in the caudal 65% of the BF distribution range (95% of which were restricted to the caudal 50% of the BF range), while those supplying BFa were located in the rostral 85% of the BF distribution range (but 95% of which were restricted to the rostral 70% of the BF range). BFm cells were located in the 55% of BF's distribution range beginning 15% of the way from the caudal end and ending 30% of the way from the rostral end. Thus, a very prominent topographical relationship exists between the central location of motoneuronal cell bodies and the intramuscular compartments of BF to which these cells supply axons. The 1100 BF motoneurons are distributed as follows: approximately 50% to BFa, 20% to BFm, and 30% to BFp. The STp portion of ST muscle is supplied by neurons in the rostral 85% of the 12 mm of spinal cord segments S1 and L7 giving rise to ST motoneurons, leaving the distal portion (STd) of ST muscle to be supplied by the remainder. However, each of these two subpopulations of motoneurons is approximately symmetrically distributed rostrocaudally so that there is extensive overlap among the motor cells supplying this hamstring synergist.

Thus, with regard to motor nucleus organizational principles, it would appear that there is an anatomical correlate which could underlie at least the topographical (or "location") portion of the argument pertaining to intramuscular reflex organization of the monosynaptic reflex (Botterman et al., Neurosci. Let. in press).

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180.3 REFLEX PARTITIONING IN THE MOTOR NUCLEUS SUPPLYING THE CAT BICEPS FEMORIS MUSCLE. T.M. Hamm, B.R. Botterman, R.M. Reinking* and D.G. Stuart. Dept. of Physiology, Univ. of Arizona, Coll. of Med., Tucson, AZ 85724

This report describes physiological experiments which demonstrate the existence of an intramuscular localization of monosynaptic Ia reflex effects in a cat hindlimb muscle.

Intracellular recordings from biceps femoris (BF) motoneurons were made in anesthetized low spinal cats during periods of electrical stimulation of the nerve branches supplying the anterior (AB), middle (MB) and posterior (PB) portions of the BF muscle. Recordings were also made during stimulation of nerves to semimembranosus (SM) and semitendinosus (ST) in order to provide a means of categorizing MB cells as "extensors" (MBe; i.e., like AB cells) or as "flexors" (MBf; like PB). Measurements of intrahomonymous (i.e., from AB, MB or PB) composite monosynaptic Ia-EPSPs revealed that in three of four comparisons (AB nerve onto AB and MBe cells, MB onto MBf and PB, PB onto MBf and PB) the AB, MB and PB nerve branches contributed larger EPSPs to their "own" motoneurons than to motoneurons supplying other "compartments" of the muscle. In the fourth case, MB input appeared to have similar effects onto AB and MBe cells. A normalization was performed to eliminate the possibility that the differences in EPSP sizes were due to differences in cell type within the four cell groupings (i.e., differences in the number of cells supplying FF, FI, FR and S muscle units). This normalization confirmed the presence of localization in the first three comparisons and, in addition, suggested that MB may indeed have greater effects on MBe than AB cells. In addition to the asymmetrical effects of AB and MB nerves onto AB and MBe motoneurons, it was shown that while ST and PB contributed larger EPSPs to MBf than to MBe cells, the AB and SM nerves contributed equally to the two MB groups. By analysis of cell location in the spinal cord, pair-wise testing of neighboring motoneurons of different category, and rostro-caudal differences in group I volley sizes and Ia afferent distributions, it is argued that somatotopic factors contribute importantly to the observed localization. However, neuronal recognition factors were also evident.

Supported by U.S.P.H.S. grants NS 07888, HL 07249, RR 94575, NS 05871 and NS 06462. Present address of B.R. Botterman: Dept. of Cell Biology, Univ. of Texas Health Sciences Center, Dallas, TX 75235.

180.4 INTRAMUSCULAR REFLEX LOCALIZATION IN RELATION TO MUSCLE DESIGN AND ACTION. B.R. Botterman, T.M. Hamm, R.M. Reinking* and D.G. Stuart. Dept. of Physiology, Univ. of Arizona College of Medicine, Tucson, AZ 85724.

By measurement of composite Ia-EPSPs, it has been shown that there is an intramuscular localization of monosynaptic Ia reflex effects in the unifunctional (hip extensor and knee flexor) components of the cat biceps femoris (BF) muscle (Botterman et al., Neuroscience Letters, In Press) but, as measured by single Ia-fiber EPSPs, not in the fellow hamstring muscle, semitendinosus (ST) (Nelson and Mendell, J. Neurophysiol. 41: 778-787, 1978). This report summarizes a composite Ia-EPSP study (152 motoneurons/12 experiments) that confirms the ST result. In addition, the atypical gross architecture (at least for a hindlimb muscle) of ST, with a proximal compartment (STp) in series with a distal compartment (STd), is emphasized by showing that spindles are unloaded by twitch of their "own" compartment but loaded by twitch of the in-series compartment. Conversely, tendon organs are loaded by twitch of both their own and the in-series compartment. It is argued that intramuscular reflex localization within ST would be mechanically unsound; in contrast to that in BF, whose fibers more typically extend largely in parallel from aponeurosis of origin to insertion. An important similarity between BF and ST is that the fibers of the anterior (AB) portion of BF and of STp are more proximally situated than the fibers of the middle (MB) and posterior (PB) portion of BF and of STd, respectively. An organization corresponding to this peripheral somatotopicity is present in the spinal motor nucleus of BF but not that of ST. As based on locations of cells identified antidromically during intracellular recording, the AB, MB and PB cells all overlap to some extent but the AB and PB population are concentrated in the rostral and caudal ends of the spinal motor nucleus, respectively, while the MB cells are concentrated toward the middle. In sharp contrast, the ST cells supplying STp and STd are co-extensive in the spinal cord.

These results suggest that somatotopic factors contribute to the localized Ia effects observed in BF and that the lack of such factors is a neuronal specialization that helps prevent the appearance of similar localization in ST.

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

180.5 LACK OF SYNCHRONY BETWEEN MUSCLE RECEPTOR AFFERENT SPIKE TRAINS IN THE PASSIVE MUSCLE AT FIXED LENGTH: SIGNIFICANCE FOR INTERPRETATION OF SPIKE-TRIGGERED AVERAGING EXPERIMENTS. R.M. Reinking*, D.D. Roscoe, T.M. Hamm, B.R. Botterman, W.E. Cameron and D.G. Stuart. (SPON: W.A. Sibley). Dept. of Physiology, Univ. of Arizona, Coll. of Medicine, Tucson, AZ 85724.

Spike-triggered averaging (STA) experiments with the test muscle passive and at fixed length have revealed a monosynaptic excitatory spindle group II connection with homonymous and heteronymous motoneurons. This report describes a method which tests for the possibility that such an averaged EPSP is attributable to the impulses of a Ia afferent fiber, firing in synchrony with a reference spindle group II spike.

Spindle afferent discharge was analyzed in nine deeply anesthetized cats. In six cats, the nerve to medial gastrocnemius (MG) was intact (whole nerve experiments). In the remaining three cats, only a single intramuscular nerve branch (Letbetter, Anat. Rec. 178: p402, 1974) was left in-continuity with the spinal cord (nerve branch experiments). Simultaneous recordings for a 60-120 sec epoch were made of the discharge of 1-4 muscle receptor afferents and of the neurogram signal from the MG nerve or one of its branches. The MG muscle was de-efferented and held at fixed length under moderate (100g) tension. In two cats, the muscle was subjected to brief stretches that evoked a synchronous volley of Ia afferent discharge. A quantitative synchronization index (SI) of synchrony between each recorded afferent and other afferent signals in the neurogram was computed by comparing averages of rectified and unrectified versions of the neurogram (Roscoe, Ph.D. thesis, 1980). The SI and PPSTH tests revealed synchronized muscle receptor discharge during brief stretches of particularly small amplitude (20μm). In contrast there was no evidence of any synchronization between spindle group II and other afferents in the passive muscle at fixed length even when the recording epochs were extended well beyond those used in previous STA studies to establish the monosynaptic spindle group II connection. The results confirm the validity of this connection with homonymous and synergistic motoneurons, at least in experiments that featured use of closely comparable test muscle conditions (e.g., Stauffer et al., 1976; Lüscher et al., 1979; Munson et al., 1980; Sypert et al., 1980).

Supported by U.S.P.H.S. grants NS 07888, HL 07249, RR 05675, NS 05871 and NS 06462. Present addresses: D.D. Roscoe, Dept. of Orthopedics-REC, Cleveland Metro General Hospital, Cleveland, OH 44109; B.R. Botterman, Dept. of Cell Biology, Univ. of Texas Health Sciences Center, Dallas, TX 75235; W.E. Cameron, Dept. of Physiology and Biophysics, Univ. of Washington School of Medicine, Seattle, WA 98195.

180.6 "SENSORY PARTITIONING" OF CAT TRICEPS SURAE MUSCLES BY TENDON ORGANS. D.G. Stuart, T.M. Hamm, D.D. Roscoe, B.R. Botterman, and R.M. Reinking*. Dept. of Physiology, Univ. of Arizona, College of Medicine, Tucson, AZ 85724.

This report summarizes data on Ib afferent behavior in both "passive" and "active" muscles with an emphasis on the detailed description of Ib firing patterns during spontaneous motor activity in the unanesthetized decerebrate cat. For passive muscles, data from MG (Cameron, Ph.D. thesis, 1979) and SOL (Stauffer, Ph.D. thesis, 1974) reveal that force sensitivity values (pps/g) progressively increase when based on responses to contraction of the whole muscle, an intramuscular compartment, and single motor units. This finding offers compelling indirect evidence that tendon organs respond to intramuscular forces directly coupled to them rather than to whole muscle force recorded at the tendon. Similarly, we have found Ib firing patterns in active muscles that are: 1) a discontinuous function of whole muscle force (Fig. 1); and, 2) not synchronized to the discharge of other afferents. However, such firing patterns give a reasonably representative picture of total muscle force as if to suggest that localized intramuscular forces and single Ib spike trains are a relatively but not fully representative version of the muscle's total force output.

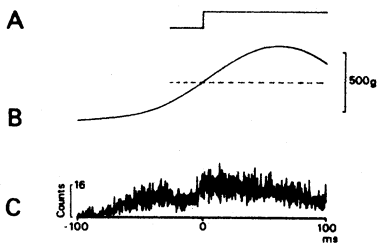

Fig. 1. Pre- and post-"stimulus" time histogram of a Ib afferent's discharge (PPSTH) during rhythmic MG activity (Roscoe, Ph.D. thesis, 1980). The Ib spike train was recorded while MG was undergoing spontaneously rhythmic (approx. 2/sec) contractions (>500g). A shows a force gate pulse enabled when the force signal exceeded 430 g (broken horizontal line). B shows averaged force profile (2048 sweeps) of MG triggered by A. C shows a PPSTH (200μ sec bin width, 1000 samples) of Ib discharge again with A as the reference event.

Supported by USPHS grants NS 07888, HL 07249, RR 05675, NS 05871 and NS 06462. Present addresses: D.D. Roscoe, Dept. of Orthopedics-REC, Cleveland Metro General Hospital, Cleveland, OH 44109; B.R. Botterman, Dept. of Cell Biology, Univ. of Texas Health Sciences Center, Dallas, TX 75235.

180.7 IDENTIFICATION OF THE HUMAN ANKLE STIFFNESS TRANSFER FUNCTION. R.E. Kearney and I.W. Hunter, Biomedical Engineering Unit, McGill University, Montreal, Quebec, Canada H3G 1Y6.

The dynamic relationship between joint position and torque (stiffness) or its inverse (compliance) is an important constraint on the motor control system since it will ultimately limit the performance that can be attained. The objective of the present study was to identify the stiffness transfer function of the human ankle using the techniques of engineering systems analysis.

A high-performance, servo-controlled, electro-hydraulic actuator was used to drive ankle position according to a computer-generated, repeated, stochastic pattern. Ankle position and the associated torque were sampled at 200 Hz for periods of up to 90 s. The resulting position input was found to have significant power in the range 0-50 Hz. Subjects were instructed not to oppose the displacement but were required to maintain a tonic contraction of either tibialis anterior or triceps surae at a level ranging from 0 to 20% of the maximum voluntary contraction.

Position and torque records were first ensemble averaged over 10-15 stimulus repetitions to reduce noise. Subsequently, the portion of the torque record due to the actuator dynamics was subtracted to leave only the component generated by the subject's ankle. The dynamic relationship between ankle position and ankle torque was then studied using both frequency and time domain analysis techniques.

The coherence between ankle position and torque was found to be greater than 0.9 over the range 0.2 to 50 Hz indicating that the dynamic relationship between the two variables is nearly linear. The stiffness gain curves were relatively flat from DC to about 20 Hz and thereafter rose at 40 db/decade. However, at intermediate frequencies (5-15 Hz) there were systematic, significant departures from the behaviour expected of a second-order system. Consequently, a more complex model is required to describe the observed behaviour. It is possible that this complexity arises from the interaction between torque generated by passive joint properties and that due to reflex activity. If so, the reflex contribution could serve to extend the range of frequencies over which ankle stiffness remains approximately constant thus simplifying the control task faced by more central motor structures.

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180.8 COMPARISON OF EMG RESPONSES TO JOINT DISPLACEMENT IN HUMAN ANKLE FLEXORS AND EXTENSORS. C.W.Y. Chan and R.E. Kearney, School of Physical & Occupational Therapy, Biomedical Engineering & Aviation Medical Research Units, McGill University, Montreal, Quebec, Canada, H3G 1Y5

Evidence that reflex mechanisms are organized differently in forelimb flexors than in extensors (Lenz, et al., Canad. Physiol., 11 (1980): 98) motivated us to carry out a comparative study of reflex responses to joint displacement of human ankle flexors and extensors.

Subjects lay supine with their left foot attached to the pedal of an electro-hydraulic actuator. They were instructed not to oppose perturbations of ankle positions but were required to maintain a tonic contraction of either the ankle flexors (tibialis anterior, TA) or extensors (triceps surae, TS). Ramp displacements of various amplitude and velocity were applied in random order while the joint position and smoothed, rectified surface EMGs from the ankle muscles were recorded. The data were subsequently sorted and ensemble averaged prior to analysis.

The results revealed 5 principal differences in the behaviour of ankle flexor and extensor muscles as detailed below:

1. The pattern of the TS response was typically a short synchronous burst of activity starting at about 40 ms and lasting no more than 30 ms. In contrast, the TA response (at similar latency) lasted much longer and frequently could be resolved into two distinct components.
2. The threshold of the TA response was much higher than that of TS.
3. Increasing displacement velocity increased the magnitude of the response in TS as well as the early component of the response in TA but decreased the late TA component.
4. Increasing the level of tonic activity augmented the magnitude of the TS response initially, but saturation soon occurred. In contrast, both components of the TA response increased with increasing tonic TA activity.
5. Finally, shortening of the tonically active TS evoked no consistent response, whereas shortening of the active TA evoked a significant pause in its activity.

The contrasting behaviour of the ankle flexors and extensors appears to indicate that there are significant differences in their reflex organizations. These findings are consistent with the hypothesis that muscle spindles in the flexor muscles are subject to a greater static fusimotor drive than are their counterparts in the extensors.

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180.9 TREMOR ASSOCIATED WITH RELAXATION-BREATHING PROCEDURE. R. S. Pozos, L. E. Wittmers*, and N. Nathan*. Dept. of Physiol., Sch. of Med., Univ. of Minnesota, Duluth, MN 55812.

Overt physiologic tremors are usually associated with fatigue induced states or cold exposure--shiver. Presently a system for relaxing subjects using a modified breathing maneuver has been used to cause an overt tremor in normal subjects. It became of interest to investigate if the tremor seen during the relaxing maneuver was similar in frequency to that reported for tremor induced by fatigue and shiver.

Subjects laid on their back and surface electrodes were placed on the following muscles: adductor longus, extensor digitorum, and the frontalis. To follow the respiratory function, the following parameters were monitored: 1) expired O_2, 2) expired CO_2, 3) minute ventilation, and 4) chest movement.

The surface EMGs were initially full wave rectified and demodulated and analyzed using traditional autocorrelation and power spectral techniques. During initial breathing, there was the presence of demodulated EMGs seen in the thighs. Eventually as the process continued there was tremor in the thighs, forearms, and forehead. This occurred early on in the experiment, usually within 5 minutes. The frequency for the thigh tremor was in the 5-7 Hz range and the forehead tremor was in the 7-8 Hz range. During the relaxing maneuver, there is an increase in minute ventilation paralleled by a decrease in end expiratory CO_2 and an increase in end expiratory O_2. The minute ventilation increased early on and returned to normal value during the relaxing procedure. The onset of the overt tremor seems to occur at the maximal increase in minute ventilation; however, this tremor continues even when minute ventilation returns toward normal levels. Interestingly, this tremor pattern of thigh, arm, and forehead seems somewhat different than that observed during shiver.

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180.10 RECIPROCAL EXCITATION: OBSERVATIONS IN NORMAL ADULTS, INFANTS, AND CEREBRAL PALSY PATIENTS. Barbara M. Myklebust, Gerald L. Gottlieb, Gyan C. Agarwal, Bernice Kaufman*, James Poyezdala*, Laura Baldwin*, Dennis Vaccaro*, Richard Penn. Dept. Neurosurg. and Physiol., Rush Med. Coll., Chicago, IL 60612.

In an earlier paper [1], we contrasted the amount of simultaneous EMG activity that could be evoked in muscle antagonists by stretch stimuli in normal and CNS injured patients. Such activity we have called "reciprocal excitation" [2]. Reciprocal excitation is caused by an abnormality of the segmental neuronal circuitry. Its presence may be a sign of abnormal growth of synaptic connections in the presence of supraspinal pathology or it may be sign of maturational failure.

Surface EMG activity from tibialis anterior and gastrocnemius-soleus muscles was assessed with tibial nerve stimulation (H-reflexes), quick stretches applied to dorsiflex the ankle, and tendon taps applied to the soleus tendon in normal subjects, from infancy to adulthood, and patients with spasticity.

Preliminary data shows a quantitative difference in the amount of simultaneous reciprocal activity evoked in soleus and tibialis anterior muscles following these stimuli between different classes of subjects. According to these measures, it may be possible to differentiate between normal infants, normal adults and patients (aged greater than 7 years) with cerebral palsy.

[1] Myklebust, B.M.; Penn, R.D.; Gottlieb, G.L.; Agarwal, G.C.: Abnormal spinal reflexes contrasted in cerebral palsy and adult CNS injuries. Society for Neuroscience 9th Ann. Mtg., p.380, abs.#1276, 1979.

[2] Gottlieb, G.L. and Agarwal, G.A.: "Load Compensating Reactions in Normal Man and in Patients with Cerebral Palsy." In: Motor Control in Man: Mechanisms and Clinical Applications, Desmedt, J.E., Ed. New York, Raven Press, in press.

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180.11 TORQUE-ANGLE RELATIONSHIPS AT THE HUMAN THUMB INTERPHALANGEAL JOINT. PATRICK E. CRAGO AND KEN SUTIN* Rehabilitation Engineering Center, Case Western Reserve University, Cleveland, Ohio 44106.

Joint rotation in response to a torque disturbance is determined by the mechanical properties of the muscle fibers recruited prior to the disturbance and the reflexively induced change in muscle activation. Both are affected by other fixed parameters such as the initial torque and angle, and the disturbance time course and amplitude. We are cataloging these dependencies in order to provide a basis for quantitative evaluation of normal and abnormal motor function.

Torque-angle relationships were measured at the interphalangeal joint of the thumb of normal human subjects. The subjects established the initial torque and angle conditions by flexing their thumb against a load provided by a torque servo motor. Torque and angle at the interphalangeal joint, and EMG of the flexor pollicus longus were measured continuously before, during and after step increases or decreases in torque. Subjects were instructed not to intervene voluntarily to correct for changes in position caused by the disturbance (Crago, Houk, and Hasan, 1976, J. Neurophysiol.,39, 925-935). Any response with an obvious voluntary component was excluded from this analysis.

The change in angle elicited by a step change in torque was also steplike, with the majority of change taking place before any reflex response could be detected in the EMG. There were small position variations in the period between 50 and 250 ms, after which both the EMG and angle remained steady.

For fixed values of initial torque and angle, the magnitude of the steady-state angular change increased more than proportionally with the magnitude of the torque disturbance. Thus, the incremental stiffness (defined as the amplitude of the disturbance divided by the change in angle) decreases as the amplitude of the disturbance increased. The greatest effects were seen with angular changes less than one degree.

Steady-state stiffness was measured as initial torque was varied with a constant amplitude disturbance. For torques less than approximately 15 N-CM, stiffness was nearly proportional to initial torque. For larger torques, stiffness increased less than proportionally.

A data base including the experiments described above is being established to assess the normal range of responses to load change. This data base will allow the evaluation of motor-servo theories such as stiffness regulation and evaluation of stretch reflex function in patients with movement disorders.

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180.12 A COMPARISON BETWEEN LOAD COMPENSATING REACTIONS AT THE WRIST AND ANKLE IN HUMANS. G.C. Agarwal, G.L. Gottlieb, and R.J. Jaeger. Dept. of Physiology, Rush Medical College, Chicago IL 60612, and Bioengineering Program, Univ. of Ill., Chicago IL 60680.

The large data base concerning load compensating responses from many muscles and paradigms has made it difficult to formulate a general classification scheme for these responses. Most classification schemes are based on latency alone. We have performed nearly identical studies at the wrist and the ankle in the same group of subjects.

Step torque perturbations, randomized in amplitude, were applied to flex and extend the wrists and ankles of normal human subjects. The effects of various instructions, torque directions, and resting muscle tensions (bias) were investigated. Subjects were asked to: 1) restore the joint to its starting position, 2) overcome and reverse the torque with a maximal response, 3) assist the torque, and 4) do not react. Subjects knew the direction of torque would be the same (simple) or unpredictable (choice).

The EMG activity was divided into four intervals: myotatic, late myotatic, postmyotatic, and stabilizing response. Our myotatic and late myotatic responses are equivalent to the M1 and M2-M3 respectively of Lee and Tatton (Can. J. Neurol. Sci. 2:285, 1975). Responses showed similar patterns in wrist flexor and extensors and the ankle flexor (tibialis anterior). The ankle extensor (soleus) lacked the late myotatic response. The myotatic and late myotatic responses were more dependent on bias than on instruction; the reverse was true for the postmyotatic and stabilizing responses. The postmyotatic response did not exhibit a latency difference between simple and choice situations and exhibited a proportional response when a maximal response was requested.

Each interval appears to have its own set of functional properties, and these may be more suitable classification criteria than latency alone. In a companion abstract, evidence is presented that each of these load compensating responses may depend on different afferent information.

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180.13 THE EFFECT OF CORTICAL LESIONS ON REFLEX REPONSES TO TORQUE PERTURBATIONS IN THE SQUIRREL MONKEY FORELIMB. Lenz, F.A.,* Tasker, R.R.,* Tatton, W.G., (Spon: H.C. Kwan). Playfair Neuroscience Unit, University of Toronto, Toronto, Canada.

Previous work has demonstrated that in the relaxed limb the EMG response of Flexor Digitorum Profundus (FDP) to imposed joint displacement consists of a medium latencey (M2) component without a short latencey (M1) component equivalent to that seen in Flexor Carpi Ulnaris (FCU) and Short Head of Biceps (SHB) (Lenz et al, The Pysiologist 22:23, 1980). The purpose of this study was to examine the effect of motor cortical lesions on reflex activity in these different muscles.

Forelimb motor cortex was delineated by: 1) surface anodal stimulation at less than 400 uA under combined light halothane/ N02 general and local anesthetic 2) comparison with cytoarchitectonic maps for this species. This area was ablated by subpial aspiration. Postoperatively, animals had pronounced weakness of the forelimb contralateral to the ablation. The weakness resolved within one month but the animals had not regained normal manipulative ability in the hand over a period of up to four months. Graded torque loads were applied about the metacarpo-phalangeal, wrist and elbow joints of these animals. The resulting EMG response in FDP, FCU, and SHB was digitized, rectified, sorted by baseline EMG level and initial velocity of displacement and finally averaged. After some trials the animals were sedated with Ketamine and the M response to supramaximal nerve stimulation determined for the EMG electrode location used in the individual reflex study. In these cases, the M2 response (normalized to our measure of maximal motoneuron output, the M response) was plotted against initial velocity as an indication of reflex "gain". These results were compared with normals and the contralateral limb.

M2 responses contralateral to the lesion were decreased or absent in FDP of all animals studied. Reflex "gain" was decreased and threshold was increased at a series of increasing baseline EMG levels. These responses did not reach normal levels even at the highest baseline EMG levels examined. No consistent difference from normals was found in FCU or SHB.

The results in finger flexors can be most easily explained either by the interruption of a reflex pathway traversing forelimb motor cortex or by the loss of descending modulation of segmental reflex activity. In any case, the results indicate that the central and/or peripheral mechanism generating the M2 response in the FDP differs from those in FCU and SHB in terms of cortical dependency.

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180.14 RELATION OF VOLUNTARY LIMB ACCELERATION TO INCREASES IN LONG LATENCY STRETCH RESPONSES PRIOR TO MOVEMENT. James A. Mortimer & Thomas G. Dukich* Geriat.Res.Educ.Clin.Ctr., V.A. Med. Ctr., Minneapolis, MN 55417 & Dept. Neurol., Univ. MN, Minneapolis, MN.

Prior to the initiation of voluntary EMG associated with rapid flexion movements of the forearm, there is a large, transient increase in the amplitude of long latency (50-100 ms) stretch responses of the agonist muscle (Mortimer et al., Soc. Neurosci. Abstr., 1979). One function that such an increase may serve is to provide enhanced facilitation to α-motoneurons during the initial phase of movement when limb inertia must be overcome. To examine this possibility, the relationship between the increase in gain of long latency pathways and the peak velocity and acceleration of the subsequent movement was investigated in 18 normal subjects.

The experimental technique has been described previously (see above ref.). Briefly, subjects were asked to respond to a tone by flexing their forearm at maximum velocity from an initial position of 90° flexion. Two Nm torque pulses of 500 ms duration stretching the biceps were presented at 8 different time intervals relative to tone onset. For each interval 20 torque pulses were given, and the tone was presented without torque perturbation on 20 trials. Biceps and triceps EMG responses corresponding to the 9 modes of stimulus presentation were sorted and computer-averaged with torque and position. The average EMG response to the tone in the absence of torque pulses was subtracted from the average EMG response for each time interval to obtain the response to the torque pulse at that delay. Motor response indices were calculated from integrals of the rectified EMG waveforms from 50 to 75 ms (M2) and 50 to 100 ms (M2-M3) following the onset of the torque pulse. Velocity and acceleration were obtained by computer differentiation of position.

For the 18 subjects, the mean interval between the initiation of voluntary motor activity and the time of peak acceleration was 140 ms, which allows sufficient time for fusimotor-induced discharge of spindle afferents to be conducted through long latency pathways and influence motor discharge prior to peak acceleration. To assess the association between the gain of long latency pathways and movement parameters, the peak values of the motor response indices prior to the time of maximum acceleration were divided by the values of these indices before tone onset. The ratios obtained in this manner were found to be significantly correlated with both peak velocity and peak acceleration.

The results of this study are consistent with the transient increase in long latency stretch responses before rapid movements providing increased excitatory input to α-motoneurons at a time when a rapid increase in EMG is needed to overcome inertia.

180.15 LONG-TERM STABILITY AND SHORT-TERM VARIABILITY OF THE PRIMATE M1 RESPONSE. V. A. Kieffer*, J. R. Wolpaw, D. J. Braitman and M. G. Sanders* (SPON: D. R. Livengood). Dept Physiol, Armed Forces Radiobiology Res Inst, Bethesda MD 20014; Div Labs Res, NYS Dept Health, Albany NY 12201.

In a study to determine if monkeys can control the amplitude of the largely monosynaptic M1 component of the muscle stretch reflex (Wolpaw et al., this vol), we measured M1 amplitude for continued periods of up to 5 mos. Rhesus monkeys were trained to maintain elbow angle at 90° (±1.5°) against steady extension force. If correct angle was held for a randomly selected 1-2s period, and if the average absolute value of biceps EMG (from chronic intramuscular electrodes) for the final 0.5s was 1.0-1.5 X a preset value, a 20ms pulse of additional extension force occurred. This stimulus extended the elbow 3-4°. M1 amplitude was the average absolute value of biceps EMG 12.5-21.5ms after stimulus onset minus pre-stimulus EMG amplitude. Under the Control condition, reward occurred 70ms after stimulus onset. Under the M1↑ or M1↓ condition, reward occurred only if M1 was greater (M1↑) or less (M1↓) than a criterion value. Each monkey normally completed 2500-6000 trials daily. Electrodes, pre-stimulus EMG, steady extension force, stimulus amplitude, and stimulus-induced movement were stable throughout.

Control condition data were obtained from six animals over 1-7 wks. For all six, average M1 amplitudes over the control periods were 120-150% of the pre-stimulus EMG amplitudes. No steady increases or decreases in M1 amplitude occurred during the control periods. For a given monkey, the standard deviation of daily M1 amplitudes was 10-15% of the average M1 amplitude for the entire control period.

When a monkey increased M1 amplitude under the impetus of the M1↑ condition, a marked diurnal variation of up to 50% in M1 amplitude often became apparent. M1 was typically greatest around midnight and least around noon, even though monkeys worked in constant light. This 24-hr rhythm was superimposed on the steady rise in M1 amplitude that occurred over 1-2 weeks.

The results indicate that in awake monkeys with constant muscle length and constant alpha motorneuron tone as measured by pre-stimulus EMG amplitude: (1) M1 amplitude remains stable over months under the Control condition, although day-to-day variations do occur, and (2) under the M1↑ condition, a marked 24-hr rhythmic change in M1 amplitude may be superimposed on the long-term progressive increase in M1 amplitude. Possible causes of the observed changes in M1 amplitude include change in gamma motorneuron tone, humoral effects on muscle spindle sensitivity, change in alpha motorneuron recruitment, and change in Ia synaptic function.

180.16 MODULATION OF STRETCH REFLEXES DURING LOCOMOTION IN THE DECEREBRATE CAT. J.W. Aldridge, R.B. Stein, K. Akazawa* and J.D. Steeves. Dept. of Physiology, Univ. of Alta., Edmonton, Alberta, Canada. T6G 2H7.

Environmental inputs that result in a stretch reflex could either assist or disturb locomotion, depending on the phase of the step cycle. Thus, the central pattern generator for locomotion may also control reflex pathways to provide adaptive control. This possibility was studied using the premamillary decerebrate cat. The left hindlimb of the cat was denervated, except for the nerves to the soleus muscle, and rigidly fixed. Locomotion was induced by stimulating the mesencephalic locomotor region with a constant current (50-200 μA) at 30 Hz and 0.5 ms duration. Brief stretches (10-100 ms) of about 1 mm were applied to the muscle at all phases of the step cycle. Isometric tension, soleus EMG and soleus nerve activity were recorded. In most experiments H-reflexes elicited by shocks to the sciatic nerve were also studied. Stimulation levels were monitored by using the EMG and nerve recordings. The levels used produced a constant afferent volley below that which produced M-waves. In a later computer analysis each step was divided into 14 periods equivalent in time and phase. The responses were averaged over several steps with the time at which the stimulus occurred determining in which of the 14 periods the response was placed. Generally there was about one stimulus per step and usually 100 to 500 steps were analysed. The reflex contribution to the total tension or EMG was calculated by subtracting the mean tension or EMG in each of the 14 time periods.

The results demonstrated a marked modulation of reflex responses as a function of the step cycle phase. The greatest EMG reflex response occurred at the time of the peak EMG produced as a result of locomotion. The reflex output diminished to zero during the time at which the soleus EMG due to walking was low (i.e., flexion phase). Between these extremes there was a gradation of reflex responses correlated to the step cycle. The results for tension paralleled the EMG responses. Thus, the central pattern generator for locomotion influences the response of stretch reflex pathways. Furthermore, the control it exerts is such that the reflex system is utilized to assist the behavioral goal.

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

The nerve innervating the cat medial gastrocnemius (MG) muscle generally divides into four to seven intramuscular branches. The muscle fibers innervated by a branch occupy a relatively distinct volume or "compartment" within the muscle and the Ia afferent fibers innervating spindles within such a compartment are contained in the same nerve branch (Farina and Letbetter, J.S.C. Med. Assoc., 73: 15, 1977). It has recently been shown that muscle receptors in MG are more responsive to contractions of their "own" intramuscular compartment than to those of neighboring and more distant compartments (Cameron et al., J. Neurophysiol., in press). These findings invite the question as to whether the topographic organization of the MG muscle is preserved in the pattern of its muscle afferent-motoneuronal connectivity (Binder and Stuart, Prog. Clin. Neurophysiol., 8: 72, 1980). The present series of experiments were specifically designed to determine if the homonymous Ia input to a motoneuron is "topographically weighted", reflecting a disproportionate contribution from spindles located within its muscle unit's territory. The MG nerves of anesthetized cats were cut away from the muscle after dividing into their natural branches. Each of three branches was placed on a separate stimulating electrode and a fourth electrode was placed proximally on the whole muscle nerve. MG motoneurons were impaled with glass microelectrodes while the muscle nerve and the individual branches were stimulated sequentially at 2 X threshold. Dorsal root volleys were recorded continuously. In most cases, it was necessary to hyperpolarize the motoneuron to prevent antidromic invasion consequent to stimulation of either the muscle nerve or the branch containing the motor axon. For each motoneuron studied, the relative contribution of each of the branches to the maximal (whole muscle) group I EPSP was calculated. This method permits a direct comparison of the synaptic input from a given branch to its "own" motoneurons (branch containing motor axon) versus that to other motoneurons within the MG pool. Moreover, this analysis obviates problems associated with variations in branch "size" and in maximal group I EPSP amplitude in different motoneurons. Thus far, in 11 of 12 successful experiments (81 motoneurons) we have found that the fraction of the maximal EPSP produced by a branch in its own motoneurons is significantly greater than that produced by the same branch in other motoneurons. These results are consistent with the hypothesis that homonymous Ia input to motoneurons is topographically weighted.

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180.18 ANALYSIS OF HETERONYMOUS GROUP IB SYNPATIC INPUT TO THE CAT MEDIAL GASTROCNEMIUS MOTONEURON POOL. Randall K. Powers* and Marc D. Binder. Dept. of Physiol. and Biophys., Univ. of Washington Sch. of Med., Seattle, WA 98195.

Previous work on cat medial gastrocnemius (MG) motoneurons has shown that the amplitudes of homonymous and heteronymous Ia EPSPs as well as those of disynaptic Ia IPSPs are largest in type S, smaller in type FR and smallest in type FF motoneurons (Burke et al., J. Neurophysiol. 31:447, 1976). Although Eccles et al. (J. Physiol. 138:227, 1957) found that Ib IPSPs were larger in soleus than in MG motoneurons, there has been no report of the relative amplitude of Ib input to different unit types within the MG motoneuron pool. The present study was designed to examine Ib input to different MG motoneurons, as well as to compare the synaptic potentials from a variety of other peripheral inputs onto the same cells. Intracellular recording and current injection were used to determine the input resistance, rheobase current (I_{Rh}), axonal conduction velocity (CV) and duration of afterhyperpolarization (AHP) of MG motoneurons in low spinal, chloralose-anaesthetized cats. In the same cells, heteronymous Ia EPSPs, sural PSPs (1.1, 1.5, 2 and 5 x threshold), flexor digitorum longus Ib PSPs and quadriceps Ib PSPs were evoked by stimulation of the respective peripheral nerves. MG motoneurons were divided into two groups on the basis of the pattern of synaptic input from the sural nerve (5 x T) (Burke et al., J. Physiol. 207:709, 1970). Thus far we have found that motoneurons with predominantly excitatory sural nerve input have significantly higher I_{Rh}s and CVs, shorter AHPs and smaller Ia EPSPs than motoneurons with mixed or predominantly inhibitory sural nerve PSPs. These results suggest that the former group is probably composed of type FF units while the latter group is probably composed of type FR and S units (Munson et al., Neurosci. Abstr. 6:714, 1980). The amplitude of Ib IPSPs is significantly smaller ($p<.01$) in these presumed type FF units than in the presumed FR and S units, and thus Ib inputs appear to show the same general pattern of relative efficacy that has been observed for group Ia input.

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180.19 Convergence from Golgi tendon organs and Mechanoreceptors in Muscle onto Force Sensitive Interneurons in the Spinal Cord of the Cat

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Convergence from different sensory receptors in muscle onto spinal interneurons has been traditionally investigated by electrically stimulating muscle afferents and recording from interneurons and motoneurons. This approach, however, does not directly address how interneurons respond to natural patterns of sensory stimulation. In order to investigate the natural response of interneurons to muscle receptor input, we have used physiologically relevant stimulation of muscle receptors to evaluate modal and spatial convergence onto spinal interneurons.

The activity of interneurons in the intermediate nucleus was extracellularly recorded in decerebrated/spinalized (T12) or chloralose anaesthetized cats. Interneurons were driven by stimulation of muscle receptors in the triceps surae and plantaris muscles. Static and dynamic changes in muscle force, produced by stimulation of the ventral roots or peripheral nerve at 5-30 Hz, were used to identify force sensitive interneurons. Convergence from primary spindle afferents was demonstrated if the interneuron was excited by longitudinal vibration (160 Hz, 90 um amplitude). Golgi tendon organ convergence if the interneuron was excited by electrical stimulation of the peripheral nerve at group I strength but not excited by vibration. Secondary spindle afferent convergence if the interneuron was excited by stretch at short muscle lengths. Mechanoreceptor input if the interneuron was excited by mechanical probing, especially light stroking, of the muscle. Interneurons were distinguished from tract neurons by tract stimulation at T12.

Many force sensitive interneurons in both decerebrated/spinalized and chloralose anaesthetized cats received input from Golgi tendon organs and mechanoreceptors but not from primary or seconday spindle afferents. Their discharge paralleled static changes in force, showed dynamic sensitivity at the onset of stimulation, and a prolonged afterdischarge at the cessation of stimulation. Unexpectedly, most responded vigorously to light stroking of the muscle and tendon surfaces. Discharge rates often equalled their maximum response to increases in muscle force. Although Golgi tendon organs are linear force transducers, the potency of mechanoreceptor input, which bears an uncertain relation to force, suggests that these interneurons may not always provide accurate information about muscle force.

180.20 REFLEX ACTIVITY OF THE RAT SPINAL CORD FOLLOWING SEGMENTAL APPLICATION OF PENICILLIN.
(SPON: M.H. BENITEZ)

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Mono- and polysynaptic discharges evoked by peripheral nerve stimulation (tibial and peroneal nerves) were recorded in decerebrate and spinal transected rats (at 10) following local application of Na-Penicillin G (PNC) to exposed lumbar segments. The tip of a glass micropipette (diameter about 20 μ) filled with a penicillin solution (5.10^5 I.U./ml) was placed upon the lateral aspect of the spinal cord between L5 and L6. By means of a microsyringe 4 μl of the solution were delivered. Reflex discharges into the ventral root of L5 and L6, as well as cord dorsum potentials were analyzed. A few minutes following drug application both mono- and polysynaptic discharges increased. Polysynaptic reflex facilitation was clearly dominant. Also after discharges appeared, triggered at latencies of 20 - 80 ms. Input-output curves for the monosynaptic reflex showed increased temporal summation. Posttetanic potentiation was decreased and shortened as penicillin effects set in. Recurrent inhibition was reduced by about 20%. Excitability changes in the monosynaptic reflex pathway tested with paired stimulus. Analyses at varying intervals displayed the following sequence: at the shorter intervals (up to about 20 msec) there was a decrease of the strong inhibition usually seen in control measurements; at longer intervals (up to 100 msec) a prolonged depression was observed. These results suggest that topically applied penicillin a) brings about clear cut qualitative and quantitative changes in the reflex activity of the rat spinal cord and b) in particular, influences inhibitory processes in a complex way.

181.1 ANTICIPATORY NEURONAL ACTIVITY IN THE MONKEY CORTEX DURING REACTION TIME FOREPERIOD. J.C. Lecas*, L. Mistler* and J. Requin. Inst. of Neurophysiol. and Psychophysiol., CNRS, Marseilles, France.

A number of single-cell recordings in monkeys trained to perform sensorimotor tasks have shown that neurons in precentral motor areas and prefrontal associative areas are activated prior to movement. The purpose of this study was to systematically investigate these anticipatory neuronal discharges using a paradigm in which the level of preparation for response was controlled.

Four monkeys (Macaca fascicularis) were trained to perform a between-hand, choice-reaction time (RT) task initiated by a warning signal (WS), in which the animal was required to point at a target. The probability of each hand performing this movement was varied within each session in blocks of trials. An inverse relationship between RT and response probability was found, while movement duration remained constant. A double chamber was then attached to the skull, allowing simultaneous microelectrode recording in both the precentral cortex and either the posterior parietal or prefrontal cortex.

Preliminary results were based on an extensive analysis of 20 cells which were activated during the pointing movement. They show that a small number of neurons, especially in the precentral areas, have discharges which are modified during the foreperiod, in correlation with the decrease in RT produced by an increase in response probability. That some of these changes appeared prior to ipsilateral movement - but without correlation with RT - could be further evidence for selective presetting processes triggered by the WS.

181.2 SINGLE UNIT ACTIVITY IN CAT PRECRUCIATE CORTEX DURING ALTERNATING MOVEMENTS. M. E. Melnick* and M. W. Rogers* (SPON: N. J. Pantazis). Graduate Programs in Physical Therapy, Univ. of Iowa, Iowa City, IA 52242.

Alterations in the pattern and rate of unit activity have been demonstrated in the cat motor cortex prior to the onset of movement and prior to the onset of EMG activity. A study by Neafsey et al. (Electroenceph. Clin. Neurophysiol. 44: 706, 1978) showed that the changes in unit activity of neurons in the medial precruciate cortex (projecting to axial and proximal musculature) occurred prior to changes in the lateral precruciate cortex (projecting to distal limb musculature). The present study is directed towards examining further functional differences in regions of the motor cortex.

Cats were trained to release one of two levers in an alternating sequence. Between trials, the animal was required to depress both levers for a minimum of 3 sec. After training, the cats were prepared for single unit recording in both precruciate cortices and for EMG recording from the triceps, brachialis, varying proximal muscles and the paraspinal muscles. The cat precruciate cortex was divided into three divisions: a medial region corresponding to area 6; an intermediate region corresponding to that portion of area 4 projecting to the proximal and axial muscles; and a lateral region corresponding to that portion of area 4 projecting to the distal limb muscles (Hassler and Muhs-Clement, J. Hirnforsch. 6: 377, 1964). Neural unit activity in all areas were classified as: 1) related to contralateral paw movement only, 2) ipsilateral paw movement only or 3) bilaterally related. Units were also classified as "early" or "late". "Early" units exhibited neuronal activity changes more than ½ sec preceding movement; "late" units, less than ½ sec before the movement. The results showed that 75% of the movement related units in the medial region, 47% of the units in the intermediate region and 14% of the units in the lateral region were "early" units. Over half of the units in area 6 were bilaterally related whereas only one-third of the units in both parts of area 4 were bilateral. The bilateral units in area 6 often had initial changes in activity which were in the same direction prior to movement of the contralateral and ipsilateral paw. Bilateral units in area 4 usually showed reciprocal changes. There were no consistent early EMG activity changes even in the axial muscles which could be correlated with the early neuronal activity changes.

These data are consistent with the hypothesis that the medial-intermediate regions of the motor cortex are preferentially involved in the preparatory adjustments for movement. They also suggest further functional differences between these two regions.

181.3 CODING OF TARGET AND RESPONSE VARIABLES IN CAT MOTOR CORTEX. J. Martin*, H. Yumiya*, and C. Ghez. Ctr. for Neurobiology and Behavior, Columbia P&S, New York, N.Y.

The arm area of cat motor cortex includes two functional subdivisions. Neurons in caudal (MCc) and rostral (MCr) regions differ in their receptive field properties and in the timing of their activity during tracking performance (Neurosci. Abstr.6:125, 1980). Task related activity in MCc lags force production. In MCr activity may lead but is better timed to target shifts than to motor behavior. This study further characterizes the coding of target and response variables in task related neurons.

The activity of single neurons in MCc and MCr was recorded in cats performing extensor or flexor adjustments in force applied to a lever with their forearm to match shifts in a target. The animals were provided with a compensatory display of their force error that moved to the right or left. To dissociate the coding of target and response variables in correlated unit activity, the animals were trained to respond appropriately when the display polarity was inverted or its gain altered. Additionally, the effects of display shifts not eliciting responses, receptive fields, antidromic activation from the cerebral peduncles and microstimulation from recording sites were routenely checked.

Three classes of task related units were identified. One showed reciprocal changes in activity according to response direction which were better timed to response onset but with a lag. Such neurons were located in MCc and had receptive fields on the responding limb. They could contribute either to tonic force production via feedback mechanisms or to other late response events. A second class showed a consistent relation to a given direction and to the time of occurence of display shift rather than to the response. Such neurons appeared to code target variables contingent on the occurence of a response in either direction. The third and major class showed reciprocal and contingent changes in activity with the direction of force production while bearing a more consistent relation to the time of occurence of the target shift than to the response. Thus, a neuron active with a given direction of force could be driven (or suppressed) at short latency by display shifts either to the right or the left according to the polarity of displayed error. These neurons could contribute to response initiation since their acitivity preceded and correlated with the direction of force production. The latter two classes of neurons were intermingled in areas of MCr where microstimulation produced contraction of arm muscles.

It is concluded that gating mechanisms operating presynatic to neurons in MCr enable the efficient transfer of behaviorally relevent target information leading to the short latency learned motor responses in our tracking task. (Supported by NS 52750)

181.4 TOPOGRAPHY OF DIFFERENTIAL PROJECTIONS TO ROSTRAL AND CAUDAL CAT MOTOR CORTEX. H. Yumiya* and C. Ghez (SPON: F. Salazar) Center for Neurobiology and Behavior, P&S, Columbia University, New York, NY, 10032.

Physiological data suggest that neurons in rostral and caudal portions of the arm area of cat motor cortex play predominant roles respectively in the initiation and control of ongoing motor responses. This study examines the differences in topographic organization of thalamocortical and corticocortical projections to the cat motor cortex. Single small injections were made intracortically in area 4γ of ten cats and the distribution of retrogradely labeled neurons was examined following processing with TMB.

In the thalamus, labeled cells were found in VL nucleus, VL-VB border zone and in CL nucleus. Within VL, clusters of labeled neurons formed lamellae with a lateral curvature in roughly parasagittal planes extending rostrocaudally. There were clear topographic relations between the location of the cortical injection site and the lamella of labeled VL neurons. Thus, the medial part of VL projected to medial precruciate area 4γ, lateral part of VL to medial postcruciate and intermediate regions projected to portions of pericruciate cortex in between. Postcruciate injections labeled neuron clusters in the VL-VB border zone in areas which were roughly co-planar but separate from those in VL proper. In contrast, precruciate injection sites yielded significantly fewer labeled neurons in VL-VB border zone. No specific topographic arrangement was observed in CL nucleus.

Clear differences between pre- and postcruciate injections were also found in the locations of labeled neurons within ipsilateral cortices (layers III and II). Following postcruciate injections, labeled neurons were abundant in areas 3a, 2, 5 and 2pri with a topographic arrangement compatible with known somatotopic representations of arm and leg in these areas. Additionally labeled neurons were present in separate portions of area 4γ remote from the injection sites and in other areas (especially medial part of area 5b and bank of anterior suprasylvian sulcus). In contrast, precruciate injections labeled many neurons in remote portions of area 4γ but only rare and scattered neurons in areas 2, 5, and 2pri. A few neurons in the banks of orbital and cingulate sulci, areas 6, 4δ, 4fu, 4sfu, 3b and 7 were labeled after some injections. These observations suggest that differences in the sources of afferent input to rostral and caudal area 4γ may give rise to the observed differences in physiological properties of neurons in these two areas.

181.5 CORTICAL MECHANISMS OF TWO-DIMENSIONAL AIMED ARM MOVEMENTS. III. RELATIONS OF PARIETAL (AREAS 5 AND 2) NEURONAL ACTIVITY TO DIRECTION OF MOVEMENT AND CHANGE IN TARGET LOCATION.
J.F. Kalaska*, R. Caminiti and A.P. Georgopoulos (SPON: A.Sastre) Dept. of Physiology, The Johns Hopkins Un. School of Medicine, Baltimore, Maryland 21205.

We hypothesize that frontal (4 and 6) and parietal (5 and 2) areas cooperate closely in the control of arm movements aimed at targets in space. An important spatial attribute of aimed movements is their direction. We showed previously (Soc. Neurosci. Abstr. 6:156,1980) that the activity of precentral motor cortical cells is related to the direction of movement in an orderly but non-exclusive manner, so that movement of a given direction engages the activity of neurons with overlapping directional preferences. We studied similarly the effects of the direction of movement on the activity of 164 single neurons electrically isolated in 39 microelectrode penetrations in areas 5 and 2 of two rhesus monkeys. A two-dimensional tracking apparatus was used in which animals were trained to move a manipulandum over a plane surface and capture with it lighted targets in a reaction task, as described before (Fed. Proc. 39:601,1980). This experimental arrangement provides 8 directions of movements which cover the whole circle with a resolution of 45^{o}. The cells studied in the task discharged with spontaneous arm movements, and most responded to passive manipulations of the arm, mainly to rotations at joints and/or palpation of deep tissues; for some cells the activation during movement was more intense than during passive manipulations. We found that the direction of movement affected the magnitude, latency, sign and time course of the cell response. These response characteristics varied in an orderly fashion with the direction of the movement. In another task the target changed during the reaction or movement time: the cell activity also changed in a systematic way, in that the pattern of activity related to the movement towards the first target was promptly interrupted and replaced by that appropriate to the movement towards the second target.

These results show that cell activity in parietal areas 5 and 2 is intimately related to the direction of movement and its changes that ensue following change in target location. Although the neuronal changes in the parietal areas generally occurred later than in frontal areas, the quantitative relations to the direction of aimed movements were similar in both of these cortical areas. This and the similar responses observed during change of target location strengthen the hypothesis of close cooperation of these frontal and parietal areas in the control of aimed arm movements. (Supported by USPHS Grants 5-RO1-EY03167-02 and NS-07226-11.)

181.6 CORTICAL MECHANISMS OF TWO-DIMENSIONAL AIMED ARM MOVEMENTS. IV. EFFECTS OF ARM POSITION IN SPACE.
R. Caminiti, J.F. Kalaska* and A.P. Georgopoulos. Dept. of Physiology, The Johns Hopkins Un. School of Medicine, Baltimore, Maryland 21205.

The direction of aimed movements is a major factor to which cell activity in precentral and parietal (5 and 2) areas is related (Soc. Neurosci. Abstr. 6:156,1980; and this Meeting). We identified another important factor that influences neuronal activity in these areas; namely, the position of the arm in space. Monkeys were trained to hold steadily a manipulandum at different points in a two-dimensional space. The desired position was indicated by a lighted target which the animal had to capture with the freely moving manipulandum and hold there for a variable period of time. The apparatus has been described before (Fed. Proc. 39:601,1980). We studied under these conditions the activity of 134 precentral and 53 parietal (areas 5 and 2) single neurons electrically isolated in 52 penetrations in two rhesus monkeys. These cells discharged during spontaneous arm movements; most parietal and some frontal neurons also responded to passive manipulations of the arm, mainly to rotations at joints and/or palpations of deep tissues. The activity of 72% precentral and 77% parietal cells was influenced by the active maintainance of the arm at different positions in space. Cells discharged at higher rates when the manipulandum was held within certain parts of the two-dimensional space; the discharge decreased as positions at increasing distances from this part were occupied. Maxima and minima of activity occurred at different parts of space for different cells.

These results show that position of the arm in space is an important determinant of cell discharge in both frontal and parietal areas studied. This underscores the participation of these areas in the control of aimed movements since a basic spatial attribute of these movements concerns the position of the arm in space, in addition to the direction of movement. Different mechanisms may account for the effects observed in frontal and parietal areas: a relation to motor commands in the first and a peripheral input in the second, but a peripheral input to precentral cells and a corollary motor output to parietal cells may also contribute. We propose that the similarity of the relations of frontal and parietal neuronal activity to arm position in space and the direction of movement indicates the use by these cortical areas of a common "language" in processing information for the spatial control of aimed movements. The nature of this common system of reference remains to be elucidated.
(Supported by USPHS Grants 5-RO1-EY03167-02 and NS-07226-11.)

181.7 CORTICAL MECHANISMS OF TWO-DIMENSIONAL AIMED ARM MOVEMENTS. V. INTERACTIONS BETWEEN DIRECTION OF MOVEMENT AND ARM POSITION IN SPACE.
A.P. Georgopoulos, J.F. Kalaska* , R. Caminiti and J.T. Massey. Dept. of Physiology, The Johns Hopkins Un. School of Medicine, Baltimore, Maryland 21205.

The spatial characteristics of aimed movements comprise the direction of the movement and the arm position in space (which defines the trajectory of the movement). Both of these factors influence the neuronal activity in precentral and parietal cortical areas (Soc. Neurosci. Abstr. 6:156,1980; and this Meeting). It is important to know whether these factors influence the same or separate populations of cells, and if the latter is true, how they interact at the single cell level. We investigated this problem by training monkeys to make equal amplitude (8 cm) movements that were either (a) of opposite directions but made between the same points (e.g., from 12 o'clock to center, and vice versa), or (b) of the same direction but made between different points in space (e.g., from 12 o'clock to center, and from center to 6 o'clock). A two-dimensional tracking apparatus was used (Fed. Proc. 39:601,1980). Starting and final target positions were varied in a randomized block design. We studied the activity of 117 precentral and 53 parietal (areas 5 and 2) arm-related single neurons in 2 rhesus monkeys. 1) The activity of 70% frontal and 74% parietal cells was related to both the direction of the movement and the actively maintained arm position in space; smaller percentages were related to direction only (23%, 21%), arm position only (1%, 4%), or to neither (6%, 1%) in frontal and parietal areas respectively. 2) The directional and positional effects were additive in some cases but not so in others. Generally, the response for a preferred direction of movement was stronger if its trajectory lay within a part of space for which the cell discharged at higher rates during steady holding. 3) Movements of opposite directions made between the same points elicited responses that differed strongly when one of the two directions was the most preferred for a given cell; for other pairs of directions differences were less pronounced.

These results show that the spatial characteristics of aimed movements are not handled in these areas by separate "directional" or "positional" populations; instead, information concerning both of these spatial attributes is processed to a large extent by the same single neurons. The exact use of this information at the single cell level remains to be elucidated. We believe that more detailed investigation of the interactions between direction of movement and arm position in space may contribute to this elucidation, as well as lead to a better understanding of the specific contribution of each area and of the nature of their cooperation in the spatial control of aimed movements.

181.8 CORTICAL MECHANISMS OF TWO-DIMENSIONAL AIMED ARM MOVEMENTS. VI. ELECTROMYOGRAPHIC ANALYSIS.
J.T. Massey, R. Caminiti, J.F. Kalaska* and A.P. Georgopoulos. Dept. of Physiology, The Johns Hopkins Un. Sch. Medicine, Baltimore, Maryland 21205.

The direction of aimed arm movements and the arm position in space are two important factors to which neuronal activity in precentral and parietal cortical areas is related (Soc. Neurosci. Abstr. 6:156,1980; and this Meeting). We investigated how these movements are performed by muscles acting on joints of the arm, and particularly how each muscle is related to the factors mentioned above. EMG activity of 21 muscles acting on shoulder, elbow and wrist joints, and of paraspinal musculature, was recorded intramuscularly in 3 rhesus monkeys while they made movements of various directions in a two-dimensional tracking task (Fed. Proc. 39:601,1980), and also when they were actively maintaining different arm positions in space. These tasks were accomplished by muscles acting on the upper arm (teres major, anterior and posterior deltoid, pectoralis, latissimus dorsi, and caudal part of trapezius); other muscles were generally inactive. The earliest onset of EMG activity preceded constantly the beginning of movement (as judged from the velocity record) by at most 150 ms; decrease of activity in antagonists occurred earlier, at most 170 ms before the onset of movement. Reaction times were 250-300 ms. For the muscles that were active in the task, the magnitude, latency, sign, and time course of the EMG response varied with the direction of movement in an orderly manner. However, the directional specificity of any single muscle was rather low, with excitatory sector widths (A.P. Georgopoulos et al., Soc. Neurosci. Abstr. 6:156,1980) of at least $170\text{-}180^{o}$. (By comparison, about 75% of precentral motor cortical neurons studied in the same animals with the same task had sectors below 180^{o}.) Muscles that were active with movements were also differentially activated during active maintainance of arm position in space. These effects were spatially congruent, so that, for example, a muscle that was phasically activated when moving from the center to an outer target (e.g. at 12 o'clock) was also tonically active when holding steadily at that target position. These results show that any two-dimensional movement performed by action of muscles at the shoulder joint is accomplished by a patterned activation of these muscles; this pattern varies in an orderly fashion with the direction of movement. We conjecture that precentral neurons, under certain circumstances, may indeed relate to the pattern rather than (or in addition) to individual muscles alone. (Supported by USPHS Grants 5-RO1-EY03167-02 and NS-07226-11.)

181.9 INPUT-OUTPUT RELATIONSHIPS IN AREA PREINSULARIS (2PRI) OF THE SECOND SOMATIC SENSORY CORTEX. A Mori*, R. S. Waters, H. Asanuma. The Rockefeller University, New York, N.Y. 10021.

Efferent projections from area preinsularis (2 pri) of the second somatic sensory cortex to limb and trunk muscles were examined in unanesthetized or lightly sedated (ketamine,1-2mg/kg) cats using the same microelectrode for intracortical microstimulation (ICMS) and recording cellular activities. In addition, connections from area 2 pri to area 4γ were examined using the electrophysiological technique. Under halothane anesthesia, areas 4γ and 2 pri were exposed and a double barreled chamber was installed over the skull. Through the anterior chamber, 8 tungsten in glass microelectrodes were inserted into area 4γ and they were positioned at depth of 1.2mm below the surface of the cortex. Receptive fields of neurons around the electrodes and muscle responses to ICMS through each electrode were examined. Through the posterior chamber, an electrode used for both ICMS and recording was inserted into area 2 pri. The motor effects were examined either by recording EMG or observing movements. The receptive fields of neurons were examined by using natural somesthetic stimulation. The spikes were monitored on the oscilloscope and also fed into a speaker through a window discriminator.

ICMS through the 2 pri electrode could elicit flexion of fingers, flexion of elbow, extension of elbow, flexion of hindlimb joints, movement of shoulder and abdomen on the contralateral side of the body. The minimum threshold current for movement was 2μa and the maximum current used was 30μa. The low threshold sites for a particular movement were confined to a small area of the cortex and constituted a columnar shape with a diameter of 200-1000μm. Neurons in the effective area received afferent input from receptors in the skin or deep structures in the region of the body which was related to the movement. To examine whether the pyramidal tract mediates the motor effects, a recording electrode was inserted into the lateral cervical cord (C_5) and large synchronized volleys were recorded in the pyramidal tract following ICMS delivered to area 2 pri.

Neurons of area 2 pri which were activated antidromically by ICMS in area 4γ had receptive fields which were similar to those of neurons around the responsible area 4γ stimulating electrode. It is concluded that neurons of areas 2 pri and 4γ are closely interconnected, but area 2 pri also has motor function which is independent of the motor cortex.

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181.10 SUSTAINED EXCITATORY SYNAPTIC INPUTS TO MOTOR CORTEX NEURONS IN AWAKE ANIMALS; A COMPARATIVE STUDY OF MEMBRANE POTENTIAL IN ANESTHETIZED AND UNANESTHETIZED STATE. M. Matsumura (SPON: E. E. Fetz). Department of Physiology and Biophysics, and Regional Primate Research Center, University of Washington, Seattle, WA 98195.

A considerable difference has been noted in membrane potentials of cortical neurons observed in awake and anesthetized animals. In awake animals investigators tend to observe smaller (more depolarized) membrane potentials (-50 to -60mV) (Woody, C. D. et al., Brain Res. 158: 343, 1978, Matsumura, M. Brain Res. 163: 33, 1979) than those observed in anesthetized animals (typically -70mV; Phillips, C. G. Quart. J. exp. Physiol. 41: 58, 1956). This difference indicates that the level of depolarization of cortical neurons may depend on the anesthetic state of the animal.

However, measurements of membrane potential could also be affected by recording conditions, such as electrode parameters or by experimental criteria of "good" intracellular recording. These factors might skew the sample. To minimize these factors and clarify the effect of anesthesia, identical recording techniques and selection criteria were applied in recording the membrane potentials of cortical neurons in the same animals, under awake and anesthetized conditions.

Chronic intracellular recordings (Woody, C. D. et al., J. Neurophysiol. 36: 1104, 1973) were obtained from motor cortex neurons of cats. Microelectrodes were beveled to a tip diameter of about 1μ to reduce the probability of dendritic penetration. After several hours of recording from the awake cat (restrained by a blanket), sodium pentbarbital (35mg/kg) was administered intraperitoneally, and the same recording procedures were repeated at the same cortical site. Approximately 1 week later the contralateral side of the motor cortex was similarly investigated.

To date, 50 neurons from pre- and postcruciate gyrus have been recorded intracellularly. The average membrane potential of the neurons was -62±7(S.D.)mV (n=30) in the anesthetized state and -54±7mV (n=20) in the awake state. The difference between these two groups was significant (t-test, $p<0.001$). Results were similar across animals for the same anesthetic state. Action potential amplitude was also significantly different between these two groups. Spikes recorded in the anesthetized state exhibited a greater overshoot (+5.4mV) than those in the awake state (+1.2mV). This observation suggests a difference in membrane conductance of cortical neurons in awake vs anesthetized preparations. (supported by NIH Grants NS12542 and RR00166).

181.11 EFFECTS OF SINGLE INTRACORTICAL MICROSTIMULI ON ACTIVITY OF INDIVIDUAL FORELIMB MOTOR UNITS IN BEHAVING MONKEYS. S. Sawyer and E. E. Fetz, Dept. of Physiol. and Biophysics and Regional Primate Research Center, University of Washington, Seattle, WA 98195.

The effects of single intracortical microstimuli on the firing probability of isolated forelimb motor units (MU) were investigated in monkeys generating isometric ramp-and-hold torque responses about the wrist. One objective was to determine whether the output effects elicited from a given cortical site were similar on all MUs of a muscle, or whether particular MUs were preferentially affected. Widespread effects would be expected if corticomotoneuronal (CM) cells terminate ubiquitously on all motoneurons of a pool, similar to Ia afferent fibers (1); selective effects would be consistent with the observation that CM-EPSPs were evoked from different cortical sites for different motoneurons of the same hindlimb muscle (2).

As previously described (3), effective output sites in precentral cortex were first identified by the appearance of post-stimulus facilitation (PStF) in stimulus-triggered averages of rectified multiunit EMG activity, recorded via electrode pairs in 5-6 identified synergist muscles. During active muscle contraction single intracortical microstimuli of 5-10 μa were subthreshold for evoking overt EMG responses; stimulus pulses were delivered slowly enough to preclude temporal summation (15/s). After identifying an effective output site, single MUs in the facilitated muscle(s) were recorded with tripolar tungsten microelectrodes, positioned in the muscle with a remotely controlled microdrive. Motor units were identified by the characteristic size and shape of their waveform in MU-triggered averages of multiunit EMG activity.

The majority of MUs identified as belonging to a particular muscle were individually facilitated from the same cortical site. Stimulus-triggered averages of rectified action potentials of single MUs showed briefer peaks than averages of multiple MUs. The post-stimulus time histogram of MU spikes exhibited peaks with half-widths in the range of .25-1.75ms, indicating that the variance in the latency of MU firing could not account for the duration of PStF of multiunit EMG. These results suggest that the cortical output effects evoked from single cortical sites may affect most, if not all, the motoneurons of a muscle.
1. Mendell & Henneman, J. Neurophysiol. 34:171; 2. Jankowska et al., J. Physiol. 249:637; 3. Cheney & Fetz, Neurosci. Abst. 3:269; Sawyer et al., Neurosci. Abst. 5:385.

181.12 INTRACORTICAL MICROSTIMULATION AND SINGLE NEURONE RECORDING DATA RELATED TO FACE, JAW AND TONGUE REPRESENTATIONS IN SENSORIMOTOR CORTEX OF MACACA FASCICULARIS. M. Sirisko* and B.J. Sessle. Fac. of Dentistry, Univ. of Toronto, Toronto, Canada M5G 1G6.

Since little information is available of the organization in the cerebral cortex of face, jaw and tongue sensorimotor representations, the present study was designed to delineate the "excitable" cortex related to orofacial movements with the use of intracortical microstimulation and, in the same animals, to determine the presence of cortical neurones that receive cutaneous or muscle afferent inputs and/or project to the vicinity of the cranial nerve motor nuclei. The motor representation of the face jaw and tongue was determined in six unanaesthetized monkeys by examining the twitch movements and EMG responses evoked by microstimulation in a series of microelectrode tracks made 1 mm. or less apart in the sensorimotor cortex; intracortical sites were stimulated (<30 μA) every 250 μ or less over vertical depths of 3-5 mm. Each animal was then anaesthetized with barbiturate, and stimulating electrodes were placed on the facial skin, oral mucosa, and the temporalis (V), facial (VII) and hypoglossal (XII) muscle nerves to produce short-latency (4-20 msec) orthodromic activation of cortical neurones; corticobulbar neurones were identified by their antidromic response evoked from stimulating electrodes located in the V, VII or XII motor nuclei. Lesions were placed in selected cortical tracks, and histologica reconstruction of the tracks was subsequently carried out.

The motor representation in the precentral cortex was characterized as a central core of tongue (and sometimes jaw) movement surrounded on its anterior, medial and posterior sides by a horseshoe-shaped area that predominantly represented facial movements. Field potential and single neurone recording data indicated that antidromically identified corticobulbar neurones were largely confined to this "excitable" cortex. Some corticobulbar neurones could be activated by stimulation of low-threshold muscle afferents in V, VII or XII nerves, but the predominant focus of muscle afferent input occurred more posteriorly, in the depths of the central sulcus. This region especially involved area 3a, although some neurones further up the posterior bank of the sulcus also appeared to receive this input. Convergence of cutaneous and muscle afferent inputs was not uncommon, since about 25% of neurones excited by muscle afferent electrical stimulation and muscle stretching could also be activated by electrical or tactile stimulation of afferents supplying facial skin or oral mucosal sites. The present findings have provided fundamental information on input-output features related to orofacial movements that will be utilized in studies of these features in chronic recordings made from monkey cortex.

181.13 MOVEMENTS EVOKED BY INTRACORTICAL MICROSTIMULATION IN THE SUPPLEMENTARY MOTOR AREA (SMA). J. M. Macpherson, C. Marangoz*, T.S. Miles* and M. Wiesendanger (SPON: J.S.McIntosh). Institut de Physiologie, Univ. de Fribourg, CH-1700 Fribourg, Switzerland.

In this study, the supplementary motor area (SMA) in the mesial cortex of area 6 was investigated using intracortical microstimulation. Each of three monkeys (Macaca fascicularis) was implanted with a cylinder over the exposed SMA using standard techniques. Perpendicular electrode penetrations were made into the SMA on a 1 mm grid and stimulation (12 pulses at 300 Hz, 0.2 mm duration) was applied every 250 um to a depth of 10 mm. After completion of mapping, horseradish peroxidase (HRP) was injected into the spinal cord at cervical or lumbo-sacral levels.

Discrete movements of the contralateral forelimb and hindlimb were evoked by SMA stimulation at currents less than 30 uA. The thresholds were, however, higher than those in the hindlimb area of the primary motor cortex. Movements of the forelimb, at both proximal and distal joints, were evoked from a small patch of mesial cortex anterior to the precentral hindlimb and tail area. Some hindlimb responsive points were found in area 6 ventral and posterior to this forelimb region. No hindlimb representation was found in the dorsal bank of the cingulate sulcus ventral to area 4. Movements could not be evoked in muscles of the face and head.

HRP-filled cells were found in the SMA mesial cortex and the dorsal and ventral banks of the cingulate sulcus. The zone of labelled cells in the SMA corresponded very well to the microexcitable region. Following the lumbar injection, the area of filled cells in mesial area 6 was surprisingly large, extending well anterior to the end of the giant Betz cell zone.

In conclusion, the SMA is microexcitable, suggesting a relatively tight coupling between this cortical area and spinal motor nuclei. Proximal muscles and, to a lesser degree, distal muscles appear to be controlled by the SMA.

182.1 A NEURAL BASIS FOR AGE DEPENDENT RESISTANCE TO HERPES SIMPLEX VIRUS INFECTION. Robert R. McKendall* (Spon: J.R.Baringer). Dept. Neurology, University of California and VA Medical Center, San Francisco, CA 94121.

Following birth, mice develop increasing resistance to neurologic disease from herpes simplex virus type 1 (HSV-1). This resistance has been attributed to maturation of cellular immune mechanisms, particularly macrophage ability to restrict virus replication. Previous studies have shown that 7-week old mice require a higher dose of virus in the footpad to cause neurologic disease than 4-week old mice (McKendall, RR, J. Med. Virology 5:25-32, 1980). The dose of virus required correlated with the dose necessary to cause viral spread along sciatic nerve.

To determine whether neural factors or immunologic factors were involved, viral tissue titers and viral spread was studied following footpad inoculation of $10^{5.5}$ pfu HSV-1 in young (4-week) and old (22-week) Balb/c mice. Viral titers in footpad were the same in both groups and remained elevated for several days after infection. Appearance of virus in sciatic nerve and spinal cord was rapid (48 hours post infection) in young mice and delayed in old mice (day 5). This delay was confirmed using a very sensitive technique for virus recovery from spinal ganglia explants. Spinal ganglia explants from 12/12 (100%) young mice contained virus by 24-48 hours after infection, while none of 12 (0%) old mice were positive through 62 hours post infection ($P < 0.01$). Thus, virus spread along nerves to spinal ganglia and spinal cord was greatly delayed in 22-week old mice despite equivalent levels of virus present in footpad. The latter finding is incompatible with an immunologic basis of resistance and suggests that neural development after birth has a major impact on virus spread and neurovirulence.

182.2 ULTRASTRUCTURAL RECOVERY IN NON-MYELINATED NEURONS DAMAGED BY ENERGY DEPRIVATION. N.A. Dahl, J.K. Marling*, G.A. Looney*. Univ. of Kansas, Lawrence, KS 66045

The reversibility of ultrastructural changes seen in nerve axons following oxygen-glucose deprivation was examined. Rabbit vagus nerves were pulled into a multicompartment perfusion chamber, stimulated 5/sec and deprived of energy by substituting nitrogen and deoxyglucose for oxygen and glucose in the Locke's perfusate. At the end of two hours or four hours of insult, the chamber was reperfused with oxygen-glucose Locke's to allow two hours of recovery. At the end of the recovery period nerves were prepared for electron microscopy. Cross sectional areas were measured for 100 randomly selected C fiber axons per nerve. Control neurons had a mean area of .85 μm^2 (S.D. $\pm$ .47). As previously reported (Dahl, Looney, Black, The Physiologist, 22: 25, 1979), two hours of energy deprivation causes significant shrinking. When this insult is followed by two hours of control Locke's, there is structural recovery, with the size and physical appearance not significantly different from controls. All C fibers are contained within normal looking Schwann cells. Four hours of energy deprivation causes startling changes, both extreme shrinking and swelling, and it is the most shrunken axons that are interrupted by huge ballooned regions. When this disruption is followed by two hours of energy substrates, this trend is not significantly reversed and the distribution of the axonal cross sectional areas is still bimodal. The smallest axons have no microtubules but are packed with neurofilaments. The huge axons are also devoid of microtubules, but contain dispersed neurofilaments and flocculant material, and have disrupted membranes. Thus, it appears that the initial shrinking of axons seen after two hours of oxygen-glucose deprivation is reversible when followed by control conditions but the extreme shrinking and swelling seen at four hours is too disrupting to allow structural recovery. (Supported by Biomedial Sciences Support Grant #RR07037).

182.3 DYSTROPHY OF CEREBELLAR AXONS OCCURS IN THREE STRAINS OF MYELIN DEFICIENT MICE. Jeffrey Rosenfeld and Victor Friedrich, Jr. Lab. of Neuromorphology, Dept. of Biobehavioral Sciences, University of Connecticut, Storrs, CT 06268.

In addition to the widespread deficit of myelin, quaking and shiverer mice exhibit neuronal abnormalities in the cerebellum. We now report that jimpy, another myelin deficient mutant strain, exhibits pathological axonal and synaptic changes similar to those in quaking and shiverer.

In Golgi impregnations of jimpy mice, twelve Purkinje cells were found with the minimal criteria that the cell body and at least 40 μm of axon were impregnated. The axons of four of these cells exhibited smooth focal elongate swellings, from 1.5-4 μm in diameter and 2-6 μm long. In three cases the swellings occurred 35-40 μm distal to the cell body; in one instance multiple swellings were seen on several recurrent collaterals branching away from the primary axon.

The swellings are of varied fine structure. Some examples contain increased amounts of endoplasmic reticulum, distributed mainly as narrow flattened cisternae, and numerous membrane vesicles, some as small as synaptic vesicles. Microtubules and neurofilaments are present but disoriented in these swellings. Other examples of the swellings are largely depleted of endoplasmic reticulum, mitochondria, microtubules and neurofilaments and contain a prominent, coarsely flocculent ground substance. It is not clear at this time whether this heterogeneity in the fine structure of the axonal swellings reflects different stages of dystrophy, or the involvement of other neuron types.

Striking abnormalities occur in the Purkinje cell terminals in the cerebellar nuclei, including enlargement of the terminals, increased occurrence of large multivesicular bodies, membrane whorls and irregular endoplasmic reticulum. In addition to these dystrophic changes, some of the Purkinje cell synaptic terminals in jimpy mice contain unusual clusters of very tightly packed small vesicles. These clusters appear identical to those in quaking and shiverer (Brain Res. 192:209).

Focal axonal swellings and abnormal synaptic fine structure have now been demonstrated in three disparate myelin disorders. This coincidence suggests that the axonal and synaptic dystrophy are related directly or indirectly to dysmyelination.

(Supported by U.S.P.H.S. Grant NS-09904).

182.4

WITHDRAWN

182.5 ULTRASTRUCTURAL CYTOPATHOLOGY AND CYTOCHEMISTRY OF TRIMETHYLTIN NEURONAL TOXICITY. T.W. Bouldin*, N.D. Goines*, C.R. Bagnell* and M.R. Krigman. Dept. of Pathology, Univ. of North Carolina, Chapel Hill, North Carolina 27514.

The organotin compounds, trimethyltin (TMT) and triethyltin (TET) produce very different types of cellular injury. Whereas TET produces widespread intramyelinic edema in the CNS, TMT produces neuronal necrosis that preferentially involves the hippocampal formation and pyriform cortex. To define the ultrastructural basis and evolution of the cell injury that occurs in TMT neuronal toxicity, we acutely intoxicated adult rats with single daily doses of TMT (5 mg/kg). After perfusion-fixation, hippocampus and pyriform cortex were examined by electron microscopy. The first signs of cell injury, which occurred 24 hours after 2 daily doses, were multifocal collections of dense-cored vesicles and tubules and membrane-delimited vacuoles in the neuronal perikaryon and proximal dendrite. Ultrastructural cytochemistry revealed that the dense-cored vesicles and tubules had acid phosphatase (AcPase) activity analagous to GERL; the membrane-delimited vacuoles occasionally had thiamine pyrophosphatase activity analagous to the trans aspect of the Golgi complex. Many of the collections of GERL-like smooth membranes appeared to have no spatial relationship to the Golgi apparatus. By 24 hours after the appearance of dense-cored vesicles and tubules, numerous autophagic vacuoles and polymorphic dense bodies accumulated in the perikaryon. Some dense bodies appeared to arise directly from the dense-cored tubules. The dense bodies also demonstrated AcPase activity. Neuronal necrosis was frequently present 24 hours after 4 daily doses of TMT and was much more prevalent in granule cells of the fascia dentata than in pyramidal neurons of the cornu ammonis. Necrotic neurons had electron dense cytoplasm, usually numerous cytoplasmic dense bodies, and large, electron-dense intranuclear masses. A similar series of subcellular pathological changes was observed in chronically intoxicated adult rats (1 mg/kg/day for 14-16 days) and neonatal rats (1 mg/kg/alternate day from the 3rd to the 29th day of life). Early ultrastructural alterations were NOT found in neuronal mitochondria or other neuronal organelles.

The multifocal cytoplasmic accumulations of GERL-like smooth membranes that characterize the early neuronal injury in TMT toxicity are unique. The frequent association of autophagic vacuoles and dense bodies with the GERL-like membranes suggests that the initial hypertrophy of GERL-like membranes may be a reactive change in the neuron to the TMT-induced increase in autophagy and lysosome formation. (Supported by U.S.P.H.S. Grants ES02083 and ES01104).

182.6 REDUCED ACTIVITY OF METHIONINE ADENOSYLTRANSFERASE IN ERYTHROCYTES OF EARLY ONSET SCHIZOPHRENICS. J. R. Kelsoe, Jr.*, L. C. Tolbert, E. L. Crews* and J. R. Smythies. Neurosciences Program & Department of Psychiatry, University of Alabama in Birmingham, Birmingham, Al. 35294.

Abnormalities of methylation have been repeatedly implicated in schizophrenia particularly because of the exacerbation of symptoms associated with methionine loading in some schizophrenics. Methionine adenosyltransferase (MAT), because of its key role in methylation, has been studied in erythrocytes from schizophrenic patients with differing results. One group found no significant differences in the activity of this enzyme between erythrocytes from normals and schizophrenics using low methionine concentrations. However, another group did find a difference using higher methionine concentrations in the assay.

The present study examined kinetic parameters of MAT activity in erythrocytes from normal and schizophrenic patients (diagnosed by Feighner) using methionine concentrations from 0.6 to 100 μM in an effort to examine this discrepancy. The results demonstrate differences in the enzyme from schizophrenics compared with normals. Vmax was significantly decreased in the samples from the early onset schizophrenics (1.71 pmoles/mg protein/hour) as compared with controls (2.65 pmoles) ($p < 0.025$, Mann-Whitney U Test). A significant difference in Km between schizophrenics (2.94 μm) and controls (3.95 μm) was also observed ($p < 0.05$, Mann-Whitney U Test). There was a significant difference between the ages of the schizophrenic patients and controls but no significant correlation was obtained between age of the patient and MAT activity. No correlation was observed between the sex of the subject and MAT activity. Although all the schizophrenic patients were receiving medication, most commonly dopamine blockers of various types, the patients with the lowest Vmax values were receiving only lithium carbonate and reserpine, which suggests that neuroleptic therapy may not have a significant effect on MAT activity.

The lower Km, supportive of a higher affinity, and a lower Vmax enzyme in schizophrenics could explain the discrepancy in previous investigation. This study lends support to the hypothesis that decreased methylation may be an important component in some schizophrenic diseases.

182.7 SCANNING ELECTRON MICROSCOPIC STUDIES OF COVERSLIP IMPLANTS IN THE RAT CEREBRAL CORTEX. C.H. Phelps, J.R. Norris* and J.C. Pearson*. Department of Anatomy, Wright State University School of Medicine, Dayton, Ohio 45435.

To examine and compare the migration of various types of inflammatory cells into wounds in the brain and skin after injury, small pieces of plastic or glass coverslips ($3mm^2$) were implanted in the parietal cortex and under the skin of adult Sprague-Dawley rats. At selected time intervals from 1 to 10 days following implantation the coverslip fragments were removed, fixed in 2% glutaraldehyde, stained with either toluidine blue or Giemsa stain, and examined with the light microscope (LM). After classification and enumeration of cell types, typical cells were photographed and the coverslip fragments were then dehydrated in ethanol, critical point dried, coated with gold and examined with the scanning electron microscope (SEM). Using these procedures it was possible to confirm the identification of the cells seen with SEM by using the nuclear and cytoplasmic characteristics demonstrated with LM. Coverslip fragments removed during the first three days after implantation contained many monocytes and young macrophages, but very few polymorphonuclear cells in either the brain or skin wounds. With SEM the monocytes appeared to have few surface specializations except for a single central enlargement containing the cell nucleus. The young macrophages were larger and had many more surface irregularities including blebs and pits. The thinner margins of the cells exhibited lamellipodia and filopodia. By the fourth day many mature macrophages characterized by large surface pits were prominent and the formation of multinucleated giant cells by fusion of macrophages were noted in both skin and brain. In early stages of fusion adjacent macrophages contacted each other by numerous cell projections and the cells then appeared to gradually merge. With time more cells were added until polykaryons containing up to 100 nuclei were observed. These cells resembled "foreign-body-type" giant cells. Spindle shaped cells were occasionally observed in the brain implants but positive identification of these cells awaits further clarification.

182.8 STAINING OF ALZHEIMER'S NEUROFIBRILLARY TANGLES WITH ANTISERUM AGAINST THE 200,000 MOLECULAR WEIGHT COMPONENT OF NEUROFILAMENT. Y. Ihara*. (SPONS: A. Pope).

To clarify the nature of the paired helical filaments (PHF) which accumulate in selected neurons in senile dementia of the Alzheimer type (SDAT), we employed an immunocytochemical approach using an antiserum against the 200K component of neurofilaments and an antitubulin antiserum. Paraffin sections of hippocampus from a brain with SDAT were processed for immunocytochemical staining (PAP method). After staining with diaminobenzidine, sections were counterstained with hematoxylin. Neurofilaments were purified from rat spinal cords with a modification of the method of Schlaepfer and Freeman (1978). Antisera were raised against each gel purified component of the neurofilament triplet. Purified tubulin was obtained from in vitro reconstituted rat microtubules by phosphocellulose column chromatography. Antiserum raised against tubulin was further purified by affinity chromatography.

Alzheimer's neurofibrillary tangles were clearly and intensely stained with the anti-200K protein antiserum by the PAP method. Some neurons contained immunolabeled granules scattered in the perikaryon which may correspond to granulovacuolar degeneration. Clusters of small dark granules, presumably representing degenerating neurites, were also clearly visible in the periphery of senile plaques. No tangles and senile plaques were immunostained with normal rabbit serum or with anti-200K serum preabsorbed with purified neurofilaments.

With antitubulin antibody, dendrites of pyramidal cells were intensely stained and normal neuronal perikarya were diffusely and lightly stained, but tangles and senile plaques were not immunolabeled. These observations strongly suggest that PHF present in Alzheimer's disease originate from neurofilaments, not from microtubules. Staining with antisera against 68K or 160K protein is now underway in our laboratory.

182.9 ULTRASOUND INDUCED LOCALIZED HYPERTHERMIA IN THE CENTRAL NERVOUS SYSTEM: THERMAL DOSIMETRY AND DAMAGE THRESHOLD STUDIES. B.E. Lyons*, R.H. Britt and D.W. Pounds* (SPON: L.H. Ostrach). Div. Neurosurg. R155., Stanford Med. School, Stanford, CA 94305.

The application of ultrasound (US) for induction of localized hyperthermia in normal brain tissue was investigated. In 9 cats lesion size as generated by US was studied by correlating thermal dosimetry data with neuropathological findings. Proper system design for localized heating in the CNS is necessitated by the lethal effects of whole brain temperatures elevated above 42.2°C. Evaluation of ultrasonic energy in penetrated tissue as offset by thermal conduction and perfusion was examined to determine damage threshold levels in normal brain tissue.

In 9 pentobarbital anesthetized cats the overlying scalp and muscle tissue were retracted and a large craniotomy was performed to allow proper transducer alignment. Temperature within the US field was continuously monitored by multiple thermocouples embedded in 26 gauge needles (Bailey Instr.). Electrical energy was converted to US by a piezoelectric Lithium Niobate crystal transducer, operating at 2.060 MHz, driven by a function generator operating in the sinewave mode. The operating frequency was monitored by an electronic frequency counter. The transducer housing provided a water-path coupling ("cuff") of US energy between the crystal and the intact dura membrane. Transmission gel was used as a contact medium for US impedance matching. Tissues were irradiated at a distance of 0.4cm from the cortical surface-transducer interface. Incident power was varied as a function of treatment duration to achieve desired temperatures.

Although threshold values defining thermal properties in the cat's brain have not been obtained to date, the authors have histologically quantitated discrete spherical thermal lesions (0.5cm in diameter) in highly perfused normal gray matter when heated to 47.0°C for 2 hrs. The spatial uniformity of the thermal lesion correlates precisely with the temperature profile recorded during US treatment. These studies suggest that the irradiated field corresponding to the lesion size caused by the thermal effects of US at this source-target distance is directly dependent on the acoustical intensity as a function of tissue temperature and the duration of treatment.

The correlation of temperature gradients with cellular viability in the CNS of the cat demands an aggressive investigation evaluating the thermal response of tumor cells in the brain. The potential role of US hyperthermia as a treatment modality by itself or as an adjunct to conventional modalities for malignant intracranial neoplasms is currently being analyzed using experimental models. (Supported by Amer. Cancer Soc. Inst. Grant, Appert Mem. Fund and the Neurosurg. Res. Fund)

182.10 CLINICAL SIGNIFICANCE OF CHANGES IN SUPPRESSOR T-CELL (S-CELL) FUNCTION IN CHRONIC PROGRESSIVE MULTIPLE SCLEROSIS (CPMS) DURING PLASMAPHERESIS (PP) AND IMMUNOSUPPRESSIVE DRUG THERAPY. B. Khairi*, S. Koethe*, K. Mezra*, and M. McQuillen. Depts. of Neurology and Pathology, Med. Coll. of Wis., Milwaukee, WI 53226.

Alterations in S-cell function in 24 consecutive patients with CPMS correlate with clinical improvement, as measured by a modified Kurtzke disability status scale (DSS) and functional systems scale (FSS) (Neurology 15:654, 1965), during weekly PP given in conjunction with cyclophosphamide (1-1.5 mg/kg/day) and prednisone (1 mg/kg/alternate day). The patients segregated into two groups, depending upon the degree of their clinical improvement. Group I (n=8) showed the greatest improvement (in DSS by >3 steps; on FSS by >6). Group II was subdivided according to the pattern of improvement -- Group IIA (n=4) showing steady improvement commencing with the first PP; and Group IIB (n=12) following a pattern of slight improvement during the first 5 PP, followed by a clinical "plateau" (accompanied by depression and increasing bladder problems), and then gradual but sustained improvement thereafter.

Concanavalin A (Con A; 10µg/ml) was used to induce S-cells, with their activity expressed in a mitogen response assay using Con A for further stimulation. The Mann-Whitney test for significance of non-parametric data was used to compare S-cell activity pre- and post-PP, and with 19 normal controls. Significance is at the $p<0.01$ level.

Patients with significantly low S-cell function pre-PP showed steady and sustained clinical improvement commencing with the first PP; the S-cell functional assay did not distinguish patients showing marked improvement (Group I) from those experiencing only modest clinical change (Group IIA). Patients with S-cell function indistinguishable from normal controls pre-PP (Group IIB) showed similar though modest improvement, but only after S-cell function dropped to significantly low values (generally after 5 PP). S-cell function in all patients approached normal values after their final PP.

182.11 THE EFFECTS OF INCREASING EXTRACELLULAR Ca^{++} CONCENTRATION UPON NEURONAL EXCITABILITY AND LONG-TERM POTENTIATION IN HIPPOCAMPAL SLICES FROM ALUMINUM ENCEPHALOPATHIC RABBITS. B.J. Farnell*, U. De Boni* and D.R. Crapper McLachlan, Dept. of Physiology, Univ. of Toronto, Toronto, Canada. M5S 1A8.

We previously reported a progressive decline in the CA1 population spike evoked in slices from rabbits with an aluminum induced encephalopathy (Soc. Neurosci. Abstr. #248.10 1980). This occurs in the absence of changes in the evoked population epsp and antidromic population spike and suggests an aluminum induced uncoupling between epsp input and spike output. The effects of increasing extracellular $[Ca^{++}]$ upon these phenomena are now reported. Four week old rabbits received either 5 µmoles aluminum lactate or 15 µmoles sodium lactate into each lateral ventricle. Slices were prepared from the dorsal hippocampus at 5, 10, 15 and 20 days post injection. A standard medium was used containing 2.4 mM $CaCl_2$. Using a perfusion chamber the total bath volume was exchanged in less than one minute with a medium containing 4.8 mM $CaCl_2$. Input - output curves relating stimulation strength, population epsp and population spike were analysed. Following a conditioning stimulus of 100 Hz. for 1 sec. to the stratum radiatum, long-term potentiation (LTP) was assessed in CA1. The criteria was an increase in the population spike of at least 25% above the baseline.

In contrast to control slices, after increasing the extracellular $[Ca^{++}]$ the magnitude of the evoked population spike is increased dramatically ($p < .001$) in slices from 15 and 20 day post aluminum animals in response to both apical and basilar inputs. This relationship remains when population spike amplitude is plotted against epsp amplitude, which isolates the epsp - spike coupling relationship from $[Ca^{++}]$ induced changes in the stimulation - population epsp relationship. The augmentation of orthodromic excitability is fully developed within 15 minutes after total bath exchange and is stable for at least 45 minutes. The effect is reversible. Elevated Ca^{++} increases both the percentage of slices developing LTP and the average magnitude of the potentiation ($p < .01$) in 10, 15 and 20 day post aluminum treated animals. The increase in $[Ca^{++}]$ must be present during and immediately following the tetanus in order to produce the LTP enhancement. This enhancement remains when the $[Ca^{++}]$ is returned to 2.4 mM in the 5 to 10 min post tetanus period. There appear to be at least two calcium dependant processes altered in the aluminum encephalopathy.

Supported by the Canadian Geriatrics Research Society.

182.12 PATHOPHYSIOLOGY OF EXPERIMENTAL ARTERIAL STENOSIS. P.A. Grady and O.R. Blaumanis* Dept. of Neurology, University of Maryland Sch. of Med., Baltimore, MD 21201

Hydrodynamic theory and experiments with models of arterial stenoses have suggested that abnormal flow patterns may play a role in the pathogenesis of several vascular lesions such as mural thrombus, atherosclerosis, poststenotic dilation and aneurysm. We have examined the consequences of disturbed flow patterns in experimental animals (dog, cat and rabbit). Arterial stenoses were created in the aorta and common carotid, vertebral and renal arteries by partial ligation (40-90% area reduction). Blood vessels supplying the brain were of primary interest because of the high incidence of stenosis in these vessels and the effect on perfusion. Segments of arteries consisting of several centimeters proximal and distal to the stenosis were examined with the scanning electron microscope from one hour to one year after ligation.

A number of morphological changes were seen involving endothelial cells lining blood vessels. These changes extended from nuclear disruption of endothelial cells to restructuring of luminal surfaces of vessels. Short term effects included infiltration of monocytes followed by platelet and red blood cell thrombi. Long term effects included restructuring of endothelial surfaces.

Regardless of the degree or duration of the stenosis there was invariable disruption of the endothelium, platelet aggregation and in many instances mural thrombosis. The location of endothelial disruption and thrombosis found in this study corresponds to the predictions of hydrodynamic theory and the studies of flow patterns in glass models. The lesions did not heal with time and were not atherosclerotic in nature. These experimental results confirm the theoretical and model studies and suggest that even a mild arterial stenosis has a thrombogenic potential.

182.13 EFFECTS OF PENTOBARBITAL ON ION FLUX AFTER CEREBRAL MICROEMBOLISM IN THE RAT. J.P. Hansen* and W.J. Giardina. Department of Pharmacology, Abbott Laboratories, North Chicago, IL 60064

Cerebral ischemia can be induced by intra-arterial microsphere injections (Kogure, et al., Brain, 97:103-114, 1974; Siegel, et al., Arch. Neurol., 26:73-77, 1972). The protective effects of pentobarbital against cerebral ischemia in animals have been widely reported (Michenfelder, et al., Arch. Neurol., 33:345, 1976; Moseley, et al., Neurol., 25:870, 1975). To study the effects of pentobarbital on ischemia following microembolization, cerebral ischemia was produced in male, Sprague-Dawley rats, weighing 180-240 gms. The rats were anesthetized with sodium pentobarbital (40 mg/kg i.v.). The left external carotid artery was ligated. The left common carotid artery was cannulated rostrally with a 20 gauge cannula. Approximately 50,000 microspheres (3-M, 25±5 μ average diameter) suspended in normal saline (0.2 cc) were injected over 30 seconds. The cannula was removed and the artery ligated. Animals were sacrificed at 2 or 24 hours after injection of the microspheres to evaluate the effects of pentobarbital on early and late brain ischemia. The brains were removed, halved and wet weights were recorded. The brains were dried for 72 hours at 100 C. Dry weights were recorded. Dried brains were analyzed for sodium (Na) and potassium (K) content by flame photometry. Na, K and water content of microembolised (E) hemispheres was compared to that of nonembolised hemispheres.

In saline treated animals sacrificed at 2 and 24 hours, K content of E hemispheres was significantly decreased and Na and water contents of E hemispheres were significantly increased. In animals sacrificed at 24 hours, pentobarbital (40 mg/kg i.p.) (P) administered 30, 120 and 180 minutes after microembolization prevented significant changes in Na and K concentrations. P administered at 60 and 120 minutes also prevented significant changes in water content. In rats sacrificed at 2 hours, P administered at 45 minutes after microembolization did not prevent significant changes in Na concentration and water content of E hemispheres, but P prevented the significant decrease in K concentration of E hemispheres. Compared with saline treated rats K content was significantly increased in both hemispheres.

The K ion changes observed 2 hours after microembolization reflect cytotoxic events in the brain resulting from ischemia. The mechanism for increase in cellular K may be a stabilizing effect of pentobarbital on cell membranes (Astrup, et al., Acta Neurol. Scand. 64:148, 1977).

182.14 ANALYSIS OF THE E-C COUPLING: GENOTYPE RELATIONSHIP IN MOSAIC (MUSCULAR DYSGENIC, mdg ↔ NORMAL, +) MYOTUBES. Alan Peterson[1], David Cross[1]*, Rohan Bhup[2]*, and Sergio Pena[2]*. [1]Neuroscience Unit, Montreal General Hospital and [2]Montreal Neurological Institute, McGill University, Montreal, P.Q. Canada.

Mice affected by the recessive muscular dysgenesis, mdg mutant express a dramatic failure of in vivo myogenesis with skeletal muscle typically achieving only an abnormal and immature myotube stage by birth (Cluecksohn-Waelsch, S., Sci. 142:1269-1276, 1973). Newborns demonstrate no spontaneous movements and die presumably of respiratory failure. In vitro, mdg/mdg myoblasts proliferate well and form extensive myotubular plexes. However, these myotubes do not contract to ACh stimulation and a failure in normal excitation-contraction coupling mechanisms has been proposed (Powell, J. and Fambrough, D., J. Cell Physiol. 82:21-38, 1973). The contractile response and genotype of mosaic myotubes containing +/+ and mdg/mdg myonuclei was investigated to determine: (1) if such myotubes would contract either spontaneously or in response to ACh; and (2) if contraction was expressed, what proportion of +/+ myonuclei would be required.

Potentially, mosaic myotubes were derived by simultaneously plating +/+ and mdg/mdg myoblasts in the same culture plates with the expectation that both would enter common myotubes during in vitro maturation. In addition to their +−,mdg difference these myoblasts differed also in an inherited electrophoretic variant of the enzyme glucosephosphate isomerase (GPI). After assessing the spontaneous or evoked contractile phenotype of individual myotubes, they were dissected from the culture plate and the ratio of GPI types expressed in each was determined by microelectrophoretic techniques (Peterson, A., Frair, P. and Wong, G., Biochem. Genet. 16:681-690, 1978) to provide an estimate of +/+ and mdg/mdg myonuclear proportions.

Mosaic mdg/mdg ↔ +/+ myotubes do form in vitro. They contract spontaneously or in response to ACh and the apparent proportion of +/+ myonuclei necessary to confer this in vitro activity is approximately 1%. This result limits the number of theoretically possible defects underlying the mdg disease and further, demonstrates a strategy of genotype assessment applicable to mosaic myotubes derived from a wide range of species. (Supported by MDAC).

183.1 A NEUROPHARMACOLOGICAL EXAMINATION OF THE EFFECTS OF NOREPINEPHRINE ON HYPOTHALAMIC NEURONS. W.-H. Tsai, F. C. Barone, M. J. Wayner and R. Guevara-Aguilar. Brain Research Lab., Syracuse Univ., 601 University Ave., Syracuse, NY 13210 and Depto de Fisiologia, Facultad de Medicine, U.N.A.M., Mexico.

Seven barrel electrodes were utilized to record simultaneously from and apply chemicals to single neurons located in the lateral hypothalamus (LH) of urethane anesthesized rats. The center barrel, used for electrical recording, and one of the outer barrels, used for current balancing, were filled with 2 M NaCl. Two other outer barrels were filled 0.5 M L-Norepinephrine bitartrate and 2 M L-Glutamic acid. The other three barrels were filled with 0.01 M Yohimbine HCl, 0.01 M Propranolol, 0.05 M Sotalol HCl or 0.05 M L-Phenylephrine HCl. When a stable baseline discharge frequency was established for single LH neurons, chemicals were administered microiontophoretically. Appropriate control procedures were utilized to eliminate current and pH effects and dose effect relations were determined. In addition, various regions known to contain noradrenergic neurons which project to the LH such as the nucleus of the solitary tract, locus coeruleus, and the medial mesencephalic reticular formation (Barone et al., Brain Res. Bull. 7, in press; Takagi et al., Brain Res. 193: 315-338, 1980) were stimulated with small concentric bipolar electrodes, 0-500 μA of 0.5 msec single pulses, in conjunction with neuropharmacological testing. As described previously (Barone et al., Brain Res. Bull. 5: 325-332, 1980), the microiontophoretic ejection of glutamate increases neuronal discharge frequency while norepinephrine usually decreases it in this part of the brain. All other chemicals tested significantly decreased the discharge frequency of LH neurons. Yohimbine was the most effective and decreased neuronal firing at the lowest ejection currents. In some cases, if antagonists were applied for long periods (1-4 min) using low ejection currents, the blocking effects of these substances could be demonstrated. Under these conditions phentolamine and sotalol were able to attenuate norepinephrine induced decreases in neural activity. Results indicate that the effects of norepinephrine on LH neurons are probably mediated by α and β postsynaptic receptors which receive noradrenergic inputs from brain stem structures. However, since neurons were most sensitive to yohimbine, presynaptic noradrenergic mechanisms might also be significant. (Supported by NIH Grant NINCDS USPHS No. 13543.)

183.2 NORADRENERGIC SYSTEM IN CULTURE: AN ELECTROPHYSIOLOGICAL STUDY. R.Y.K.PUN, K.C.MARSHALL†, S.FITZGERALD*, P.B.GUTHRIE, P.G.NELSON. Lab. Devel. Neurobiol., NICHD, NIH, Bethesda, MD 20205; † Dept. of Physiol., Univ. of Ottawa, Ottawa, Ontario, K1N 9A9, Canada

We previously reported the development of an in-vitro system in which dissociated mouse spinal cord cells were co-cultured with locus coeruleus (LC) region of the brain stem (Marshall et al., Canada Physiol., 11: 103, 1980). Noradrenaline (NA) was suggested to mediate the slow depolarising responses evoked by the stimulation of the explant. We present evidence here to further support this hypothesis. Our data further suggest that the frequency of occurence of the depolarising responses is enhanced by the innervation by the LC neurones.

The depolarising responses evoked by stimulation of the explant and by exogenously applied NA were accompanied by a small increase (<20%) or no change in input resistance of the membrane. The membrane time constant, determined with hyperpolarising pulses, was increased at the peak of the evoked responses.

Both the neurally evoked response and the response to NA were antagonised by the α-adrenoceptor antagonists piperoxan (7 cells, 5 and 50 μM), phentolamine (2 cells, 1 μM) and phenoxybenzamine (3 cells, 1 μM). However, the antagonism of the latter compounds was more rapid and effective for the response to NA than for the neurally evoked response. The β-adrenoceptor antagonist propranolol did not antagonise the responses at the concentration tested (4 cells, 2.5 μM).

Neurones responding to the stimulation of the explant showed a high incidence of depolarising response to applied NA (69%). In contrast, spinal cord neurones not innervated by LC neurones had a very low incidence of depolarising response to applied NA (14% in cultures without explant, 11% in cultures with explants but in cells not responding to stimulation of the explant). It remains to be determined whether the presence of noradrenergic terminals 1) induces an increase in sensitivity of spinal neurones to NA or 2) promotes the survival of spinal neurones that are sensitive to NA.

P.B.G. is supported by the Muscular Dystrophy Association.

183.3 ACTIVATION OF LOCUS COERULEUS NEURONS IN CLONIDINE WITHDRAWAL DUE TO REDUCED RESPONSIVENESS OF α_2-RECEPTORS. T.H. Svensson, G. Engberg* and M. Elam*. Dept of Pharmacology, University of Göteborg, S-400 33 Göteborg, Sweden.

Sudden withdrawal of chronic treatment with the centrally active, antihypertensive α_2-adrenoceptor agonist clonidine has been associated with a rebound syndrome, including a hypertensive reaction, tachycardia and mental symptoms such as anxiety, restlessness and insomnia. It is also accompanied by increased peripheral catecholamine (CA) secretion.

Recent evidence indicates increased sensitivity of centrally located postsynaptic adrenergic receptors in clonidine withdrawal presumably due to functional denervation, since clonidine causes inhibition of central noradrenergic neuronal activity (Svensson et al., Brain Res. 92, 291, 1975). We have also shown biochemical evidence for increased activity in central noradrenaline (NA) pathways in the withdrawal phase (Svensson & Strömbom, Naunyn-Schmiedeberg's Arch. Pharmacol. 299, 83, 1977). Here we have used single cell recording techniques and microiontophoresis to characterize the function of brain NA neurons in locus coeruleus (LC) of rats after discontinuation of a chronic clonidine regimen (100-200 μg/kg/day for 14 days).

During treatment the average firing rate of LC neurons remained significantly reduced, whereas after withdrawal of the drug it was largely increased above base-line (maximum 60 per cent, 25--35 h after discontinuation). In withdrawal phase, the LC neurons showed a significantly reduced responsiveness to the inhibitory effect of systemically or microiontophoretically applied clonidine, as reflected in the larger charge (C_{50}) required to obtain a 50 per cent decrease in the rate of spontaneous firing. In contrast, the inhibitory response of the LC neurons to morphine in analogous experiments was unchanged.

The degree of reduced responsiveness of the neurons to the α_2--receptor agonist was directly correlated to their degree of activation above base-line. Thus, the LC activation in clonidine withdrawal is related to, and probably caused by, the specifically reduced responsiveness of the α_2-receptors on these cells, which seem to mediate the inhibitory influence on the LC of noradrenergic axon collaterals.

Since electrical stimulation of LC has been found associated with restlessness and increased blood pressure, the activation of central NA neurons such as those in the LC may in clonidine withdrawal, analogously to the situation in morphine abstinence, be important for the mediation of the withdrawal syndrome. (Supported by the Swedish Medical Research Council, project no. 4747).

183.4 RELATIVE POTENCIES OF ANTAGONISTS AT α_2-ADRENOCEPTORS OF THE LOCUS COERULEUS AND α_1-ADRENOCEPTORS OF THE LATERAL GENICULATE NUCLEUS. Jwaharlal Marwaha and George K. Aghajanian, Depts. of Psychiat. and Pharmacol., Yale Univ. Sch. Med., New Haven, CT 06508.

Previous studies (Cedarbaum and Aghajanian, Brain Res., 112, 413, 1976 and Rogawski and Aghajanian, Brain Res., 182, 345, 1980) have shown that the adrenoceptors of the locus coeruleus (LC) are of the α_2-type and those of the dorsal lateral geniculate (dLGN) of the α_1-type. In vitro studies in the periphery (Starke and Docherty, J. Cardiovas. Pharmacol., 2, S269, 1980) show the following order of potency for antagonists at α_1-adrenoceptors: prazosin ≥ WB-4101 > corynanthine >> piperoxane >>> yohimbine > rauwolscine; the reverse order applies to α_2-adrenoceptors. In the present study, the potencies of the above antagonists to reverse the inhibitory effects of the α_2-agonist, clonidine (i.v. and iontophoretic) in the LC or the activation of dLGN neurons by iontophoretic application of the α_1-agonist, phenylephrine were examined by single-cell recording in chloral hydrate anesthetized rats.

Clonidine (20 μg/kg, i.v.) totally suppressed LC neuronal firing for at least 20 minutes. Antagonists administered intravenously 3 minutes after clonidine inhibition of LC firing restored neuronal firing with the following order of potency: rauwolscine = yohimbine > piperoxane >>> WB-4101 ≥ corynanthine = prazosin. Rauwolscine, yohimbine, and piperoxane were able to completely reverse the effect of clonidine, but WB-4101, corynanthine and prazosin were often inactive at cumulative doses of up to 3-4 mg/kg. Similar results were obtained when clonidine was administered iontophoretically.

Phenylephrine applied iontophoretically to dLGN neurons produced a brisk activation of firing which could be maintained without attenuation for 15-20 minutes. Alpha-antagonists reversed this activation in the following order: prazosin = corynanthine = WB-4101 >> piperoxane = yohimbine = rauwolscine. The ED_{50} for WB-4101 was 20 μg/kg while that for piperoxane and yohimbine 300 μg/kg and 350 μg/kg respectively. ED_{50} values for piperoxane and yohimbine in the LC were 600 μg/kg and 350 μg/kg respectively.

Our results suggest that the order but not the magnitude of potencies for α-antagonists observed in vitro in the periphery is largely valid for the adrenoceptors of the LC and dLGN. The above antagonists of α_1-receptors are highly selective in that they have very weak activity at α_2-receptors. In contrast, piperoxane and yohimbine which have hitherto been considered selective α_2-antagonists block α_1-receptors at a similar dose as that required to block α_2-receptors.

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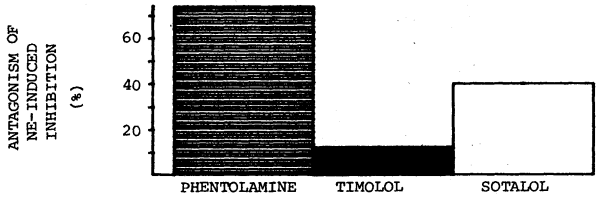
183.5 NORADRENERGIC RESPONSES IN RAT HIPPOCAMPUS: EVIDENCE FOR THE PARTICIPATION OF α AND β COMPONENTS IN VIVO. Alan L. Mueller*, Michael R. Palmer, Barry J. Hoffer*, and Thomas V. Dunwiddie. Dept. of Pharmacology, Univ. of Colorado Health Sciences Center, Denver, CO 80262.

Utilizing the in vitro rat hippocampal slice preparation, we have previously demonstrated that noradrenergic responses can be pharmacologically separated into distinct α- and β-receptor-mediated components (Mueller, Hoffer and Dunwiddie, Soc. for Neurosci. Abstracts 6: 607, 1980; Mueller, Hoffer and Dunwiddie, Brain Research, in press). Activation of α-receptors and β-receptors leads to a decrease and an increase, respectively, in hippocampal pyramidal neuron population spike size. In this study, the effect of locally applied norepinephrine (NE) on single action potentials of identified pyramidal (P) cells in region CA1 of the in vivo rat hippocampus was examined. Pressure ejection of NE from one barrel of a multibarreled pipette generally produced depressions of P cell single unit activity. These NE-induced inhibitions were effectively antagonized by the concurrent pressure ejection of the α-receptor antagonist phentolamine. In contrast, the β-receptor antagonist timolol was without effect. Another β-receptor antagonist, sotalol, was found to occasionally elicit a partial but inconsistent antagonism of the NE-induced inhibition. In approximately 25% of the cases, micropressure ejection of NE resulted in an increase in P cell single unit discharge. These NE-induced excitations were effectively antagonized by timolol and sotalol and were largely unaffected by phentolamine. Finally, local application of the β-receptor agonist isoproterenol and the α-receptor agonist clonidine, by micropressure ejection, usually resulted in increases and decreases, respectively, in P cell activity. Taken together, these results provide additional support for the hypothesis that exogenous NE is able to interact with both α- and β-adrenergic receptors to decrease and increase, respectively, pyramidal cell excitability. (This work was supported by DA-02702 to T.V.D.)



183.6 METHYLXANTHINE EFFECTS ON NE NEURONAL ACTVITY ASSOCIATED WITH THE QUASI-MORPHINE WITHDRAWAL SYNDROME. S.J. GRANT, D.E. REDMOND, Jr. Dept. of Psychiatry, Yale School of Medicine, New Haven, CT 06510.

The reversal by clonidine of increases in noradrenergic (NE) neuronal function and of the clinical manifestations of the opiate withdrawal syndrome have been demonstrated. These studies were undertaken because of the observation that increased NE function in monkeys was similar to opiate withdrawal. Since certain non-opiate compounds, primarily methylxanthines, precipitate withdrawal-like syndromes which are potentiated by naloxone and blocked by opiates, it has been suggested that a common mechanism underlies true morphine withdrawal and the "quasi-morphine withdrawal syndrome" (QMWS) produced by methylxanthines. The present study was designed to test whether brain NE systems may, in fact, represent such a common mediator. If so, methylxanthines should increase noradrenergic function, as is observed during opiate withdrawal, and clonidine should reverse such an increase. In addition, the behavioral signs associated with the QMWS should be attenuated by the alpha-2 adrenergic agonist clonidine.

Single unit activity was recorded from the principal brain NE nucleus, the locus coeruleus (LC) using conventional extracellular recording techniques in chloral hydrate anesthetized rats. Isobutylmethylxanthine (IBMX) (1-10 mg/kg I.V.) markedly increased unit activity in the locus coeruleus. Clonidine (5-15 microgram/kg I.V.) restored unit activity to baseline or below. Clonidine also reduced the behavioral effects of IBMX in a pilot behavioral study. The QMWS was precipitated in drug naive rats by a combination of IBMX (10-15 mg/kg I.P.) and naloxone (1 mg/kg I.P.). The subjects were pretreated with either clonidine (50 microgram/kg I.P.) or saline. Seventeen withdrawal symptoms were rated by one or two observers blind to the drug treatment. Clonidine pre-treated rats exhibited significantly less diarrhea, ptosis, salivation, rhinorrhea, lacrimation, jumping, rearing, wet dog shakes, grooming and restlessness compared with saline treated animals. However, squeaking on touch was increased after clonidine pre-treatment.

These results are consistent with the hypothesis that brain noradrenergic neurons may mediate the QMWS induced by methylxanthines, as well as some aspects of true opiate withdrawal. This possibility is supported by other biochemical evidence of interactions of methylxanthines with NE function based on changes in cyclic adenosine monophosphate or on effects at adenosine receptors, both of which may alter NE function.

This work was supported by USPHS grants DA02321, MH31176, MH14276. RSCDA Grant KO2-DA00075 to DER.

183.7 ACTIVATION OF LATERAL GENICULATE NEURONS BY NORADRENERGIC PATHWAY STIMULATION: SELECTIVE BLOCKADE BY THE α_1-ADRENOCEPTOR ANTAGONIST PRAZOSIN. Michael A. Rogawski and George K. Aghajanian. Departments of Pharmacology and Psychiatry, Yale University School of Medicine, New Haven, CT 06510.

Previously we reported that electrical stimulation in the region of the locus coeruleus (LC) facilitates the firing of single dorsal lateral geniculate nucleus (LGN) neurons (Nature 287: 731-734, 1980). Iontophoretic norepinephrine (NE) mimics this effect (Brain Res. 182: 345-359, 1980) and both the responses to stimulation and to locally applied NE are blocked by the α_1-antagonist WB-4101. These observations suggested that LC stimulation activates LGN neurons via the release of NE from coeruleo-geniculate fibers, although inadvertant activation of other pathways could account for the data as well. There are two extraretinal brainstem inputs to the LGN in addition to the LC projection: (1) a serotonergic pathway from the raphe nuclei and (2) a cholinergic pathway from the dorsal midbrain tegmentum (Hoover and Jacobowitz, Brain Res. 170: 113-122, 1979). Serotonin suppresses the activity of LGN neurons whereas acetylcholine (ACh) and the non-hydrolyzable cholinergic agonist carbachol (CCh) are facilitatory. The present study provides evidence against the participation of ACh in the effects of LC stimulation.

Extracellular recordings were carried out in the LGN of rats lightly anesthetized with chloral hydrate. Pulse trains (1 ms, 10 Hz, 0.3-0.8 mA) applied in the vicinity of the LC produced a marked acceleration in the spontaneous firing of LGN neurons; activations were unassociated with the individual stimuli and often were delayed in onset and persistent beyond the termination of the train. Similar effects were obtained with stimulation of the LC axons in the dorsal NE bundle (DB) except that lower currents were effective (0.1-0.3 mA). Intravenous or iontophoretic WB-4101 antagonized the effects of LC stimulation. However, WB-4101 also diminished the response to iontophoretic ACh indicating that it had cholinergic blocking activity and would not discriminate between a NE and ACh pathway. The antihypertensive α_1-antagonist prazosin selectively blocks NE but not CCh in the LGN (Menkes, Baraban & Aghajanian, submitted). In contrast to WB-4101, prazosin (120 μg/kg, i.v.) blocked the response to LC or DB stimulation (up to 30 min) without affecting responses to iontophoretic CCh. In addition to confirming that LC stimulation activates LGN neurons via a NE pathway, these experiments demonstrate that systemically administered prazosin can influence central α-adrenergic transmission in clinically relevant doses.

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183.8 EFFECT OF YOHIMBINE ON RAT SERUM PROLACTIN LEVELS, H. Y. Meltzer*, M. Simonovic and R. So. Department of Psychiatry, University of Chicago Pritzker School of Medicine, Chicago, IL. 60637.

Pharmacologic and binding studies have established that yohimbine (Y), a naturally occuring alkaloid, is a selective antagonist of adrenergic alpha$_2$ receptors. In addition, Y has been reported to increase dopamine (DA) turnover in the brain suggesting that it may also be a DA receptor blocker. At least two types of DA receptors are believed to exist in the brain: D_1 which is coupled with adenylate cyclase and D_2 which is not linked to adenylate cyclase. It has recently been proposed that Y blocks D_2 receptors (Scatton et al., J.Pharmacol. Exp. Ther. 215:494,1980).

Rat prolactin (PRL) secretion is under tonic dopaminergic inhibitory control. Thus, blockade of pituitary DA receptors results in a prompt rise in circulating PRL levels. The DA receptors involved in the regulation of PRL secretion are believed to be of the D_2 type. In order to test the postulated ability of Y to block D_2 receptors, we have examined its effect on rat PRL secretion and its ability to displace ^{3}H-spiroperidol from bovine anterior pituitary membrane preparations. The ability of neuroleptic drugs to stimulate rat PRL secretion in vivo is correlated with these ability to displace ^{3}H-spiroperidol from rat pituitary. Y(1, 5 or 10 mg/kg,ip) was administered to male rats 30 min before decapitation. The two higher doses produced marked increases in serum PRL levels. The rise in serum PRL levels due to Y (5 mg/kg,ip) lasted for more than two hours, and was completely blocked by apomorphine (3 mg/kg,sc). The ability of Y to stimulate rat PRL secretion is not due to its alpha$_2$ antagonist properties since another selective alpha$_2$ antagonist, piperoxane (1 10 or 20 mg/kg) had no effect on serum PRL levels. Clonidine (0.1 mg/kg,ip), which can effectively antagonize the presynaptic effects of Y, had no effect on Y-induced increase in serum PRL levels. Thus, stimulation of PRL secretion by Y is not due to the blockade of presynaptic alpha$_2$ receptors. Y does not effectively compete with ^{3}H-spiroperidol for binding sites on membrane preparations from anterior pituitary membranes. This could mean that Y exerts its effect on PRL secretion by binding to a separate population of DA receptors on pituitary lactotrophs not labeled by ^{3}H-spiroperidol. It is also possible that Y stimulates PRL secretion through a non-dopaminergic mechanism. This research was supported by USPHS MH 30,938 and MH 29,206.

183.9 EVIDENCE FOR A NEW TYPE OF ALPHA-ADRENERGIC RECEPTOR IN THE CEREBRAL (PIAL) MICROCIRCULATION. S. Lassoff* and B.M. Altura. Dept. of Physiol., SUNY Downstate Med. Ctr., Brooklyn, NY 11203.

Recent biochemical studies on isolated brain preparations suggest the presence on cerebral microvessels of binding sites for alpha-and beta-adrenergic stimulants. However, no quantitative in vivo functional studies exist. With this in mind, we undertook quantitative in situ studies on pial terminal arterioles (PTA) in the anesthetized rat in order to determine the structure-activity relationships (SAR) of alpha-adrenergic stimuli. Animals of both sexes, weighing 150-350g, were subjected to craniotomy and removal of the dural membrane. High-resolution closed-circuit television microscopy (at magnifications up to 3000x) allowed measurement of pial vessel diameter down to 0.02 μm. The animals remained normocapnic (PCO_2 = 29-34 mm Hg) and normotensive (systolic average pressure = 115-120 mm Hg) throughout testing. Neither epinepherine (E), α-methylnorepinepherine, nor isoproterenol (up to 10^{-1} mg), when applied perivascularly, elicited any constrictor or dilator responses in PTA, either in the absence or presence of phentolamine (PH) and propranolol (PR). Phenylephrine (PE), however, produced potent concentration-dependent constriction of PTA (7-30%, 10^{-8}-10^{-2} mg) while norepinephrine (NE) and dopamine (D) elicited equi-potent constrictions (5-20%, 10^{-6} to 10^{-1} mg); these were less potent then those elicited by PE. PH (5-10 pg), but not PR, shifted the concentration-response curves for PE, NE, and D rightward in a parallel manner. Intracarotid infusion of all adrenergic agents (10^{-8} mg to 10^{-1} mg) produced no significant change in PTA size. Although α-adrenergic receptors exist in PTA, the SAR (i.e., where PE $>$ NE $\cong$ D $\gg$ E) is atypical of that found in other microvasculatures and on peripheral arteries and veins. (Supported in part by PHS Grants HL-18015, DA-02339 and T32 NS 07117-02).

183.10 (Na^+,K^+)-ATPase, OUABAIN BINDING, AND NORADRENERGIC ACTIVITY. A.C. Swann, S.E. Hattox, S.J. Grant, J.W. Maas. Dept. of Psychiatry, Univ. of Texas Med. Sch., Houston, TX 77025, and Yale Univ. Sch. Med., New Haven, CT 06510.

The molecular mechanism of the hyperpolarization produced by norepinephrine (NE)$_+$ has not been established. NE has been shown to stimulate (Na^+,K^+)-Adenosine triphosphatase (ATPase) in vitro; this is consistent with neurophysiologic effects of NE, but may result from nonspecific properties of NE rather than receptor binding. We have shown, however, that acute NE stimulation in vivo increases ATPase activity (Life Sci 28, 251). If ATPase mediates any NE effects, its adaptation to chronic or repeated changes in NE activity may be important in NE regulation. The experiments reported here examine 1) the effects of acute NE stimulation and inhibition on ATPase activity and on NE impulse flow as indicated by the NE metabolite 3-methoxy-4-hydroxyphenylglycol (MHPG) and 2) the effects of long term NE changes, produced by denervation, hyperinnervation, and repeated stimulation, on ATPase activity and on the binding of ouabain to the cardiac glycoside receptor associated with ATPase.

Acute NE inhibition by clonidine (50 μg/kg) or debrisoquin (10 mg/kg) decreased ATPase activity. NE stimulation with piperoxane (5 mg/kg) increased enzyme activity. Cerebral cortex MHPG and ATPase activity were strongly correlated ($r=0.85$; $P<.005$).

Denervation and hyperinnervation were examined by comparing ipsilateral and contralateral cerebral cortex and cerebellum, respectively, after unilateral 6-hydroxydopamine lesions of the dorsal NE bundle. Denervation decreased, and hyperinnervation increased, ATPase activity and ouabain binding. Daily injections of piperoxane also increased ATPase activity and ouabain binding 24 hours after the last dose.

Inhibition by Erythrosin B (Silbergeld, Neuropharmacol. 20,87) showed that the effects of denervation and hyperinnervation were accounted for entirely by changes in the nerve-specific form of ATPase (Sweadner, J. Biol. Chem. 254, 6060).

These results demonstrate parallel changes in NE impulse flow and ATPase activity. Chronic NE changes produced persistent effects on the binding of ouabain to its receptor associated with brain ATPase. The ouabain receptor has been shown to have endogenous ligands (Lichtstein and Samuelov, Biochem. Biophys. Res. Commun. 96, 1518; Gruber et al., Nature 287, 743). Changes in this receptor may be involved in long term NE effects.

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183.11 TRANSMITTER ACTIVATION OF REGIONAL BRAIN ATPases E.Undesser and D.H.Ross Division of Molecular Pharmacology Univ. Texas Health Sci. Center, San Antonio, TX 78284

Membrane ATPases regulate ionic flow in excitable cells. Recently Na-K ATPase has been shown to be stimulated by various putative neurotransmitters including norepinephrine (NE) and dopamine (DM). These transmitter activated ATPases have been suggested to play a role in transmitter release although a mechanism is not yet established. Sincetransmitters regulating ion flux may alter the release of other transmitters or neurohormones, we have investigated the interaction of two transmitters NE and DM with two membrane ATPases, Na-K, and Ca-Mg ATPase in different regions of rat brain. Regional brain tissue was prepared from male Sprague-Dawley rats(100-150gm) and crude P_2 fractions isolated by conventional techniques. P_2 fractions were lysed with TRIS-DTE buffer washed twice and resuspended to protein concentrations of 1mg/ml. ATPase activity was measured colorimetrically. Ca concentrations were regulated by EGTA buffers to 5 uM. Mg and ATP were added to final concentration of 250 uM in a HEPES buffer pH 7.4. Reactions containing 100 ug of membrane protein were started by addition of ATP and terminated 5 min later with TCA. NE (10^{-4}) had no effect on Mg ATPase. NE stimulated Na-K ATPase in the hippocampus, cerebellum, and brainstem ($P<.05$) but had no significant effect on Ca-Mg ATPase. DM showed no effects on Na-K ATPase but significantlly stimulated Ca ATPase in the cortex, striatum and hypoththalamus. These studies suggest that neurotrans mitters may differentially regulate the flow of ions in different brain regions. The ability to regulate calcium flux presynaptically may provide a mechanism for transmitters to regulate their own release as well as that of other neuromodulators.

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183.12 EVIDENCE FOR A ROLE FOR CATECHOLAMINES IN THE HEMISPHERIC CHANGES IN THE ACTIVITY OF A BRAIN ENZYME FOLLOWING A LOCAL CORTICAL INJURY. W.G. Dail, M.G. Boyeson, D.M. Feeney and H.M. Murray. Departments of Anatomy and Psychology, University of New Mexico School of Medicine, Albuquerque, NM 87131.

We have previously reported that a focal injury to the cortex of the rat results in a widespread depression or loss in histochemical staining for the enzyme alpha glycerophosphate dehydrogenase (α-GPDH) in the ipsilateral hemisphere (Dail et al., Brain Res. 211: 79, 1981). Reduction of enzyme staining, which was most evident in cortical layers II and III throughout the hemisphere, appeared as early as 36 hours after focal cortical ablation, laceration or contusion injuries. The staining pattern returned to normal by 9 days. We have investigated a possible role for catecholamines in the loss of enzyme staining since (1) amphetamine has been shown to speed behavioral recovery in injured animals (Feeney et al., Neurosci. Absts. 6: 802, 1980) and (2) small infarcts in the cortex lead to widespread reductions in brain catecholamine levels (Robinson et al., Nature 255: 332, 1979). In the present study, a single injection of d-amphetamine (2mg/kg i.p.) prevented the loss of enzyme staining which is normally seen at 4 days after an undercut laceration of the cortex. Moreover, d-amphetamine was effective only when administered 24 hours following the laceration and not when given at 18, 30, 36 and 72 hours post injury. This latter observation suggests that once the metabolic alterations are set in motion throughout the hemisphere, amphetamine cannot reverse the process. To determine if the staining pattern of the lesioned cortex would be changed in the catecholamine depleted brain, rats were studied following a unilateral radiofrequency lesion of the locus ceruleus (LC). A lesion of the locus ceruleus alone had no affect on α-GPDH staining, however, when a lesion of the locus ceruleus was followed in two weeks with a focal cortical injury, the well-defined loss of enzyme staining occurred at 12 hours, much earlier than with motor cortex injury alone. This result indicates that depletion of noradrenergic innervation to the cortex shortens the time to onset of the metabolic disturbance. The mechanisms underlying the effects of amphetamine and LC lesions on α-GPDH activity are currently not understood, although catecholamines, in particular norepinephrine, have been implicated in disturbances of oxidative metabolism (LaManna et al., Brain Res. 204: 87, 1981). Our pharmacological and LC lesion alterations of enzyme staining after cortical injury suggest an important role for noradrenergic systems in the cortical response to trauma. (Supported by NIH #NS-13684-03.)

184.1 NEUROTENSIN SELECTIVELY ACTIVATES DOPAMINERGIC NEURONS OF THE SUBSTANTIA NIGRA. Rodrigo Andrade and George K. Aghajanian. Depts. of Pharmacology and Psychiatry, Yale University School of Medicine, New Haven, CT 06510.

Neurotensin is a tridecapeptide originally isolated from bovine hypothalami by Caraway and Leeman (1). Subsequent studies have shown it to be located in nerve cell bodies and terminals (3). Neurotensin is released by K+ depolarization through a Ca^{2}+ dependent mechanism (2) and has an heterogeneous distribution in the mammalian CNS (3). In the midbrain the highest density of neurotensin receptors is present in the zona compacta of the substantia nigra (4). By immunohistochemistry, neurotensin-like immunoreactivity has been detected over the zona compacta but not over the zona reticulata (3). In the present study we have examined by single-cell recording and microiontophoresis the effects of neurotensin on dopaminergic and non-dopaminergic neurons in substantia nigra and compared its effects to that of substance P, an undecapeptide present in high concentrations in the substantia nigra.

Sprague-Dawley rats anesthetized with chloral hydrate were used in these experiments. Extracellular single-unit recordings were obtained through the center barrel of a 5 barrel micropipette. The remaining 4 barrels were used for current balancing and peptide microiontophoresis. Low currents of neurotensin (1-20 nA; 3 mM in 20 mM sodium acetate, pH 4.5) consistently excited dopaminergic neurons of the zona compacta. In contrast, non-dopaminergic neurons of the zona reticulata showed no response to similar currents of neurotensin. Unlike neurotensin, substance P (1-15 nA; 5 mM in 50 mM sodium acetate, pH 4.5) had no effect on dopaminergic neurons and excited only a small proportion of non-dopaminergic zona reticulata neurons.

These results support a neurotransmitter or neuromodulator role for neurotensin and suggest the existence of an excitatory neurotensin input to substantia nigra which could selectively activate the dopaminergic neurons in this region.

1. Caraway, R., Leeman, S.E., J. Biol. Chem. 248 (1976) 6854-6861.
2. Iversen, L.L., et al., Nature 273 (1978) 161-163.
3. Uhl, G.R., et al., Brain Res., 167 (1979) 77-91.
4. Young, W.S. III. and Kuhar, M.J., Brain Res., 206 (1981) 273-285.

Supported by USPHS Grants MH-17871, MH-14459, GM-07324, and the State of Connecticut.

184.2 POSSIBLE ELECTROTONIC COUPLING BETWEEN SETS OF RAT NIGRAL DOPAMINERGIC NEURONS. A. A. Grace and B. S. Bunney. Depts. Pharmacology and Psychiatry, Yale Univ. Sch. Med., New Haven, CT 06510

The activity of dopaminergic (DA) neurons in the mammalian substantia nigra is known to be affected by several inherent properties, including inhibitory autoreceptors for their own neurotransmitter. We report here data which suggests that an excitatory interaction, mediated by electrical synapses between two or more DA neurons, may also be present.

In vivo intracellular recordings were obtained from DA neurons in the substantia nigra zona compacta of albino male rats. In order to identify these cells as DA neurons, one of tnree compounds was injected intracellularly to increase the catecholamine fluorescence of the DA neurons studied. The compounds were: (1) L-DOPA (to load cell with DA precursor); (2) enzyme cofactor (to activate tyrosine hydroxylase) or (3) colchicine (to cause DA accumulation). Antidromic activation from the striatum further confirmed their identity as nigral DA neurons.

Presumptive anatomical evidence of coupling was obtained by intracellular injection of the dye Lucifer Yellow, which is known to cross gap junctions. Out of a total of 20 DA neurons injected with Lucifer Yellow, 6 neurons demonstrated coupling to one neighboring DA cell, and 2 neurons demonstrated 3-way coupling. However, as this dye has been observed to be taken up by damaged fibers when injected extracellularly in vivo, intracellular recording was necessary to confirm an electrotonic interaction.

Spontaneously occurring small (3-15 mV) potentials have been described in intracellular recordings obtained from DA neurons (Grace and Bunney, Science 210: 654-656, 1980). As these potentials exhibit a rate and firing pattern (1-8 Hz, irregular with occasional burst firing) similar to that seen extracellularly in DA neurons, the possibility that they arise from electrical coupling was investigated. These potentials could be activated antidromically from the caudate nucleus at a constant latency, follow high frequency antidromic stimulation, and collide with spontaneous small potentials but not reliably with somatic spikes. Depolarizing and hyperpolarizing current injection resulted in an increase and decrease, respectively, in their rate of spontaneous occurrence. The activity of these potentials also could be inhibited by i.v. apomorphine and excited by i.v. haloperidol.

This fast excitatory interaction between DA cells may be important in controlling synchrony between functionally related cells and may be related to their frequently observed burst firing. (Supported by USPHS Grants MH-28849, MH-25642 and the State of Connecticut.)

184.3 PROJECTION FROM AMYGDALA TO GLOBUS PALLIDUS VIA NUCLEUS ACCUMBENS: ELECTROPHYSIOLOGICAL STUDIES. C.Y. Yim* and G.J. Mogenson. Dept. of Physiology, University of Western Ontario, London, Canada N6A 5C1.

Anatomical studies have shown that the nucleus accumbens (NA) receives projections from the amygdala (AMY) and in turn projects to the globus pallidus (GP). Previous electrophysiological studies demonstrated that stimulation of the AMY produced short latency excitations in NA frequently followed by prolonged inhibition; stimulation of NA produced short latency inhibitions in GP that seemed to be GABA-mediated. The present study investigates whether output from the AMY is relayed to the GP via the NA and whether this relay is modulated by activity of the mesolimbic dopamine (DA) projection.

Extracellular single unit recordings were obtained from neurons in GP of urethane anesthetized rats using glass micropipettes filled with 0.5 M sodium acetate. The effect of electrical stimulation of AMY on neurons in the ventral part of the GP known to receive heavy projections from NA was investigated.

GP neurons had discharge rates of 10-30 spikes/sec. In 12% (10/82) of the neurons electrical stimulation of the AMY elicited inhibitory responses having a mean latency of 17 ms. The inhibitory response was followed in 6 cases by a prolonged period of excitation. Stimulation of the ventral tegmental area (VTA) with a train of 10 pulses (600μA, 0.15 ms delivered at 10 Hz) 1.1 sec before stimulation of the AMY attenuated the inhibitory response in 4 cases and in each case, the excitatory response that followed was not affected. Procaine hydrochloride (20% solution) injected into NA (0.3 μl) attenuated both the inhibitory response and the excitatory response that followed.

The response of GP neurons to AMY stimulation appears to be the reciprocal of the response of NA neurons. This is consistent with the hypothesis that output from the AMY may synapse directly on an inhibitory GABAergic neuron projecting to the GP. Results of microinjection of procaine HCl into NA support the hypothesis as well. It has previously been shown that stimulation of the VTA with a train of pulses attenuates the excitatory response of NA neurons to AMY stimulation but not the inhibitory response. The present study shows that responses of GP neurons to AMY stimulation can be affected in a similar way indicating that activity of the mesolimbic DA pathway was altering the response of output neurons from NA. NA has been considered as a possible interface between the limbic and motor systems. Results of the current study support the hypothesis, but the precise functional role of this pathway requires further investigation.

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184.4 STUDIES OF DOPAMINE AS A NEUROTRANSMITTER IN THE TURTLE OLFACTORY BULB. Martha C. Nowycky, Norbert Halász* and Gordon M. Shepherd. Sec. of Neuroanatomy, Yale Univ. Sch. of Med., New Haven, Ct. 06510.

Several recent studies have indicated that dopamine (DA) is present in part of the periglomerular cell population in the rat olfactory bulb, and may serve a neurotransmitter or neuromodulatory function (Hökfelt et al, Neurosci. Letters 1: 85, 1975; Halász et al. Brain Res. 154: 253, 1978; Priestley et al, Brain Res. 165: 149, 1979). In the course of physiological studies of the isolated turtle olfactory bulb, we have carried out preliminary studies of the possible role of dopamine in this structure.

Single volleys in the lateral olfactory tract (LOT) or olfactory nerves (ON) generate large field potential responses in the olfactory bulb (Nowycky et al., Soc. for Neurosci. Abstrs. 4: 583, 1978; Waldow et al. Brain Res. in press). Dopamine was added to the Ringer solution in the bath in concentrations of 10^{-6}M to 10^{-3}M. In the presence of DA, there was an increase in latency, and a slight decrease in peak amplitude, over most of the concentration range, with a steep fall in amplitude at the highest concentrations. The conduction velocity of the ON was not affected by DA. Using paired volleys, there was a profound and prolonged depression of responses to the test volley, over periods of several seconds, as previously reported. In the presence of DA there was less depression of the test response. The dopamine agonist apomorphine (10^{-5}M) caused similar effects as DA. The dopamine antagonist fluphenazine (10^{-3}M) in contrast was associated with a decreased latency and an increase in amplitude of single responses; with paired volleys, there was increased suppression of test responses, especially with LOT stimulation.

The uptake of ^{3}H-dopamine in the isolated turtle olfactory bulb has been studied with autoradiography. At the light microscopic level, the greatest amount of labelling was seen in the glomerular layer, within the glomerular neuropil and also in cell bodies surrounding the glomeruli. Heavy labeling was also seen over terminals in the granule layer. These results are similar to those previously reported in the rat. Additional labelling was found in cells of the periventricular region of the turtle. The labelled structures showing affinity for catecholamines may modulate the inhibitory responses as revealed in the physiological studies.

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184.5 DOPAMINE MODULATES GLOBUS PALLIDUS RESPONSES TO IONTOPHORETICALLY APPLIED GABA. J.R. Walters and D.A. Bergstrom. NIH, NINCDS, Bethesda, MD 20205.

Previous studies in our laboratory have demonstrated that systemically administered apomorphine (APO) or d-amphetamine markedly enhances the activity of spontaneously active neurons in the rat globus pallidus (GP). To determine whether stimulation of dopamine (DA) receptors located on GP neurons plays a role in mediating these effects, pallidal responses to direct application of DA were analyzed. We also examined whether DA, applied iontophoretically, modulates pallidal responsiveness to GABA's inhibitory actions.

Extracellular single unit responses of GP neurons were recorded in gallamine-paralyzed, locally anesthetized and artificially respired rats. Drugs were iontophoretically applied to spontaneously active cells via 5-barrel micropipettes: DA HCl, 0.2M, pH4; l-norepinephrine bitartrate (NE), 0.2M, pH4; acetylcholine HCl (ACH), 0.2M, pH4; GABA, 0.001M in 0.2M NaCl, pH4.

Direct application of DA increased baseline firing rates of 7 of 22 cells tested by an average of 23±2%, decreased the rates of 4 cells by 25±2%, and had no significant rate effect on 11.

Responses to iontophoretic pulses of GABA (30 sec duration at 30 sec intervals) were examined before and during ejection of DA (10nA, 3-5 min). Currents of GABA (0-10nA) were selected which, when administered alone, depressed neuronal firing by approximately 80%. When DA was simultaneously iontophoresed with GABA, GABA was approximately half as effective at inhibiting pallidal activity; DA significantly attenuated the inhibitory effects of GABA by 47±5% (n=22). DA antagonized GABA's effects on 3 cells by more than 75%. Similar reductions of GABA's ability to inhibit pallidal firing were observed when APO (80 µg/kg) was given i.v. and GABA applied directly. APO markedly reduced GABA's effects on 5 of 7 cells by an average of 48±9%. Subsequent administration of haloperidol (0.2 mg/kg, i.v.) reversed APO's effects.

To investigate the specificity of this DA-GABA interaction, effects of NE and ACH on GABA-inhibition were assessed. NE (10nA) exerted no consistent effects on GABA-induced decreases in pallidal activity: NE attenuated GABA's effects on 4 cells by an average of 22±8%, potentiated GABA's effects on 1 cell by 18.5% and had no marked rate effects on the remaining 4 cells. Moreover, ACH (10nA, n=6; 15nA, n=2) had no significant effect on GABA's ability to inhibit pallidal activity.

These results suggest that pallidal DA receptor stimulation plays a role in mediating APO-induced excitation of pallidal activity. Further, in addition to their other actions in the basal ganglia, DA agonists and, to the extent that DA fibers innervate the GP, DA itself may affect basal ganglia function by modulating GABAergic transmission in the globus pallidus.

184.6 APOMORPHINE INCREASES THE ACTIVITY OF RAT GLOBUS PALLIDUS NEURONS; EVIDENCE THAT THE SCHEDULE OF ADMINISTRATION DETERMINES THE MAGNITUDE OF EXCITATION. D.A. Bergstrom, S.D. Bromley* and J.R. Walters. NIH, NINCDS, Bethesda, MD 20205.

Dopaminergic agents may interact with several dopamine (DA) receptor types in the basal ganglia and substantia nigra to affect the neuronal activity of these nuclei. How the actions of DA agonists at different DA receptor subtypes and at different anatomical regions summate to affect information processing in the basal ganglia is not clear. In this study we hoped to gain some insight into the net effects of these agonists in the basal ganglia by investigating the effects of systemic administration of apomorphine (APO) on the tonic activity of the globus pallidus (GP). Low doses of APO which appear to preferentially stimulate DA presynaptic receptors and higher doses which stimulate DA postsynaptic receptors were studied.

Extracellular single unit responses were recorded from spontaneously active cells in the GP of rats paralyzed with gallamine, locally anesthetized and artificially respired; one cell was monitored per rat. All drug injections were given i.v. In general, nonsignificant rate increases were noted after 5 µg/kg (n=15) and 20 µg/kg (n=10) APO; only 1 cell showed a decrease in activity after 20 µg/kg APO. Significant increases in pallidal activity were observed with 80 µg/kg, 160 µg/kg, 320 µg/kg and 1 mg/kg (32±13%, 71±17%, 111±12%, 115±16%, respectively, n=7-18). APO-induced excitations were blocked by pretreatment with haloperidol (HAL) (0.4 mg/kg, n=5) or reversed by subsequent administration of HAL. The ergot derivatives, lisuride and pergolide (1µmol/kg, a dose equivalent to 320 µg/kg APO), also caused marked increases in activity (113±25%, 127±25%, respectively, n=5,5).

To determine whether different schedules of APO administration affect the magnitude and/or direction of neuronal firing changes in the GP, 3 different schedules of i.v. APO administration were compared. A single 320 µg/kg dose increased GP activity by 111±12% (n=18). Significantly attenuated responses to APO were observed when this dose (320 µg/kg) was administered in divided doses (5, 35, 280 µg/kg) 3-5 min apart or when a non-excitatory dose (20 µg/kg) of APO preceded the excitatory dose (320 µg/kg). Average increases of 39±19% (n=15) and 35±17% (n=9) were observed for these 2 dose schedules, respectively.

These data indicate that APO, 0.08-1.0 mg/kg, administered systemically, markedly enhances firing of spontaneously active GP neurons. Low doses (5, 20 µg/kg), which preferentially stimulate presynaptic DA receptors, did not cause (24/25 cells) effects opposite to those observed with higher doses. Moreover, the results suggest that the expression and magnitude of APO-induced excitation are influenced by the schedule of APO administration.

184.7 DOPAMINE EFFECTS ON STRIATAL NEURON ACTIVITY: A PUSH-PULL PERFUSION STUDY. E.P. Schoener and D.P. Elkins*. Department of Pharmacology, Wayne State Univ., Sch. Med., Detroit, MI 48201.

Effects of discretely perfused dopamine (DA) were studied on extracellular single unit activity in the rat striatum. Male Sprague-Dawley rats (250-300 gm) anesthetized with urethane (1 gm/kg, I.P.) were cannulated in order to monitor heart rate and blood pressure on a Grass 7C polygraph. The animal's head was fixed in a DKI stereotaxic apparatus and a 3 x 8 mm burr-hole was made in the skull at coordinates .5 - 3.5mm lateral of the midline and 1mm posterior - 7.0mm anterior to bregma (A Stereotaxic Atlas of the Rat Brain, Pellegrino, L.J. and A.J. Cushman, Meredith Pub., N.Y., 1967). A push-pull cannula (0.8mm O.D.) was stereotaxically placed in the caudate nucleus (CN) at a 20° angle and the region of perfusion was probed with a glass coated Pt-Ir electrode. Perfusion of mock CSF (Merlis, J. Physiol., 13(2):231, 1940) was established and maintained at 50 µl/min by means of a pressure-damped peristaltic pump circuit (Harvard, model 1203). While varied concentrations of DA were added to the perfusion fluid, the neuronal firing rate was recorded on a Bell and Howell CPR 4010 FM recorder. A continuous count-rate analysis of neuron activity was performed on-line.

When DA was administered by single trial (3 min exposure) to naive units at 10^{-9}M (10), 10^{-8}M (9) and 10^{-7}M (12) there was an initial increase in the firing rate (10% above control) at 10^{-7}M and a significant dose-dependent decrease over the range of test concentrations (avg. = 58, 72 and 76% decrease, respectively). Neuronal effects were noted within 0.1 - 9.0 min of discrete drug application depending upon drug concentration and the cannula to electrode distance. The average duration of depression was 8 min at 10^{-9}M, 22 min at 10^{-8}M and 13 min at 10^{-7}M. Progressive loss of sensitivity was observed with repeated administration of DA at 10-20 min intervals. The initial increase in firing rate did not occur with a second application of 10^{-7}M DA, but decreases were observed at 10^{-8}M and 10^{-7}M (33 and 21%, respectively). The average duration of response was 8 min at 10^{-8}M and 7 min at 10^{-7}M. A third application of 10^{-8}M DA produced a 13% decrease of firing rate (duration = 8 min) but no response was seen following a third application of 10^{-7}M DA. Administration of the DA antagonist Sulpiride (Ravizza, Milan, Italy) was followed by a prolonged polyphasic change in firing rate with applications as low as 10^{-10}M.

184.8 The action of dopamine on rat cortical neurons recorded intracellularly.
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Previous results have shown that dopamine (DA) has a complex action on mammalian striatal neurons. This catecholamine induces a slow depolarization of the membrane potential, inhibits the "spontaneous" and evoked action potentials without evident changes of the membrane conductance. Since there is anatomical evidence for a mesencephalic dopaminergic projection to rat cerebral cortex, the purpose of this study was to investigate the effects of dopamine on rat cortical neurons.

63 cortical neurons were recorded intracellularly ; only 3 out of 24 cells that responded to DA application were monosynaptically activated by stimulation of the SN or ventral tegmental area with a latency of 5-7 msec. In 21 neurons, DA, applied iontophoretically (50-200nA) slowly depolarized the membrane potential and decreased the "spontaneous" and evoked action potentials. The amplitude of the EPSP-IPSP sequences evoked by SN or ventral tegmental area stimulation decreased. No clear change of the membrane conductance was detected. The block of the firing rate was not due to the Na+ inactivation, since the shape of the action potential was not changed during DA application and Glutamate applied during the plateau phase of DA could restore the firing.

In 3 neurons DA induced an hyperpolarization of the membrane potential and decreased the firing rate. Sometimes the initial hyperpolarization in these neurons was followed by a slow depolarization. DA action on rat cortical neurons overlaps the effect of this catecholamine on rat and cat striatal neurons (Bernardi et al. 1978 ; Herrling and Hull, 1980), in which high frequency stimulation of the SN elicit similar effects. In conclusion this work support evidence for on inhibitory role of DA on rat cortical neurons.

G. Bernardi, M.G. Marciani, C. Morocutti, F. Pavone and P. Stanzione. Neurosci. Lett., 8 : 235, 1978.
P.L. Herrling and C.D. Hull. Brain Res., 192 : 441, 1980.

184.9 DIFFERENTIAL SUBSENSITIVITY OF PRE- AND POSTSYNAPTIC DOPAMINE RECEPTORS FOLLOWING LONG-TERM TREATMENT WITH APOMORPHINE. George V. Rebec and Eunjee H. Lee. Dept. Psychol., Indiana Univ. Bloomington, IN 47405.

Dopamine (DA) autoreceptors in the substantia nigra pars compacta (SNC), which participate in dendrodendritic synapses on DA neurons, appear to be more sensitive to DA agonists than postsynaptic DA receptors in the neostriatum (Skirboll et al., Science, 1979, 206:80). To determine if these receptors change their sensitivity with repeated stimulation, we examined the response of neurons in the SNC and neostriatum to apomorphine, a direct acting DA agonist, following its long-term administration. Adult, male rats received subcutaneous injections of saline, 0.05, 0.5 or 2.0 mg/kg apomorphine twice daily for 5 consecutive days. Approximately 12 hours after the last injection, single unit activity was recorded by conventional means after the animals had been immobilized and locally anesthetized. Recordings were obtained from neurons in the SNC whose firing characteristics resembled those previously described for DA neurons and from spontaneously active units in the neostriatum. Apomorphine was administered via a previously implanted jugular catheter in increasing incremental doses.

Consistent with a growing body of evidence, neurons in the SNC of saline pretreated animals were more sensitive to apomorphine than neostriatal neurons. Thus, apomorphine inhibited SNC activity at doses as low as 0.01 mg/kg, but neurons in the neostriatum typically required doses of 0.32 mg/kg to elicit the same response. Following chronic treatment, there was a progressive dose-dependent decrease in the sensitivity of DA neurons to this drug. Pretreatment with 0.5 mg/kg, for example, raised the mean effective dose of apomorphine-induced inhibition in the SNC to 0.32 mg/kg. A similar, though less dramatic, shift in sensitivity occurred following chronic administration of 0.05 mg/kg. In the neostriatum, however, repeated administration of 0.05 or 0.5 mg/kg apomorphine did not significantly alter the response to challenge injections compared to saline controls. Only long-term treatment with 2.0 mg/kg decreased the sensitivity of neostriatal neurons to apomorphine. The results of these experiments indicate that in comparison with postsynaptic DA receptors, DA autoreceptors are not only more sensitive to apomorphine but they are also more likely to develop subsensitivity following repeated administration of this drug.

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184.10 DOPAMINE(DA), NOREPINEPHRINE(NE) AND SEROTONIN(5HT) INCREASE THE EFFICACY OF EVOKED EPSPs IN CAT HIPPOCAMPAL PYRAMIDAL CELLS. Paul L. Herrling* (SPON.: B.H. Gähwiler). Pharmaceutical Division, Preclinical Resarch, Sandoz LTD., CH-4002, Basle, Switzerland.

Intracellular recordings were made in halothane anaesthetised cats with K^+-citrate filled electrodes while the fornix was stimulated (0.2 to 0.5 mA, 100 μs, 0.5 Hz) to produce an EPSP-IPSP sequence. With stimulation intensity adjusted just below threshold for the generation of action potentials on the evoked EPSPs, iontophoretically applied DA, NE or 5HT (≈100 nA) reversibly increased the amplitude of the EPSP and evoked an AP in 9 of 13 cells, the membrane was hyperpolarized by 3 to 12 mV and the rate of spontaneous or carbachol-induced firing was markedly reduced. Intracellular current pulses (-0.1 to-0.3 nA, 100 ms) applied at the time of the evoked EPSP did not induce APs. With stimulus intensity above threshold, NE, DA or 5HT had neither no effect or increased the amplitude of the AP in 9 of 10 cells. In contrast, GABA applied to the same cells shunted the evoked AP for at least 5s and caused membrane hyperpolarizations of 5 to 10 mV in 10 of 11 cells.

The observed effects of DA, NE and 5HT on the EPSP and AP might be due either to the increase of the membrane resistance (Herrling, P.L., Brain Res., in press) and the hyperpolarization and/or to additional pre- or postsynaptic effects.

The present results are in partial agreement with findings reported since the early '70s and recently reviewed by Woodward et al. (Fed.Proc. 38: 2109-2116, 1979) from which it was conjectured that one important role of NE in the brain is to decrease background firing activity while enhancing synaptic signals.

184.11 THE EFFECT OF BUSPIRONE, A NOVEL ANXIOLYTIC DRUG, ON RAT BRAIN DOPAMINERGIC NEUROTRANSMISSION. B.A. McMillen, M.K. Sanghera, R.T. Matthews and D.C. German. Depts. Pharmacology, Physiology and Psychiatry, U. Texas Health Science Ctr. Dallas, TX. 75235.

Stanton et al. (1981) reported that buspirone is a weak inhibitor of apomorphine (APO) stereotopy and conditioned avoidance responding, but does not cause catalepsy. Buspirone lacks clinical efficacy for treatment of schizophrenia in doses over 2.0 g/day, but buspirone is equi-potent to diazepam for relief of anxiety in clinical trials. These authors tested buspirone in a battery of radioligand receptor assays and found significant displacement only of N-n-propylnorapomorphine and spiperone (20 and 290 nM, respectively). Because of the higher affinity for APO binding, they suggested buspirone may be a presynaptic dopamine (DA) agonist. However, we have found that buspirone does not inhibit tyrosine hydroxylase (Tyr-OH) activity in striatal synaptosomes and does not reverse the 'in vivo' activation of Tyr-OH (determined by 30 min accumulation of L-DOPA after inhibition of DOPA-decarboxylase) caused by γ-butyrolactone (GBL). These results with functional receptor systems indicate that buspirone is not a DA autoreceptor agonist. Buspirone shifts the APO inhibition curve for synaptosomal Tyr-OH activity to the right with a calculated K_B=2.1 μM and reverses 'in vivo' APO inhibition of GBL-induced Tyr-OH activation, similar to classical anti-psychotic drugs. Buspirone, from 0.03 to 3.0 mg/kg s.c., causes a dose related increase of DA metabolism (30% to 400%) and 'in vivo' Tyr-OH (determined by L-DOPA accumulation). During extracellular recording from single DA cells in the substantia nigra pars compacta, systemic buspirone (0.01-0.05 mg/kg I.V.) reverses APO or d-amphetamine induced inhibition of impulse flow. Systemic and iontophoretically-applied buspirone inhibits the reduction in DA neuronal impulse flow caused by iontophoresis of DA onto nigral DA cells. These data demonstrate that buspirone is not a presynaptic agonist, but rather an inhibitor of presynaptic DA autoreceptors. Buspirone at 3.0 or 10 mg/kg causes neither catalepsy nor inhibition of APO-induced turning in rats with unilateral nigral lesions, which indicates a low affinity for the classical postsynaptic DA receptor. A further observation is that buspirone, unlike diazepam, does not decrease noradrenergic impulse flow determined by single cell recordings from the locus coeruleus. Whether these effects are related directly or indirectly to the anxiolytic properties of buspirone is unclear. (Supported by USPHS grants MH-30546 and MH-33513)

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184.12 ACTIONS OF ZOXAZOLAMINE AND RELATED CENTRALLY ACTING SKELETAL MUSCLE RELAXANTS ON NIGROSTRIATAL DOPAMINE NEURONS. M.K. Sanghera, P. Shepard*, H.S. Jarrah*, D.C. German and P.A. Shore. Depts. of Physiology, Psychiatry and Pharmacology. Univ. of Texas Health Science Center, Dallas, TX 75235.

Zoxazolamine (ZOX) and related centrally acting skeletal muscle relaxants, chlorzoxazone and mephenesin, in doses producing loss of righting reflex (100-120 mg/kg) greatly lowered striatal dihydroxyphenylacetic acid (DOPAC) concentrations in the rat with less effect on homovanillic acid (HVA). Diazepam (5-20 mg/kg) produced no significant lowering. ZOX inhibited the haloperidol-induced elevation of DOPAC and, again to a lesser degree, HVA. ZOX, in combination with haloperidol, tended to raise dopamine (DA) levels compared to haloperidol alone, and ZOX inhibited the DA lowering seen after the combination of haloperidol and the tyrosine hydroxylase inhibitor, α-methyltyrosine. The DA lowering and marked DOPAC elevation seen after the combination of haloperidol and amfonelic acid, a DA uptake inhibitor, was greatly lessened by ZOX, while HVA elevations were less affected. In this system as well, diazepam had little effect. The effects of ZOX are not due to MAO blockade since ZOX did not block DA lowering by the tetrabenazine analog, Ro-4-1284, whereas pargyline did so. ZOX did not significantly affect normal tyrosine hydroxylase activity in vivo (dopa accumulation method), but lessened the enhanced enzyme activity seen after haloperidol. ZOX did not alter spontaneous or K^+-induced DA efflux from striatal synaptosomes. Unlike other agents which lower DA metabolism in vivo by decreasing DA neuronal activity (e.g. apomorphine), electrophysiological experiments showed little or no effect of systemic ZOX on the firing rates of nigrostriatal DA cells.

Thus ZOX and related agents have a unique effect on DA neurons in vivo. The drugs appear to influence DA metabolism by altering the normal intraneuronal flux of DA such that the main DA storage pool is stabilized. This results in lessened availability of stored DA for release and intraneuronal DA metabolism. These effects occur in the absence of significant changes in DA neuronal impulse flow.
(Supported by USPHS Grants MH 05831, 30546 and 33513.)

185.1 MECHANISM OF INHIBITION OF FIRING BY OPIATES OF NEURONS IN RAT LOCUS COERULEUS. J.T. Williams*, T.M. Egan* and R.A. North (SPON: R.D. Wurster). Neurophysiology Laboratory, Department of Pharmacology, Loyola University Stritch School of Medicine, Maywood, Illinois 60153.

Intracellular recordings were made from locus coeruleus neurons contained in slices cut from rat pons. Membrane potentials ranged from -45 to -60 mV and all cells showed spontaneous overshooting action potentials (frequency 1-10 Hz) throughout recording periods of up to 5 hours. Normorphine and D-Ala^2-D-Leu^5-enkephalin were applied by perfusion in concentrations from 10 nM - 3 μM. These opioids caused a concentration-dependent inhibition of firing in almost all neurons. This inhibition was accompanied by a membrane hyperpolarization and conductance increase in only about one-third of neurons. Action potentials induced by passing repeated depolarizing currents (10-25 Hz for 100-1000 ms) were followed by a hyperpolarization of up to 10 mV which lasted 0.5 to 5 s. This post-tetanic hyperpolarization (PTH) was associated with an increase in membrane conductance and was greatly reduced or abolished in calcium-free or cobalt containing solutions. The opioids prolonged the duration of the PTH 1.5 to 10 fold in the majority of neurons, including many in which they had no effect on resting membrane potential. The results indicate that the inhibition of firing of locus coeruleus neurons may be due not only to an increase in the resting membrane potential but also to an enhancement of the calcium-dependent hyperpolarization which follows the action potential.

185.2 DO OPIATES PLAY A ROLE IN POTENTIATION OF HIPPOCAMPAL FIELD POTENTIALS? M. A. Linseman and W. A. Corrigall. Neurobiology Section, Addiction Research Foundation, Toronto, Canada, M5S 2S1.

We have previously shown, both in vitro and in vivo, that opiates have an excitatory effect on hippocampal field potentials recorded in the CA1 pyramidal cell layer following stimulation of the stratum radiatum. This is manifest as an increase in size of the primary spike, including a reduction of threshold, and by the appearance of additional population spikes in the field potential response. It has also been shown that the size of hippocampal field potentials may be similarly enhanced, more or less permanently, by the application of one or more short bursts of high frequency stimulation, a process referred to as potentiation.

In behavioral experiments, morphine has been shown to be readily self-administered (i.e. acts as a reinforcer) as well as to enhance learning, both in appetitive and aversive conditioning paradigms, when administered immediately post-trial, an effect generally interpreted to reflect an enhancement of the consolidation process. Potentiation is believed by many to be a possible neurophysiological model of the process of learning. It was of interest therefore to determine if the administration of opiates or their antagonists might affect the process of potentiation within the hippocampus.

Rats were implanted with chronic stimulating and recording electrodes in the stratum radiatum and CA1 areas respectively. The experiment took place following a one-week recovery period. Initially, to study low frequency potentiation, changes in responses were measured during trains of 2 and 10 Hz separated by a 2 min interval. The 10 Hz train was then considered the first in a series of 6 trains from 10-100 Hz (100 pulses/train) intended to induce long term potentiation (LTP). Potentiating current in both cases was chosen so as to produce a spike that was 50% of maximum. LTP was measured at 3 equally spaced stimulus intensities along the input/output curve from just above threshold to maximum for the spike, 10 min following each train in the series and 24 hours later.

In the first experiment, 5 mg/kg naloxone s.c. was administered 10 min prior to the start of the session in one half of the animals, saline in the other half. There were no differences on any of the measures of potentiation between the 2 groups, suggesting that endogenous opiates are not involved in potentiation within the hippocampus. To assess whether exogenously administered opiates might nevertheless affect the process, an experiment in which potentiation is measured similarly following 10 mg/kg i.v. morphine is currently in progress, and these results will also be presented.

185.3 ENKEPHALINS RELEASE INTRINSIC BURST GENERATION IN HIPPOCAMPAL CELLS THROUGH DISINHIBITION. L.M. Masukawa and D.A. Prince. Dept. Neurology, Stanford Univ. Sch. of Med., Stanford, CA 94305.

Enkephalin-induced excitation in the hippocampus has been attributed to augmentation of EPSPs and blockade of IPSPs by different investigators. CA1 and CA3 pyramidal cells of guinea pig hippocampal slices maintained in vitro were examined electrophysiologically to determine contributions of intrinsic membrane currents and synaptic transmission to the increase in excitability produced by enkephalins. Five-100 μM D-alanine^2-D-Leucine^5-enkephalin (DADL), an analogue of leucine-enkephalin, or leucine^5-enkephalin dissolved in physiological solution was applied to slices by pressure ejection or leakage from a micropipette, or by superfusion. Enkephalins increased the number of field potential population spikes from 1-2 to 5 in the CA1 and CA3 pyramidal layer in response to stimulation of stratum radiatum. These effects were seen at concentrations as low as 5 μM DADL and were reversed by 10 μM naloxone.

Enkephalins hyperpolarized the resting membrane potential by 1.5 ± 1.8 mV (n = 23). However, they did not significantly affect anomalous inward rectification exhibited by these cells. The Ca^{2+}-dependent afterhyperpolarization was unaffected by 100 μM DADL. These results suggest that changes in intrinsic conductances do not underlie increases in the number of population spikes seen with enkephalins.

The effects of enkephalins on EPSP-IPSP sequences evoked in CA1 and CA3 pyramidal neurons by stratum radiatum stimulation were studied. Following application of 10-100 μM enkephalin, IPSPs were decreased or blocked, leading to a prolongation of the decay phase of the EPSP and often to development of a burst of spikes. IPSPs evoked by alvear stimuli were also blocked. Recovery occurred within minutes when leucine^5-enkephalin was applied by pipette. Hyperpolarizing responses with a reversal potential of -75 to -80 mV were evoked by pressure application of 50 μM GABA in physiological solution. 100 μM DADL, which blocked IPSPs, had no effect on nonsaturating GABA responses. Therefore, enkephalin is not acting as a GABA antagonist. Effects of enkephalins on EPSPs were tested during superfusion with 5 μM bicuculline, which completely blocked alveus-evoked IPSPs. Under these conditions, EPSPs did not increase in amplitude or duration after application of 100 μM DADL.

The increase in excitability of hippocampal pyramidal neurons in the presence of enkephalin is consistent with a presynaptic block of inhibitory interneurons in both the CA1 and CA3 areas of the hippocampus, and is apparently not related to direct effects on EPSPs on voltage-dependent conductances.

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185.4 METHOD FOR QUANTITATING OPIOID EFFECTS ON SYNAPTIC TRANSMISSION IN THE RAT HIPPOCAMPAL SLICE. Rita J. Valentino* and Raymond Dingledine, Dept. Pharmacol., Univ. N. Carolina, Chapel Hill, NC 27514.

Extracellular recordings were made with separate microelectrodes of the evoked field EPSP and population spike in the CA1 region of the hippocampal slice preparation. The sigmoidal relationship between the slope of the EPSP and spike amplitude (input-output curve) was plotted before and 30 min after perfusion of the slice with 300 nM morphine. Morphine shifted the input-output curve to the left, and this effect was only partially reversed by subsequent perfusion with 1 μM naloxone. The input-output curves were normalized by expressing the population spike as a percent of its maximum amplitude, and the EPSP as a ratio to that value producing a half-maximal population spike in control conditions. Drug effects were expressed as the change in the normalized EPSP required to produce a standard population spike that is 50% of its maximum amplitude. This procedure allows the pooling of data from different experiments since the drug effect measured is relatively independent of the orthodromic response characteristics of individual slices. Using this method we determined that 300 nM morphine decreased the EPSP required to produce a standard population spike to 60 ± 11% of the control value, and in 1 μM naloxone the EPSP recovered to only 73 ± 10% of its control value (n = 5 slices). We are using this method to construct dose-response curves to various opioid agonists and antagonists.

This technique was also used to study opioid pharmacology in slices prepared from morphine-dependent rats and bathed continuously in 300 nM morphine. The addition of 1 μM naloxone to these slices produced a small but significant increase in the normalized EPSP (110 ± 2%, n = 9 slices). The magnitude of this naloxone shift was similar to that produced by naloxone in slices prepared from narcotic - naive animals following a 30 min exposure to 300 nM morphine. Thus, in contrast to the guinea pig ileum, we have not been able to demonstrate a narcotic abstinence sign in the hippocampal slice from rats chronically treated with morphine. Supported by DA02360, NS07166, and a Sloan Research Fellowship to RD.

185.5 ENKEPHALIN EFFECT ON CALCIUM SPIKES, N-METHYL-ASPARTATE RESPONSES AND SYNAPTIC INHIBITION IN THE RAT HIPPOCAMPAL SLICE, Raymond Dingledine, (SPON: Dr. J.E. Wilson), Dept. of Pharmacol., Univ. of North Carolina, Chapel Hill, NC 27514.

Previous studies suggest that the epileptiform effect of opioid peptides in the hippocampus may be due either to a reduction in non-somatic inhibition onto pyramidal cells or to a post-synaptic facilitation of excitatory synaptic transmission onto pyramidal cell dendrites. These possibilities are being tested. Intracellular recordings were made from neurons in the CA1 pyramidal cell layer in the presence of tetrodotoxin (0.5-1 μM) to suppress Na-spikes. In TTX transmembrane depolarizing current pulses triggered high-threshold, broad, slowly rising spikes; the spike risetime was further slowed by cobalt (10 mM) and verapamil (3 mM) applied in a small droplet (<1 nl) to the surface of the slice. Neither the threshold nor amplitude of these probable Ca-spikes appeared altered in [D-ala, D-leu]-enkephalin (DADL) applied by droplet in high concentration (10 μM). N-Me-Aspartate (NMA) iontophoresed into st. radiatum evoked complex depolarizations, including Ca-spikes arising from several discrete voltage thresholds and large Ca-spike after-hyperpolarizations. NMA depolarizations that were subthreshold for a frank Ca-spike were regularly associated with an apparent increase in membrane input resistance, and were reduced in magnitude as the cell was hyperpolarized by injected current. In 16 tests on 12 neurons, DADL had no consistent effect on NMA responses. These results lend no support to the hypothesis that DADL acts on pyramidal cell dendrites to enhance the coupling of dendritic potentials to the soma membrane.

With low-frequency (<0.1 Hz) stimulation in st. radiatum two forms of apparently non-somatic synaptic inhibition can be revealed by pairing orthodromic stimuli through separate stimulating electrodes. The first form of inhibition is a reduction in the extracellularly recorded field EPSP by a prior orthodromic stimulus. The second is a long-lasting inhibition of the intracellularly recorded EPSP when the amplitudes of "control" and "conditioned" dendritic field potentials are matched. Both forms of synaptic inhibition show fatigue at stimulus frequencies above ~0.5 Hz; both are obliterated by short tetanic trains and recover slowly. Both appear to be reversibly blocked by a droplet of DADL (10 μM). In other experiments it was shown that the potentiating effect of DADL on orthodromic EPSPs is voltage-sensitive, the opioid effect being reduced as the cell is hyperpolarized. The data taken together suggest that part of the epileptiform activity of DADL in the hippocampal slice may be due to blockade of synaptic inhibitions that are distinct from classical recurrent inhibition. Supported by DA02360 and FR05406.

185.6 MODULATION OF NIGROSTRIATAL DOPAMINE ACTIVITY BY OPIATES. D. Hommer*, D. P. van Kammen and A. Pert. Biological Psychiatry Branch, NIMH, Bethesda, MD 20205

There is considerable evidence to suggest that opiates exert an important influence on the nigrostriatal dopamine system. In our studies as well as in studies by Iwamato et al. (J. Pharmacol. Exp. Ther. 197:503-516, 1976), rats lesioned unilaterally in the substantia nigra (SN) with 6-OHDA rotated ipsilateral to the lesion following systemic injections of morphine. We have found this rotational behavior to increase in intensity with repetitive administration. Direct unilateral microinjections of opiates into the SN induce rotational behavior contralateral to the injection. This rotational behavior can be blocked by injections of haloperidol into the ipsilateral caudate nucleus. Unilateral injections of opiates into the SN also induce a significant elevation in homovanillic acid in the ipsilateral caudate.

Single-unit recording techniques were also used to study the effects of systemically administered morphine on the activity of rat SN dopamine (SN-DA) neurons and a specific sub-population of SN zona reticulata (SN-ZR) neurons. It has been reported that i.v. morphine increases the firing rate of SN-DA neurons but decreases the firing rate of unidentified SN-ZR neurons. Our results confirm and extend these findings. SN-DA neurons increase their firing rate an average of 70% above baseline following administration of 4.0 mg/kg i.v. morphine. This increase is completely reversed by 0.01 mg/kg of i.v. naloxone. The class of SN-ZR neurons we examined have an inhibitory influence on SN-DA neurons and were identified using previously established criteria (Grace and Bunney, Eur. J. Pharmacol. 59:218, 1979). These SN-ZR neurons were more sensitive to morphine than SN-DA neurons and decreased their firing rate an average of 26% below baseline following 1.0 mg/kg i.v. morphine. Naloxone 0.01 mg/kg i.v. led to a consistent rebound increase in firing rate of these SN-ZR neurons to 29% above baseline. No such rebound was seen in SN-DA neurons following naloxone. Data related to the effects of iontophoretically applied opiates on the basal activity of identified SN-DA and SN-ZR cells will also be described.

185.7 A COMPARATIVE MICROIONTOPHORETIC STUDY ON THE EFFECTS OF METHIONINE-ENKEPHALIN AND MORPHINE ON SINGLE UNIT ACTIVITY IN THE RAT GLOBUS PALLIDUS. J. M. Frey* and R. D. Huffman. Department of Pharmacology, Division of Neuropharmacology, The University of Texas Health Science Center at San Antonio, San Antonio, TX 78284

While the globus pallidus of the rat brain has been shown to have only moderate to diffuse opiate receptor density, it has been shown to contain the highest content of met-enkephalin found within the rat brain. In addition, it has recently been demonstrated that striatal enkephalinergic neurons send projections to globus pallidus. It has been previously demonstrated in this laboratory (Huffman, R.D. and Felpel, L.P., Neurosci. Lett., 22: 195, 1981) that the microiontophoretic application of morphine depressed the spontaneous firing of single globus pallidus neurons in morphine-naive rats. The present study was conducted in order to compare the effects of microiontophoretically applied met-enkephalin and morphine on spontaneous single unit activity within the globus pallidus of paralyzed, locally anesthetized rats. Male Sprague-Dawley rats (170-250g) were initially anesthetized with Halothane for the purpose of tracheotomy and placement of an indwelling catheter into the external jugular vein. While under anesthesia, a left craniectomy was performed to expose the cortex dorsal to the globus pallidus. The animals were then paralyzed with gallamine triethiodide and maintained on a respirator for the remainder of the experiment. Pressure points and surgically manipulated areas were anesthetized by the infusion of a local anesthetic. Drugs were applied by microiontophoresis from 5-barrel glass micropipettes with tip diameters of 10-14μm. Drug concentrations utilized were as follows: (met-enkephalin, 70mM in 70mM NaCl, pH 4.5 (Sigma); morphine hydrochloride, 100mM, pH 4.0-5.0 (MacFarlan Smith Ltd.); naloxone hydrochloride, 50mM in 50mM NaCl, pH 4.0-5.0 (Endo); NaCl, 4M for recording; NaCl, 1M for current control. The electrodes were filled by centrifugation and utilized within a short time thereafter. Preliminary results have revealed depression of spontaneous neuronal firing by both met-enkephalin and morphine on all globus pallidus neurons tested. At the injection currents used in this study (70nA met-enkephalin and 100nA morphine), the mean reductions in unit activity of 36.2% ± 2.6 (SEM) and 38.7% ± 3.8, respectively, were statistically significant. The specificity of drug effects was demonstrated by naloxone reversal. (Supported in part by Morrison Trust Foundation and NSF grant BNS-24774.)

185.8 ELECTROPHYSIOLOGICAL EVIDENCE IN FAVOR OF DIFFERENT OPIATE RECEPTOR POPULATIONS IN THE CAUDATE AND THE CENTRAL GRAY OF THE RAT. A.Schurr, B.M.Rigor and N.Dafny. Dept. Neurobiology and Anatomy and Dept. Anesthesiology, Univ. Texas Med. Sch. Houston, TX 77025.

The aim of the present study was to compare the direct actions of morphine on two brain areas which are known to be rich in their content of opiate binding sites,namely, the caudate nucleus and the central gray. Electrophysiological recordings of spontaneously active single units and morphine injections by microiontophoresis were made through a multi-barreled glass micropipettes.

Four different patterns of neuronal response to increasing currents of morphine could be followed in both brain sites. However, differences in the response to morphine were detected between the two brain areas in morphine-dependent rats. While the caudate neurons of morphine-dependent rats exhibited super-sensitivity to morphine,the neurons of the central gray of these animals displayed tolerance to the drug and in some, morphine dependence was evident when naloxone iontophoresis was used.

The distribution of spontaneously active neurons within these two brain areas was found to be generally corresponds to the known distribution of the opiate binding sites in the striatum and the periaqueductal gray matter.

The electrophysiological findings of this study support the notion that the two tested brain areas contain different sets of opiate receptors.

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185.9 TOLERANCE TO MORPHINE IN THE C-FIBER REFLEX (CFR) OF THE ACUTE DECEREBRATE SPINAL CAT. James A. Bell* (Spon: Wallace B. Pickworth). NIDA Addiction Research Center, Lexington, KY 40583.

Cats were treated for 30 days with morphine (15 mg/day, i.p.). They were given morphine (10 mg/kg, i.p.) immediately before preparation for electrophysiological recording of the CFR. CFR's were recorded from an L_7 ventral root after stimulation of the ipsilateral superficial peroneal nerve with a stimulus intensity sufficient to activate C-fibers. The magnitude of the CFR recorded from the chronic morphine preparations averaged 240% larger than the CFR recorded from cats not treated with morphine. This demonstrates tolerance to morphine because morphine (10 mg/kg, i.p.) completely abolished the CFR if administered before the experimental procedure in untreated cats. Tolerance to i.v. injections of morphine was also demonstrated. To significantly depress the CFR in the morphine-treated cat (67% of control) an i.v. dose of 10 mg/kg was required, whereas the minimal i.v. dose required to depress the CFR in the untreated cat (66% of control) was 0.15 mg/kg. The 10 mg/kg i.v. dose completely abolished the CFR in the untreated cat. We conclude from these studies that morphine tolerance of a C-fiber-activated spinal reflex arc can be produced by chronic treatment of the cat with morphine. Furthermore, these morphine tolerant CFRs can serve as a model system in which to study the neuronal mechanisms which produce morphine tolerance.

185.10 MORPHINE ANTAGONIZES GAMMA AMINOBUTYRIC ACID-INDUCED INHIBITION IN RAT CEREBELLUM. Hylan C. Moises, Hermes H. Yeh and Donald J. Woodward. Dept. Cell Biology, Univ. Tx. Hlth. Sci. Ctr., Dallas, Tx. 75235.

To determine whether opiates may exert some of their central effects by altering gamma aminobutyric acid (GABA)-mediated synaptic transmission, we examined the effect of morphine on GABA-mediated inhibition in cerebellum, a well-defined neuronal system. Direct GABAergic inputs to the Purkinje cell were activated by local stimulation (LOC) of the cerebellar surface, and pure 'off-beam' inhibitory responses of single units were recorded extracellularly in halothane-anesthetized rats. Poststimulus time histograms were used to quantitate inhibitory responses to LOC stimulation applied before, during and after iontophoretic or intravenous administration of morphine. Inhibitory responses to microiontophoretic pulses (10 sec duration at 45 sec intervals) of GABA, glycine and norepinephrine (NE) were also examined before, during and after morphine iontophoresis.

Microiontophoretic application of morphine 50 mM (10-120 sec, 5-30 nA) reversibly depressed the spontaneous discharge of 32 of 68 (47%) neurons tested and accelerated firing rate in 19 cells (28%). Antagonism by naloxone and opiate receptor stereospecificity, however; could only be demonstrated for the depressant action of morphine on Purkinje cell activity. In 8 of 12 neurons, iontophoresis of morphine reduced the 'off-beam' inhibitory response to LOC stimulation. This attenuation of synaptically-induced inhibition often occurred independent of opiate effects on Purkinje cell background activity and was observed in 3 of 5 cells following systemic injections of morphine (0.2-0.6 mg/kg, i.v.). Inhibitions produced by the direct application of GABA were reversibly antagonized during iontophoresis of morphine in 15 of 18 cells. Glycine-induced inhibitions were also blocked in 5 of 6 cells from the same population, whereas NE-induced inhibitions were not altered by morphine (8 of 8 cells). Prior administration of naloxone prevented morphine blockade of GABA-induced inhibition in 3 of 8 cells. However, levorphanol was found to be no more effective in blocking GABA responses (0 of 6 cells) than was dextrorphan, its inactive stereoisomer (0 of 4). Down-regulation by morphine of the previously described tonic noradrenergic bias on GABA action (Woodward et al., Fed. Proc. 38, 1979) was unlikely to account for the present findings, because responses to GABA were also blocked by morphine in 8 of 12 neurons from 6-hydroxydopamine pretreated animals.

In summary, these data suggest that morphine at analgesic dose levels may have non-specific effects on membrane mechanisms which result in a decreased postsynaptic efficacy of GABA action.
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185.11 ENKEPHALINS AND TRANSMISSION IN MAMMALIAN SYMPATHETIC GANGLION CELLS. M. A. Simmons, Z. G. Jiang* and N. J. Dun. Dept. of Pharmacol., Loyola Univ., Maywood, IL 60153.

The effects of met- and leu-enkephalin (met- and leu-Enk) on guinea pig inferior mesenteric ganglion cells and on ganglionic transmission were studied by means of intracellular recording techniques. Bath application of Enk (0.05-5 μM) to sympathetic neurons caused no consistent membrane potential change. A portion of the neurons showed a hyperpolarization of 1-5 mV which was associated, in some neurons, with a fall in input resistance. A depolarization (2-5 mV) was observed in a few neurons that was not accompanied by any clear change in input resistance; however, the membrane potential and resistance in the majority of neurons tested were not appreciably affected by Enk. The most consistent effect of Enk was a reversible depression of the amplitude of the fast epsp. The depressant effect of Enk was concentration-dependent and prevented by pretreatment with naloxone (1 μM) or naltrexone (1 μM). In a low Ca (0.5 mM)/ high Mg (5.5 mM) solution the quantal content was significantly reduced by Enk whereas the quantal size was not appreciably affected. While depressing the amplitude of the fast epsp, Enk caused no significant change of the amplitude of the acetylcholine (ACh) potential induced by iontophoretic application of ACh to the same ganglion cells. Increasing the extracellular Ca concentration to 5 and 10 mM partially antagonized the depressant effect of Enk on the amplitude of the fast epsp. The non-cholinergic excitatory potential, the transmitter of which may be substance P, induced by repetitive nerve stimulation was also depressed by Enk in a number of cells tested whereas the depolarization induced by bath application of substance P was not reduced by Enk. Naloxone or naltrexone potentiated the response of the non-cholinergic excitatory potential in a portion of the neurons studied. Our results suggest that the primary action of Enk in sympathetic ganglia is to regulate transmitter output, probably by affecting Ca influx into the preganglionic nerve terminals. (Supported by NS15848).

185.12 OPIATE ALKALOID EFFECTS ON GLYCINE- AND GABA- MEDIATED POSTSYNAPTIC INHIBITION: CORRELATION WITH PAROXYSMAL ACTIVITY. M.A. Werz, K. Baum*, A.B. Young, and R.L. Macdonald. Neurosciences Program and Dept. of Neurology, The University of Michigan, Ann Arbor, MI 48109.

We have investigated opiate actions on GABA, glycine (GLY), and glutamate (GLU) postsynaptic responses using intracellular recording techniques applied to murine spinal cord neurons in primary dissociated cell culture and on amino acid receptor binding to rat spinal cord homogenates.

Morphine antagonized GLY and GABA but not GLU postsynaptic responses. GLY and GABA response amplitudes were reduced to half of control (IC_{50}) at morphine concentrations of 33 μM and 475 μM, respectively. The opioid peptide, [D-ala^2]-met-enkephalinamide, did not affect GLY, GABA, or GLU responses. Opiate alkaloid interaction with GLY was weakly stereospecific with levorphanol three times as potent as dextrorphan while GABA responses were reduced equipotently by the enantiomers. Naloxone did not reverse opiate depression of GLY responses. These data suggested that opiate effects on postsynaptic inhibitory amino acid responses were not mediated by opiate receptors since these effects were not naloxone reversible, only weakly stereospecific, and not produced by the opioid peptide.

To determine if opiate effects on inhibitory amino acid responses were due to opiate alkaloid interaction with GLY and GABA receptors, receptor binding assays were performed. Morphine displaced ^{3}H-strychnine from GLY receptors and ^{3}H-muscimol from GABA receptors with IC_{50}s of 113 ± 17 μM and 315 ± 78 μM, respectively. Levorphanol (IC_{50} = 70 ± 10 μM) was three times more potent than dextrorphan (IC_{50} = 195 ± 18 μM) in displacing ^{3}H-strychnine. In contrast, the opiate enantiomers were equipotent in displacing ^{3}H-muscimol with IC_{50}s of 322 ± 21 μM and 313 ± 11 μM for levorphanol and dextrorphan respectively. Naloxone inhibited ^{3}H-muscimol binding equipotently with morphine (IC_{50} = 273 ± 88 μM) but was much weaker than morphine in displacing bound ^{3}H-strychnine (IC_{50} = 2.5 ± 0.6 mM).

These data support an opiate alkaloid action to depress inhibitory amino acid responses by interacting with GLY and GABA receptors rather than opiate receptors. Antagonism of inhibitory amino acid transmission may underlie the convulsant action of high concentrations of the opiate alkaloids. Indeed, we found opiate antagonism of GLY responses to be correlated with induction of paroxysmal depolarizations in cultured spinal cord neurons.

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185.13 EFFECTS OF OPIATES ON IDENTIFIED SEROTONERGIC AND NON-SEROTONERGIC NEURONS IN THE RAPHE MAGNUS (RM) M.W. Wessendorf, E.G. Anderson, and H.K. Proudfit, Dept. Pharmacology, Univ. of Illinois Medical Center, Chicago, IL 60612.

It has been suggested that excitation of neurons in the RM is essential for the expression of the analgesic effects of opiates. This hypothesis was tested using single unit recording of identified serotonergic and non-serotonergic neurons. Rats were anesthetized with urethane, curarized, ventilated, and single units were recorded from the RM using single barrel glass microelectrodes pulled to a tip diameter of about 0.75 µm. Conduction velocities of raphespinal units were determined by antidromic activation and serotonergic neurons were identified by their conduction velocities of <6.0 m/sec. Fentanyl (50 µg/kg) was administered i.v., and the effect on neuronal firing rate recorded.

A total of 48 units were recorded. Twenty four were non-serotonergic, and of these 29% were excited by fentanyl, 46% inhibited, and 25% showed no change. Another 24 units were serotonergic, and of these only 13% were excited, while 54% were inhibited and 33% showed no change.

It is concluded that excitation of either serotonergic or non-serotonergic units in the RM is probably not necessary for systemic opiate analgesia. On the other hand, spinal perfusion studies have shown increased serotonin release after microinjection of morphine into the periaqueductal gray. Since tissue concentrations of drugs are unknown in microinjection studies, it is possible that very high local tissue levels are attained. Thus it was decided to test the effects of very high systemic doses of morphine on serotonergic units.

Morphine was administered i.v. in cumulative doses of 3,5,10, 20,40 and 80 mg/kg, and the firing of serotonergic units was observed for 15 minutes after each dose. Only 6 units survived the entire regimen. Of these, 2/3 (4 units) showed a surprising result: higher doses of morphine would produce the opposite of the response (either inhibitory or excitatory) established at the 3-5 mg/kg dose. Naloxone 0.2 mg/kg was given in 2 cases, and in one it suceeded in reversing 80 mg/kg morphine. These effects did not appear to correlate consistantly with blood pressure changes.

It is tentatively concluded that high doses of opiates may have effects different from, or opposite to, those seen at low doses, and that this may have relevance to the interpretation of microinjection studies.

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185.14 ANTICONVULSANT EFFECTS OF ELECTROCONVULSIVE SHOCK (ECS) ON SUBSEQUENT KINDLED SEIZURES IN RATS. Y. Shavit, S. Caldecott-Hazard and J. C. Liebeskind. Department of Psychology, UCLA, Los Angeles, CA 90024.

Because naloxone shortened and morphine prolonged the post-ictal behavioral depression normally seen after a kindled seizure, it was suggested that opioid peptides are released during ictus and reduce the severity of subsequent seizures (Frenk et al., 1979). We later found that morphine (50 mg/kg, but not 20 mg/kg) and an enkephalin analogue (6 mg/kg) significantly decreased the number of behavioral seizures in kindled rats (Caldecott-Hazard et al., 1980). Since ECS is reported to cause opioid peptide release (Hong et al., 1979), we sought to determine if ECS would also inhibit kindled seizures.

Rats were kindled with electrical stimulation to the amygdala until 3 generalized seizures were produced. After a week of rest, all rats were stimulated 7 times (spaced at 2 min intervals) in the amygdala. The severity of each behavioral seizure was rated for each rat and the mean provided a baseline seizure index. A week later, half the rats were given naloxone (2 injections of 10 mg/kg, 15 min apart) and half saline. ECS was then induced through a pair of cortical electrodes. Two min after ECS, amygdala stimulation was applied as before and the seizure index recalculated. A week later, this procedure was repeated with reversed drug treatments. Another group of kindled rats was made tolerant to morphine, and then tested for baseline seizure index and the effect of ECS, as described above.

ECS significantly decreased the seizure index, and fewer behavioral seizures were seen. When generalized seizures did occur, they were seen only towards the end of the series of 7 stimulations. The inhibitory effect of ECS on kindled seizures was greater than what we previously observed with 50 mg/kg morphine. Naloxone partially reversed the inhibitory effect of ECS. The baseline seizure index of morphine tolerant rats was not different from that of controls, but the effect of ECS was reduced to the same extent as that following naloxone, providing evidence for cross-tolerance between morphine and the effect of ECS.

The present results support the hypothesis that opioid peptides released by ECS serve to reduce the severity of subsequent kindled seizures. Because the effects of naloxone and cross-tolerance were only partial, ECS appears to exert its anticonvulsant action by nonopioid as well as by opioid mechanisms. (Supported by NIH grant #NS07628 and fellowship #NS06289.)

185.15 THE EFFECTS OF INTRA-AMYGDALOID NALOXONE INFUSION ON KINDLED CONVULSIONS. A. Mansour, B. Himmelstein* & E.S. Valenstein; Depts. Psych. & Neurosci. Lab., Univ. Michigan, Ann Arbor, Mich. 48109.

Previous research in our laboratory has shown that kindling, the progressive development of behavioral convulsions of increasing severity in response to a regimen of brain stimulation, produces long-lasting changes in morphine sensitivity. These changes in morphine responsiveness may reflect some change in the endogenous opiate systems of the animal. To test this hypothesis, we examined the effects of naloxone, a specific opiate receptor blocker, on kindled convulsions. Thirteen DBA/2J mice were stereotaxically implanted with chemtrodes in the right amygdala. The chemtrodes consisted of a combined 23 gauge cannula and a teflon coated twisted wire electrode. Following recovery from surgery, all the animals were stimulated once daily with one second of 50 u AMP., 60 Hz electrical current until they reached a criterion of 5 consecutive generalized (Stage 5) convulsions. The animals were then divided into two groups: One group (N=7) was infused with naloxone (2.5 ug/0.5 ul, over a 2 minute period using a Harvard infusion pump), while the second group (N=6) was similarly infused with an equal volume of saline. Five minutes after the completion of the infusion procedure, both groups were stimulated with the kindling current. Naloxone significantly blocked the appearance of generalized kindled convulsions (chi square = 5.28 $p < .05$). Most of the kindled animals showed minor, nongeneralized (Stage 1 or 2) convulsions and greatly attenuated electrographic afterdischarges, while the saline treated animals showed generalized convulsions. These results implicate opiates in kindled convulsive activity. Infusion of naloxone prior to electrical stimulation on subsequent days did not block the kindled convulsions and may, in fact, have had a facilitory effect. These results parallel the finding that naloxone pretreatment inhibits morphine induced convulsions in rats with the first administration, but fails to do so with subsequent pretreatment.

185.16 THE EPILEPTIFORM DISCHARGES PRODUCED BY ENKEPHALIN AND THE EFFECT OF BODY TEMPERATURE. Rabi Simantov, Elias Motles and Zeev Elazar. Department of Genetics, Weizmann Institute of Science, Rehovot and Department of Physiology and Pharmacology, Sackler School of Medicine, Tel-Aviv University, Israel.

The influence of different body temperatures on the epileptiform discharges induced in hippocampus by local microinjections of leu-enkephalin was studied in rats.

The animals were divided into four groups according to rectal temperatures during the experiment: 25-26°C; 29-30°C; 37-38°C; and 39-40°C. Rectal temperature and hippocampal EEG were monitored continuously during cooling or heating the body. Once a required temperature was reached, it was maintained during the rest of the experiment.

In the absence of enkephalin, epileptiform discharges were induced by either cooling or heating the body while the rectal temperature changed. After temperature stabilization these discharges disappeared and the hippocampogram looked normal for long periods of time.

Leucine-enkephalin (30 µg in 1.5 µl saline) was infused into the dorsal hippocampus at a speed of 0.01 µl/sec. Prolonged seizure-like discharges were recorded from the microinjection area at normal rectal temperature (38°C). These discharges could be prevented by local microinjections of naloxone given ten minutes before enkephalin. At below normal temperatures (25-26°C and 29-30°C) the epileptiform episode induced by enkephalin was of longer duration. At above normal rectal temperatures (39-40°C) the epileptiform episode was less intense and of shorter duration.

An in vitro study of the effect of temperature on the degradation of leu-enkephalin by brain tissue was conducted. One nmole of leu-enkephalin was incubated for 5 minutes at four different temperatures with rat brain tissue homogenate. The enkephalin was then re-extracted and assayed by the opiate radioreceptor assay. The amount of enkephalin recovered after incubation at different temperatures was quantified. A progressive reduction in the enkephalin activity recovered with the increase in temperature between 0 and 40°C was found. This finding is in remarkable agreement with our findings in the in vivo experiments.

It is proposed that changes in the temperature of the hippocampus as a result of cooling or heating of the body, by affecting the enzymatic activity involved in the breakdown of enkephalin, played the main role in determining the strength of enkephalin's epileptogenic effect. This indicates that in conditions of hyperthermia the activity of the enkephalinergic system in the brain is reduced.

186.1 EFFECTS OF TRANYLCYPROMINE AND PHENELZINE ON RAT BRAIN 5-HYDROXYTRYPTAMINE/TRYPTAMINE RATIOS. D.F. LeGatt*, G.B. Baker, H.R. McKim*, J.L. Hay*, W.G. Dewhurst* and R.T. Coutts*. Neurochemical Research Unit, University of Alberta, Edmonton, Canada.

The trace amine tryptamine (T) and the neurotransmitter 5-hydroxytryptamine (5-HT) are derived from the amino acid tryptophan via independent metabolic pathways. T and 5-HT have been implicated in the etiology of various psychiatric disorders.

The ratios of 5-HT/T in rat whole brain were calculated for animals undergoing treatment with the monoamine oxidase inhibitors (MAOIs) phenelzine (PLZ) and tranylcypromine (TCP) for varying time periods, to monitor the effect of the antidepressants on relative amounts of T and 5-HT. Separate groups of rats were administered the drugs i.p. once daily (10 mg/kg for TCP and 15 mg/kg for PLZ) for 1, 2, 8, and 19 days. Rats were sacrificed 6 h after the final dose. Some animals which received TCP or PLZ for one day were administered tryptophan i.p. (100 mg/kg) 1 h prior to sacrifice. Analyses of T and 5-HT were based on a procedure utilizing electron-capture gas chromatography with a capillary column (Calverley et al., Can. J. Neurol. Sci. 7:237, 1980).

The control 5-HT/T ratio was > 550/1. For TCP, the ratio was 79/1 on Day 1, and varied from 77/1 to 128/1 through to Day 19. For PLZ, the 5-HT/T ratio was > 550/1 on Day 1, but a gradual decline to 380/1 occurred by Day 19. The 5-HT/T ratios after PLZ remained several times higher as compared to TCP for all time periods. A much higher increase in T was demonstrated with TCP than with PLZ, and 5-HT levels did not vary markedly between the two drugs. Supplementation of TCP (10 mg/kg) and PLZ (50 mg/kg) with tryptophan on Day 1 resulted in 5-HT/T ratios of 19/1 and 84/1 respectively. The reason for higher levels of T with TCP than with PLZ is unclear at this time, but it has been reported that TCP does cause a significant increase in brain concentrations of tryptophan, the precursor of T, over control values. This investigation demonstrates differing effects of the two MAOIs and the influence of tryptophan on 5-HT/T ratios in rat brain.

(Supported by Alberta Heritage Foundation for Medical Research and Medical Research Council of Canada).

186.2 METHYSERGIDE ANTAGONISM OF FACILITATORY BUT NOT INHIBITORY EFFECTS OF 5-HYDROXYTRYPTAMINE ON LUMBAR MOTONEURON EXCITABILITY. S.R. White and R.S. Neuman. Faculty of Medicine, Memorial Univ. of Newfoundland, St. John's, Newfoundland, A1B 3V6.

5-Hydroxytryptamine (5-HT) has recently been shown to produce a long lasting facilitation of glutamate-evoked firing of lumbar spinal motoneurons (White & Neuman, Brain Res., 188 (1980) 119). While this facilitatory effect of 5-HT is always observed, it is often preceded by a period of brief inhibition. Engberg, Flatman & Kadzielawa (Acta Physiol. Scand., 1976, 96, 137-139) reported that iontophoretic application of 5-HT, NE and a variety of other amines produced a nonspecific hyperpolarization of α-motoneurons. In the present experiment, we examined the specificity of the facilitatory and initial inhibitory effects of 5-HT on glutamate-evoked motoneuron activity using the 5-HT antagonist, methysergide. Rats were anesthetized with urethane (1.3-1.5 g/kg). The lumbar spinal cord and cauda equina were exposed and lumbar motoneurons were identified by responses to antidromic stimulation of L_4 and L_5 ventral roots. Extracellular unit recordings were made from the center NaCl barrel of a 7 barrel glass micropipette. Other barrels contained glutamate, NaCl for current balance, 5-HT, methysergide and norepinephrine (NE). Motoneurons were found which responded to application of 5-HT (15-25 nA, 30-60 sec) with a brief inhibition followed by a prolonged facilitation of glutamate-elicited firing. NE (15-30 nA, 30-60 sec), like 5-HT, also facilitated glutamate-evoked motoneuron activity and the facilitation was often preceded by brief inhibition. Methysergide (10 nA, 2 min.) antagonized only the facilitatory effect of 5-HT, sparing the inhibitory effect of 5-HT as well as both the excitatory and inhibitory effects of NE. The selective antagonism by methysergide of only the 5-HT facilitatory effect on motoneurons supports the conclusion by Engberg, et al. that the inhibition of motoneurons produced by iontophoretic application of 5-HT is a nonspecific action.

186.3 IDENTIFICATION AND QUANTITATION OF N-ACETYLSEROTONIN IN RAT BRAIN REGIONS BY GCMS. G. M. Brown, A. Porietis and N. Narasimhachari . Dept. of Neurosciences, McMaster University, Hamilton, Ontario, Canada. L8N 3Z5 and Dept. of Psychiatry, Medical College of Virginia, Richmond, Virginia.

N-Acetylserotonin (NAS) has previously been identified in the pineal and has been quantitated by GCMS using N-acetyltryptamine as internal standard (A.R. Green, S.H. Koslow and E. Costa, Brain Res. 51, 371, 1973). In previous studies we have identified NAS specific immunoreactivity in areas outside the pineal, including cerebellum, hippocampus, brain stem and retina, however, absolute identification and quantitation has not been reported in those areas. We have now identified NAS in rat hippocampus and cerebellum by GCMS. The tissue was homogenized in 5 vols of phosphate buffer (0.1 m pH 7.4) centrifuged at 2500 rpm and the supernatant separated. d_4-N-Acetylserotonin which was prepared from d_4 - serotonin by selective-acetylation was added to the supernatant as internal standard, pH adjusted to 4.5 with a few drops of 1N hydrochloric acid and extracted twice with 10 ml of ethyl acetate. The pooled ethyl acetate extract was evaporated to dryness under nitrogen, the residue heated with trifluoracetic anhydride for 15 minutes at 80°C (K. Blan, G.S. King and M. Sandler, Biomed. Mass Spect. 4, 232, 1977). The identity of this compound was established by obtaining the total mass spectrum by GCMS and comparing it with the spectrum of standard N-Acetylserotonin-TFA under identical conditions. The gc retention time and the mass spectra obtained from the brain tissue and the standard were identical. N-Acetylserotonin in the hippocampus was quantitated by selected ion monitoring in the electron impact mode using the molecular ion m/z 392 and m/z 396 for $d_4$internal standard. In another set of experiments the pentafluoropropionyl derivative was prepared, where ions m/z 492 and 496 were monitored for the sample and internal standard respectively. A standard calibration curve was obtained using 10 to 100 ng of N-Acetylserotonin and 250 ng of internal standard.

Using this technique we have quantitated NAS in rat hippocampus and examined the effect of tryptophan hydroxylase inhibition with parachlorophenylalanine (PCPA). Male Wistar rats of about 200 g were treated with PCPA (Sigma) 350 mg/kg/day or saline for three days and sacrificed 12h after the last treatment. Hippocampal NAS concentration in controls was 496 ng/g, S.E. 168, n = 4 and following PCPA was 79 ± 65, n = 4 ($p < 0.05$).

We conclude that NAS is present in rat hippocampus and that the enzyme tryptophan hydroxylase is essential in its synthesis.

186.4 EFFECTS OF PHENCYCLIDINE (PCP) ON SEROTONERGIC AND GABAERGIC SYSTEMS IN THE RAT BRAIN. M. A. Peat* and J. W. Gibb, Dept. of Biochem. Pharmacol. and Toxicol., Univ. of Utah, S.L.C., UT 84112

Behavioral and biochemical studies have indicated that PCP, like amphetamine, may act as an indirect dopamine agonist. Tyrosine hydroxylase activity was decreased after both acute and chronic treatment with PCP (10 mg/kg) (Doherty et al., Eur. J. Pharmacol. 65:139, 1980; Smith et al., J. Neural. Trans. 48:289, 1980). Doherty et al. postulated that PCP may also act by blocking transmitter uptake. PCP has been reported to have differing effects on whole brain concentrations of 5-HT and 5-HIAA; no studies of its effect on tryptophan hydroxylase have been reported. Methamphetamine and cocaine have been shown to alter the activity of this enzyme. A single dose of PCP (10 mg/kg) decreased whole brain concentrations of GABA, although it has not been reported to inhibit glutamic acid decarboxylase (GAD) activity in vivo.

In this study we investigated the effects of PCP on the concentrations of 5-HT, 5-HIAA and tryptophan (TRP) and on GAD activity in various brain regions. Rats were administered PCP (10 mg/kg i.p.) either acutely or chronically and sacrificed by decapitation; neostriatum and cerebellum were dissected. Acute administration consisted of one single injection of PCP; groups of rats were sacrificed at 10, 15, 20, 30, 60 and 120 min after injection. Chronic administration consisted of 30 daily injections; rats were sacrificed 15 min and 24 hr after the last injection. GAD activity was measured by a modified $^{14}CO_2$ trapping procedure (Hotchkiss et al., Life Sci. 25:1373, 1979) and indoleamine concentrations by an HPLC-fluorescence method.

GAD activities (expressed as % of control values) in the cerebellum after acute dosing were as follows: 10 min 104%, 15 min 98%, 20 min 104%, 30 min 96%, 60 min 108% and 120 min 110%. The increase in activity seen after 120 min is significantly different from control. The corresponding activities after chronic dosing were: 15 min 109% and 24 hr 80%. Four injections of PCP (2.5, 5 and 10 mg/kg, given at 3 hr intervals) decreased cerebellar GAD activity. The results of acute and chronic PCP treatment on striatal indoleamine levels (expressed as % of control values) are tabulated below:

	Time after injection (minutes)							
	Acute						Chronic	
	10	15	20	30	60	120	15	1440
5-HT	79	115	86	132	105	98	110	114
5-HIAA	97	151	86	151	115	85	104	91
TRP	100	-	99	113	89	92	121	106

These preliminary data indicate that PCP has an effect on the serotonergic and GABAergic systems in the rat brain regions examined. (Supported by USPHS Grant DA 00869)

186.5

WITHDRAWN

186.6 MORPHOLOGY OF DORSAL RAPHE NEURONS INTRACELLULARLY INJECTED WITH HRP. LIGHT MICROSCOPIC FINDINGS. H. Imai*,M.R. Park and S.T. Kitai (SPON: J. Thornburg). Dept. of Anatomy, Michigan State Univ., East Lansing, MI 48824.

Neurons of the dorsal raphe nucleus of the rat were intracellularly recorded and filled with horseradish peroxidase. The neurons subsequently identified resided within 300μm of the midline in the rostral part of the nucleus, dorsal to the medial longitudinal fasciculus. Classification according to axonal trajectory yielded a minimum of two neuron types: 1) neurons whose axons were ventrally projecting as they left the nucleus and 2) neurons with either dorsally or laterally directed axons which were not seen to leave the nucleus. Considering this latter class of neurons, the following soma-dendritic features were noted: Somata ranged in size from 110μm^2 to 380μm^2 in cross-sectional area. The smallest cells (110-200μm^2) had round somata and were completly spine-free. Larger cells had fusiform or polygonal somata, sparse dendritic spines and, occasionally, somatic spines. Neurons of the first type, those with ventrally projecting axons, had fusiform or polygonal somata ranging in size from 250 to 430 μm^2. These cells possessed dendritic spines and occasionally somatic spines as well. There was no obvious correlation between the pattern of dendritic arborization and axon trajectory so that the following observations apply to both neuronal classes: Neurons gave off 3 to 7 primary dendrites which branched within 10-20μm of the soma. The two daughter dendrites then continued in a relatively straight course with little or no further branching except at their extreme tips. Here, they often ended in a terminal tuft of 3-10 branches. The dendritic arbors had a preferential rostro-caudal orientation with a dendritic extent of up to 1.2mm in that plane but having a lateral extent of no more than 700μm. The averaged length of all dendrites from any neuron ranged from 250-500μm. The longest dendrites observed were 750μm in length and were directed in the rostro-caudal plane, Dendrites remained within the dorsal raphe nucleus in the medio-lateral axis. Rostrally, some dendrites extended as far as the rostral border of the principal occulomotor nucleus. Dorsally, dendritic processes could extend to the IVth ventricle and ventrally, some dendrites were seen to penetrate the medial longitudinal fasciculus. For both cell types, axons most frequently originated from a primary dendrite and less often arose directly from the soma. Axons first described a bend or loop within the dendritic field of the parent cell before becoming directed in their eventual course, be it ventral, dorsal, or lateral. The axons of both neuron types gave off a few axon collaterals which arborized within dorsal raphe. Axonal varicosities along these intrinsic collaterals suggest a synaptic interconnection among raphe neurons. (USPHS Grant NS 14866)

186.7 EVIDENCE FOR SEROTONIN CONTAINING NEURONS IN RAT RETINA. J.W. Zemp, T.N. Thomas and L.D. Middaugh. Depts. of Biochemistry and Psychiatry, Medical Univ. of S.C., Charleston, S.C. 29425.

The evidence for a 5-hydroxytryptamine (5-HT, serotonin) containing neuronal system in rat retina was studied. By high performance liquid chromatography rat retina was found to contain appreciable amounts of the precursor tryptophan, 5-HT, and its major metabolite 5-hydroxyindole-3-acetic acid (5-HIAA). In vitro incubation studies showed that homogenates of rat retina accumulates ^{3}H-5-HT by a process with properties similar to an active uptake system: it was sodium and temperature dependent, ouabain-sensitive and saturable. Inhibitors of 5-HT uptake such as chlorimipramine, desimipramine and fluoxetine were potent inhibitors of ^{3}H-5-HT uptake in rat retina whereas dopamine uptake was more sensitive to inhibition by drugs such as benztropine and d-amphetamine, thus demonstrating the accumulation of 5-HT and dopamine by distinct populations of nerve terminals. Kinetic analysis of the 5-HT uptake data demonstrates a high affinity transport system with an apparent Km of 3.95 x 10^{-7} M, which presumably inactivates 5-HT following synaptic release. The 5-HT is presumably stored in storage vesicles as the uptake was extremely sensitive to reserpine. Intravitreal injection of 5,7-dihydroxytryptamine, which produces degeneration of serotonergic terminals, produced significant reduction in 5-HT uptake by rat retina. Fractionation studies demonstrated that the 5-HT accumulating terminals are localized to elements in the inner plexiform layer, i.e. bipolar or amacrine cells. The results are consistent with the suggestion that 5-HT is a transmitter candidate in rat retina (supported by P.H.S. Grant EY03098 (T.N.T.)).

186.8 SEROTONIN-POSITIVE NEURONS IN MONKEY SPINAL CORD. C. C. LaMotte, N. C. de Lanerolle, and D. Johns*. Sections of Neuroanatomy and Neurosurgery. Yale Univ. Sch. of Med., New Haven, CT. 06510.

The possibility that serotonergic interneurons are present within the spinal cord has occasionally been suggested from physiological and pharmacological studies which indicate 5-HT does not completely disappear following transection to interrupt descending 5HT axons from the brain stem raphe (Shibuya & Anderson 1968; Anderson, 1972; Jones and Wright, 1980).

We have identified a total of 150 neurons in samples of 3 monkey (M. fascicularis and M. arctoides) spinal cords which are positive for serotonin, using a 5-HT antibody (courtesy R. Elde) with the Sternberger peroxidase-anti-peroxidase method. Most of these neurons were located in a small, sparsely-populated column of cells in lamina X, ventral to the central canal. They were either in the ventral commissural grey or lying on the midline ventral grey-white border. The cells were usually very small, 5-10 microns in diameter. EM examination revealed cytoplasmic and nuclear morphology typical of neurons and they could be readily distinguished from glia. Processes of some cells extended toward the ventral motor horn; in others, processes reached to the central canal or wrapped around large blood vessels situated in lamina X. Cells were most frequent in the cervical cord (approx 6-7/mm length of cord) and less frequent in thoracic (1.5/mm), lumbar (3/mm), and sacral (2/mm) cord. In the sacral cord only, a second set of small cells was found consistently in the medial marginal zone, approximately 2/mm length of sacral cord. Their processes could not be traced beyond the marginal and gelatinosal regions.

Immunohistochemical controls confirm the specificity of the immunoreaction used for staining these neurons. In addition, the Falck-Hillarp histofluorescence technique was applied to the lumbosacral cord from one of the 3 monkeys; results confirmed the presence of cells in lamina X with characteristic yellow color and rapid fading typical for serotonergic cells.

It is possible these cells contribute to the innervation of spinal neurons, spinal vessels, or in some cases may secrete into the CSF or may be receptors for CSF composition. Transection experiments in progress may clarify the functions of these cells. (Supported by NIH grant NS13335)

186.9 CENTRAL SEROTONERGIC NEURONS CLEARLY VISUALIZED USING COLCHICINE PRETREATMENT AND A RAPID GLYOXYLIC ACID TECHNIQUE. S. A. Rasmussen* and B. S. Bunney (SPON: J. S. Ebersole). Depts. of Psychiatry & Pharmacology, Yale Univ. Sch. Med., New Haven, CT 06510

The application of the Falck Hillarp fluorescence technique to visualize monoamine systems in mammalian brain ushered in a new era in neuroscience. However, the technique is not without problems. It is time consuming, special equipment is required, and axons and terminals are visualized only in neurons with relatively high concentrations of monoamines. The development of a rapid and more sensitive technique using glyoxylic acid resolved most of these problems but added some of its own. One of these difficulties was the fact that serotonergic neurons could not be adequately visualized in rat brain.

Colchicine, a substance which inhibits axoplasmic transport, concentrates enzymes and transmitters in cell bodies. It has been used effectively in combination with immunocytochemistry to visualize peptidergic neurons with low peptide content. We have found that intraventricular colchicine (20 ul, 0.015 M; 24 hrs before death), combined with the modified glyoxylic acid (SPG) method of de la Torre and Surgeon[1], greatly improved the visualization of rat CNS monoaminergic neurons including serotonergic cell bodies.

There are three major advantages to the use of colchicine pretreatment with the SPG method. First, we have been able to consistently visualize serotonergic neurons. The cells emit a bright yellow light with UV illumination. The intensity of their fluorescence is comparable to that obtained with the Falck Hillarp technique. In addition, although the intensity does diminish, it does so quite slowly in a manner similar to that of neighboring catecholamine neurons. Sections can be examined and photographed on multiple occasions over a four to six week period without the rapid fading of fluorescence seen in Falck Hillarp preparations. Second, visualization of the dendritic fields of catecholaminergic neurons is vastly improved. Processes can often be followed for several hundred microns. Resolution is comparable to that seen with tyrosine hydroxylase immunocytochemistry. Finally, catecholaminergic cells with low amine content, such as those found in the arcuate and periventricular nuclei, are readily visualized. The intensity of fluorescence in these cells is comparable to that seen with immunocytochemical techniques. Thus, intraventricular colchicine has been found to extend the usefulness of the SPG technique to the serotonergic system and to catecholaminergic perikarya with low amine content. (Supported by USPHS grants MH-28849, MH-25642 and the State of Connecticut.)

[1]de la Torre, J.C. and Surgeon, J.W. (1976). Histochem. 49:81-93.

186.10 INFLUENCE OF NEUROLEPTICS ON SEROTONIN- AND DOPAMINE-METABOLISM IN MESOLIMBIC AND STRIATAL AREAS OF THE RAT BRAIN. H. Gerhards*, H. Dilsky*, and A. Hullmann* (SPON: S. FIELDING). HOECHST AG, 6230 Frankfurt/M., Germany.

Levels of 5-hydroxytryptamine (5-HT), 5-hydroxyindole-acetic acid (5-HIAA), dopamine (DA), dihydroxyindole-acetic acid (DOPAC) and homovanillic acid (HVA) in rat striatum and mesolimbic areas (N. accumbens, Tub. olfact.) were determined using High Performance Liquid Chromatography (HPLC) with electrochemical detection.

The method consists of an extraction/homogenization step with formic acid/acetone (twice), a wash of the extract with heptane/chloroform, drying of the aqueous portion under a stream of nitrogen and resuspension in the HPLC-buffer (0.1 M citrate-disodiumphosphate, pH 3.5, containing 0.004 % sodium octylsulfate and 11 % methanol) and is a modification of the method described by C.C. LOULLIS et al.; Pharmacol. Biochem. & Behavior 11, (1979) 89-93. The HPLC-system utilized a C-18 reverse phase column (WATERS Ass. INC, Milford, Mass.) coupled with a glassy carbon detector (Bioanalytical Systems INC., West Lafayette, IN.) set at a potential of + 0.8 V versus the reference electrode. Different chemical classes of neuroleptics, Chlorpromazine (CPZ) and Clozapine (CLOZ) in the phenothiazine resp. dibenzazepines group, Haloperidol (HAL) and Pimozide (PIM) in the butyrophenone group and Sulpiride (SUL) in the benzamide group were given in acute (2 h) and subacute (10 days) experiments to male Wistar rats and levels of 5-HT, 5-HIAA, DA, DOPAC and HVA were determined in striatum and mesolimbic areas of the rat brain.

When compared with untreated controls, all neuroleptic drugs tested increased DOPAC and HVA-levels in both areas in the acute study, whereas only CPZ and CLOZ induced an increase of 5-HIAA-levels. Tolerance developed to both effect after 10 days of treatment.

While the increase in DA-metabolism after neuroleptics is well known and probably a result of the DA-receptor antagonism of those drugs, their effect on 5-HT-metabolism is not well understood. The possible relationship between sedative and muscle relaxant effects of neuroleptics and their effect on 5-HT-metabolism will be discussed.

186.11 METABOLITES OF DOPAMINE AND 5-HYDROXYTRYPTAMINE IN CEREBROVENTRICULAR PERFUSATES OF RATS PRETREATED WITH INTRACEREBROVENTRICULAR INJECTIONS OF 6-HYDROXYDOPAMINE OR 5,7-DIHYDROXYTRYPTAMINE. J.A. Nielsen* and K.E. Moore. Dept. of Pharmacol./Toxicol., Michigan State University, East Lansing, MI 48824.

The present study was initiated to evaluate an in vivo cerebroventricular perfusing technique for estimating the activity of dopamine (DA)- and 5-hydroxytryptamine (5HT)-containing neurons in the central nervous system (CNS) of unanesthetized freely-moving rats. Male rats were implanted with permanent push-pull cannula such that the tips were located in a lateral cerebral ventricle (Tilson and Sparber, J. Pharmacol. Exp. Ther. 181: 387, 1972). Following recovery the cerebroventricular system of each rat was perfused with artificial cerebrospinal fluid (CSF) at a rate of 20 µl/min, and perfusate samples were collected in a high performance liquid chromatography (HPLC) mobile phase (0.1 M citrate-phosphate buffer, pH 3.0, containing 8% methanol and 0.036% sodium octyl sulfate) every 15 min for up to 105 min each day. The concentrations of DA metabolites, dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA), and of the 5HT metabolite, 5-hydroxyindoleacetic acid (5HIAA), were analyzed by HPLC using a C_{18} µBondapak column coupled to an electrochemical detector. The administration of haloperidol (1 mg/kg, s.c.) increased the efflux of DOPAC and HVA but not 5HIAA, while the administration of L-tryptophan (100 mg/kg, i.p.) increased the efflux of 5HIAA but not DOPAC or HVA. Seven days after the intracerebroventricular (icv) injection of 6-hydroxydopamine (6OHDA; 200 µg) the basal and haloperidol-induced release of DOPAC and HVA were markedly reduced, while the basal release of 5HIAA was unaltered. Seven days after a second dose of 6OHDA there was a further reduction in the basal and haloperidol-induced efflux of DOPAC and HVA, while the basal efflux of 5HIAA was still unaltered. On the other hand, 10 days after icv 5,7-dihydroxytryptamine (200 µg) the basal and L-tryptophan-induced release of 5HIAA were markedly reduced while the basal release of DA metabolites were unaltered. These results reveal that the metabolites of DA and 5HT appearing in CSF perfusates originate from DA and 5HT neurons in the CNS, respectively, and suggest that this technique can be employed to monitor the activity of DA and 5HT neurons in conscious, unrestrained rats. (Supported by USPHS grant NS 15911.)

186.12 REGIONAL DIFFERENCES IN THE RESPONSE OF NEOSTRIATAL NEURONS TO SYSTEMIC AMPHETAMINE ADMINISTRATION: APPARENT INVOLVEMENT OF A SEROTONERGIC PROJECTION FROM THE DORSAL RAPHE NUCLEUS. Stephen D. Curtis and George V. Rebec, Dept. Psychol., Indiana Univ., Bloomington, IN 47405.

We have previously reported that a direct infusion of d-amphetamine into the dorsal raphe nucleus (DRN) inhibits unit activity in this site but produces a concomitant mirror-image increase in firing rate in the lateral, but not medial, neostriatum (Rebec et al., Soc. Neurosci. Abstr., 1980, 6: 233). These results are consistent with evidence that serotonergic afferents from the DRN are confined to the lateral neostriatum (e.g., Azmitia & Segal, J. Comp. Neurol., 1978, 179: 641) and that they exert an inhibitory influence on neostriatal activity (e.g., Miller et al. Brain Res., 1975, 97: 133). The present study further examined the DRN-neostriatal interaction following systemic amphetamine administration in immobilized, locally anesthetized rats. Unit activity was recorded simultaneously in the DRN and in the medial or lateral anterior neostriatum. d-Amphetamine was administered via an indwelling intraperitoneal catheter.

An injection of 1.0 mg/kg d-amphetamine inhibited the activity of single neurons in the DRN but produced a simultaneous increase in the firing rate of neurons in the lateral neostriatum. Consistent with previous reports, unit activity in the medial neostriatum was typically inhibited by this low dose of the drug. In some animals, electrolytic lesions of the DRN produced a dramatic acceleration of neostriatal activity but only if this activity was recorded in the lateral neostriatum. The results of these experiments suggest that the response of neurons in the neostriatum to amphetamine is not homogenous and that serotonin may mediate regional differences in the response to this drug.

This research was supported by USPHS Grant DA-02451-03 from the National Institute on Drug Abuse.

186.13 EFFECTS OF THE SEROTONIN AGONIST, QUIPAZINE, ON SPINAL REFLEXES IN THE RAT. Barry D. Goldstein and Edmund G. Anderson. Dept. of Pharmacology, Univ. of Illinois Coll. of Med. Chicago, IL 60612.

We have used quipazine, a serotonin (5-HT) receptor agonist, to study the role of 5-HT in modulating dorsal and ventral root reflexes in the rat spinal cord.

Male Sprague-Dawley rats were anesthetized with halothane and the spinal cord transection at C1. Following laminectomy the L4-S1, dorsal roots were bilaterally sectioned. The monosynaptic reflex (MSR), dorsal root potential (DRP), and dorsal root reflex (DRR) were recorded. The spontaneous ventral root discharge (VRD) and spontaneous antidromic dorsal root discharge (DRD) were simultaneously recorded from the L6 ventral and L5 dorsal roots.

Quipazine (1.0 mg/kg, i.v.) increased the MSR and VRD to 210 and 330 percent above control values, respectively. Conversely, quipazine depressed the DRP, DRR and DRD to 70, 55 and 50 percent of control. The maximal effect on the ventral root responses (MSR and VRD) appeared about one minute after injection. These effects remained maximal throughout the experiment. On the other hand, the depression of the dorsal root responses was delayed, requiring about 30 minutes for the maximal effect.

Cinanserin (4 mg/kg, i.v.), a 5-HT antagonist with high affinity for the 5-HT_2 receptors, was only effective in reversing the quipazine-induced excitation on the ventral root. It had no effect on the quipazine-induced depression of the DRP, DRR, and DRD. The 5-HT antagonist metergoline (0.5 mg/kg, i.p.) was tried because it has a high affinity for both 5-HT, and 5-HT_2 receptors. Metergoline had little effect on reversing the excitation of the ventral root responses, or the depression of the DRP. It did, however, reverse the depressant effect of quipapzine on the DRR, increasing it to 225 percent above control. However when metergoline alone was given it increased the DRR to 260 percent above control without affecting the MSR or VRD. The increase in the DRR by metergoline was antagonized by cinansein, suggesting metergoline may have some 5-HT agonist actions. Low doses of quipazine (0.1 mg/kg) were investigated to clarify the actions on the DRR. Low doses of quipazine, like the high dose, increased the MSR and VRD. However, unlike the high dose, this dose increased the DRR and DRD. Again, the onset of the dorsal root effects was slow.

In conclusion, quipazine appears to facilitate motoneuronal discharge, an action reversed by 5-HT antagonists with high affinity for 5-HT_2 receptors. Low doses of quipazine facilitate and high doses depress the DRR. These actions are not blocked by 5-HT_2 receptor antagonists. Metergoline also increase the DRR. The effects on the DRR may be mediated by a 5-HT_2 receptor system. (Supported by USPHS Grant PHS NS 14985)

186.14 SPINAL SEROTONIN MEDIATES A SUPRASPINAL COMPONENT OF ANALGESIA PRODUCED BY VAGINAL STIMULATION IN RATS. J.L. Steinman* and B.R. Komisaruk.

Previous studies have shown that perispinal (intrathecal) administration of phentolamine significantly attenuates vaginal stimulation-produced analgesia (VS-PA) on both tail flick and vocalization to shock tests. On the other hand, intrathecal methysergide antagonized VS-PA on the vocalization measure only. To further elucidate the role of spinal serotonin in the mediation of VS-PA, we administered 5,7-dihydroxytryptamine (DHT) intrathecally to the lumbar cord to destroy serotonergic terminals in the spinal cord.

DHT was injected through PE-10 catheters passed to the lumbar level of the spinal cord via an incision in the cisterna magna according to the method of Yaksh and Rudy (1976). VS(200g force) was applied via a calibrated glass probe assembly (modified 1cc syringe plunger). Analgesia was assessed by the latency (in sec) to withdraw the tail from radiant heat and the threshold (in mA) to vocalize in response to tail shock.

Three separate groups of ovariectomized rats received 4ug or 10ug of DHT or vehicle control in 10ul saline (with .2mg ascorbic acid/ml). Data reported were taken three days after perispinal drug administration. No significant differences were observed among groups on the tail flick measure; VS significantly increased the tail flick latency in all groups (preprobe latencies: 4ug-2.2; 10ug-2.15; Saline-2.49; after 5sec VS: 4ug-5.08; 10ug-5.42; Saline-5.31). In contrast, using the vocalization measure, both 4ug and 10ug DHT significantly depressed the effects of VS (correlated t test; *:$p<.05$). Vocalization threshold is expressed as percent increase from preprobe baseline 4ug-135% ns; 10ug-106% ns; Saline-181%*.

The present findings demonstrate that destruction of serotonergic terminals in the spinal cord with 5,7-DHT attenuates the effects of VS-PA as measured by vocalization, but not tail flick tests. Vocalization threshold was used as an indicator of a supraspinal nociceptive process and tail flick as an indicator of a spinal mechanism, since the latter is blocked by VS even in spinal transected rats. These findings indicate that a descending spinal serotonergic mechanism which is activated by VS differentially suppresses a supraspinal but not a spinal nociceptive response. These results support our earlier findings that intrathecal serotonin receptor blockers inhibit the effects of VS on vocalization tests only.

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186.15 CHARACTERIZATION OF THE ACUTE EFFECTS OF METHAMPHETAMINE ON SEROTONERGIC AND DOPAMINERGIC ACTIVITY IN SELECTED REGIONS OF THE RAT BRAIN. C. Bakhit*, M. A. Peat* and J. W. Gibb (SPON: W. Stevens). Dept. of Biochem. Pharmacol. and Toxicol., Univ. of Utah, Salt Lake City, Utah 84112.

Acute administration of methamphetamine (METH) to rats results in a dose-dependent depression of brain tryptophan hydroxylase (TPH) activity. In this study, we measured TPH activity, tyrosine hydroxylase activity (TH) and the levels of dopamine (DA), 5-hydroxytryptamine (5-HT), 5-hydroxyindoleacetic acid (5-HIAA) and tryptophan (TRP) in the cerebral cortex and neostriatum of the rat brain at 15, 30, 60 and 180 min following a single injection of METH (2.5 or 10 mg/kg, s.c.). TH and TPH activities were measured as described previously (Hotchkiss et al., Life Sci. 25, 1373, 1979). DA, 5-HT, 5-HIAA and TRP were measured concurrently by an HPLC-fluorescence method. In both the neostriatum and cerebral cortex, TPH activity was depressed at lower doses of METH and at earlier times than 5-HT and 5-HIAA. The depression of TPH was then followed at a later time by a decrease in levels of 5-HT and its metabolite. For the cerebral cortex, METH (2.5 mg/kg) produced a significant decrease in TPH activity at all time points, no change in 5-HT levels, a transient increase in 5-HIAA at 30 min and a significant rise in TRP levels at 60 and 180 min. When 10 mg/kg of METH was given, the depression of TPH was more pronounced, compared to the depression observed with the 2.5 mg/kg dose of METH, and a profound depression of 5-HT and 5-HIAA was observed at the later time points in both regions. TRP was increased at an earlier time at this dose of METH. In contrast, no change was found in TH activity at the times and doses of METH selected. Preliminary results show an increase in DA levels with the lower dose of METH. The results indicate that the primary effect of METH on the serotonergic system is on TPH activity and that the serotonergic system is more sensitive than the dopaminergic system to the effects of METH. (Supported by USPHS Grant DA 00869)

186.16 RECORDING OF RAPHE UNIT ACTIVITY IN VITRO IN MOUSE BRAIN SLICES. Gailyn A. Howell, J. W. Brandstetter*, Mary H. Frederickson, Christopher J. Frederickson and Michael E. Trulson. Dept. Psychol. Univ. of Texas at Dallas, P.O. Box 688, Richardson, TX 75080.

Serotonin-containing raphe neurons display a characteristic slow and rhythmic discharge rate (0.5-3.0 spikes/sec) in chloral hydrate anesthetized rats. However, the significance of this discharge pattern and the factors which generate it are completely unknown. Interestingly, these neurons maintain this characteristic slow and rhythmic discharge pattern when recorded from rat brain slices in vitro (Mosko and Jacobs, Neurosci. Lett. 2: 195-200, 1976). This suggested that it may be possible to develop an in vitro preparation for studying the synaptic pharmacology of raphe neurons. In the present study we explored this possibility by recording the activity of raphe neurons from mouse brain slices in vitro with and without high concentrations of magnesium in the incubation medium. Adult mice were decapitated, the brains rapidly removed, and the brainstem was sectioned into 400 u coronal slices using a Sorvall tissue slicer. The slices were incubated in standard oxygenated Yamamoto's solution at 34°C under an atmosphere of moist 95% O_2 - 5% CO_2. A glass micropipette (tip dia 1-2 u) filled with 2 M NaCl was lowered into the dorsal raphe nucleus with the aid of a dissecting microscope, and extracellular unit activity was recorded and analyzed. Raphe neurons recorded in mouse brain slices in vitro discharged spontaneously with a slow and regular rhythm (3.2 ± 0.4 spikes/sec) very similar to that observed in rat brain in vivo or in vitro. Consistent with the findings of Mosko and Jacobs (1976), a few cells with a rhythmic discharge pattern but much higher rate (8-13 spikes/sec) were found within the dorsal raphe nucleus. When magnesium ion (as the sulfate salt) was added to increase the media concentration to 20 mM, for the purpose of inhibiting all synaptic transmission (e.g., Barker and Nicoll, J. Physiol. 228: 259-277, 1973) raphe neurons continued to display the same discharge pattern and rate (3.0 ± 0.5 spikes/sec). These latter cells were recorded between 10 - 120 min post-magnesium, and several were recorded through the transition of pre- to post-magnesium. The fact that raphe neurons continue to discharge in the characteristic regular fashion in the apparent absence of any synaptic input suggests that they have an endogenous rhythm and may function as pacemaker cells in the CNS. Moreover, the fact that these cells can be identified by their characteristic firing pattern in high magnesium media makes it possible to investigate the action of drugs which are believed to act directly on raphe neurons (e.g., LSD, DMT, psilocin), i.e., independent of confounding presynaptic effects.

187.1 EFFECTS OF PERINATAL HYPOXIA ON KINDLED CONVULSIONS IN YOUNG RATS. B. J. Albala, S. L. Moshe' * and I. Katz.* Dept. of Psychiatry and Dept. of Neurology, Albert Einstein College of Medicine, Bronx, New York 10461.

Early childhood seizures are often attributed to perinatal oxygen deprivation. To evaluate this relationship of perinatal hypoxia to seizure development we exposed newborn rats to hypoxia and measured their susceptibility to electrically kindled seizures.

Two litters of Sprague-Dawley rats, bred in these labs., were randomly divided into three groups. Within the first 24 hours after birth two of the groups were removed from their mothers and placed into one of two glass bell jars equipped with a water mattress which was maintained at 34-36 C. Humidified room air or 6% oxygen in nitrogen was passed through the sealed chamber at the rate of 2-3 L/min. The levels of oxygen, CO_2, temperature and the behavior of the animals were monitored during the 4 hour experimental period. The parameters of hypoxia used have previously been reported to produce long lasting neurochemical and behavioral alterations which persist for at least 4 weeks after a single exposure (Hedner, T., *Acta Physiol. Scand., suppl.460:1,* 1978). Both groups were returned to their home cage with their mother and the undisturbed third group of rat pups and were allowed to grow until they were stereotaxically implanted at 27 days of age with a bipolar electrode in the left amygdala. After two days of recuperation each animal was given a 50 *uA*, 60 Hz 1 sec sinusoidal stimulation which was then increased by 50 *uA* every hour until an afterdischarge (AD) was elicited; this current was designated as the AD threshold. The animals were subsequently given a suprathreshold (400 *uA*, peak to peak) stimulation every hour until a generalized motor convulsion was elicited or the fortieth stimulation was completed; this procedure was conducted over four days.

During the 4 hour period of hypoxia the newborn pups became markedly cyanotic in contrast to their littermates in air. No seizures or deaths occurred. There were no statistically significant differences in body weight at 27 days of age among the three groups. Analysis of the kindling data did not reveal any significant differences in the AD thresholds, AD duration at threshold, AD duration during the first generalized convulsion and in the number of stimulations required to elicit a generalized convulsion.

These preliminary results indicate that this degree of hypoxia during the first day of life does not facilitate the development of kindling later in life. The data also suggest that neither early nutritional deprivation nor maternal separation for a period of 4 hours significantly alters later weight or any of the epileptogenic variables that were measured in the present study.

187.2 PHOSPHORYLATION OF SYNAPTIC PROTEINS IN AMYGDALOID-KINDLED RATS. Elizabeth I. Tietz* and Robert F. Berman (SPON:Joyce A. Benjamins) Dept. of Psychology, Wayne State University, Detroit, MI 48202.

Converging lines of evidence suggest that alterations in phosphoprotein metabolism may be involved in the initial formation or maintenance of a seizure focus. The kindling model of epilepsy was used to investigate possible changes in phosphoprotein metabolism occuring during the development of an amygdaloid-kindled seizure focus.

Bipolar electrodes were stereotaxically implanted in the amygdala of adult male hooded rats. Afterdischarge thresholds were determined and animals were stimulated once daily at threshold (100 Hz, biphasic, square wave, 0.1 msec pulse duration) for 1 sec until Stage 5 kindled seizures were elicited on 3 of 5 days. After reaching criterion, kindled rats were sacrificed by decapitation immediately after (n=6), or without (n=5) an elicited seizure. Non-implanted matched controls were sacrificed with each kindled animal for comparison. Brains were removed and the amygdala-entorhinal cortex, hippocampus, and frontal cortex of each animal were dissected within 1 min and homogenized at 4 C. The homogenate was centrifuged to yield a crude P_2 pellet which was resuspended for 30 min in ice-cold dH_2O and recentrifuged. The resultant pellet was resuspended and the protein concentrations were adjusted to 3-4 mg/ml. The samples were phosphorylated in vitro by incubation with 5 uM (^{32}P)-ATP for 2 min. The phosphorylated samples were subjected to SDS-polyacrylamide gel electrophoresis, stained with Coomassie Blue, and densitometrically scanned for protein concentration. Dried gels were placed in contact with No-Screen X-ray film for 3-5 days and the resultant autoradiograms were scanned to determine the amount of ^{32}P incorporation into protein.

Decreases in ^{32}P incorporation were seen in synaptic proteins isolated immediately after a kindled seizure from amygdaloid-kindled rats, compared to non-kindled controls. Specific decreases were most prominent in bands 55K and 80K daltons in membranes derived from the amygdaloid region. Less prominent decreases in ^{32}P incorporation were also observed in hippocampal-derived membranes, but not in samples from cortex. In contrast, an increase in ^{32}P incorporation was observed in amygdala-derived membrane fractions obtained from kindled rats sacrificed without seizure, with the most pronounced change occuring at 80K daltons.

These results support the possible involvement of synaptic phosphoproteins in the development and maintenance of an amygdaloid-kindled seizure focus. (Supported by NIMH Grant MH35078-01)

187.3 LONGLASTING ALTERATIONS IN INHIBITORY PROCESSES IN KINDLED RATS. L.P. Tuff, R. Racine and R. Mishra. (SPON: H. Weingarten). Dept. of Psychology, McMaster Univ., Hamilton, Ont. L8S 4K1.

Repeated intermittent electrical stimulation of many brain structures, particularly in the limbic system, eventually produces increasingly long and widespread afterdischarges and culminates in the production of major motor seizures. This phenomenon has been termed 'kindling' and is essentially permanent. As a model of epilepsy it provides a unique opportunity to examine the longlasting effects of epileptogenesis uncontaminated by the immediate sequelae of the ictal event.

Inhibitory systems, and in particular those mediated by GABA, have for sometime now been associated with seizure phenomena. We have measured GABA and benzodiazepine receptor binding in thirteen different brain areas in kindled and control rats two weeks after their last stimulation. ^{3}H-flunitrazepam binding was significantly increased ($p<.01$, n=10) in both the stimulated and contralateral amygdalae of kindled animals by 15% compared to controls. In addition, significant decreases were seen in striatal tissue on both sides of the brain which were of similar magnitude. Subsequent Scatchard analyses indicated that the number of binding sites and not their affinity was the source of the changes. ^{3}H-GABA binding in TX-100 treated tissue was unaltered in kindled animals, but in tissue that was washed without detergent ^{3}H-muscimol binding was significantly increased in the ipsilateral hippocampi of kindled rats.

In a parallel experiment, the effects of kindling on paired pulse depression of potentials evoked in the dentate by stimulation of the perforant path were examined. This phenomenon is thought to be indicative of granule cell recurrent inhibition. Kindling produced a dramatic increase in the depression effect that was not seen in unkindled control animals.

Taken together, these experiments suggest that following kindling, GABA mediated inhibition is increased in several brain areas. It would appear then, that if inhibitory failure plays a role in the established kindled response, it is probably not due to a tonic deficit.

187.4 IN VIVO AND IN VITRO INVESTIGATIONS OF KINDLING-INDUCED EPILEPTOGENESIS. E.W. Kairiss*, R.J. Racine and G.K. Smith. Dept. of Psychology, McMaster University, Hamilton, Ontario, Canada L8S 4K1.

The experiments reported here use the kindling model of epilepsy to determine which brain areas develop spontaneously discharging epileptic foci most readily.

Five experimental groups of male Long-Evans rats were prepared. Bipolar stimulating electrodes were stereotactically implanted in region CA3 of hippocampus (HPC), lateral amygdala (AMY), lateral olfactory tract (LOT), perforant path (PP) and fornix (FX). EEG and evoked potentials were recorded with monopolar electrodes from hippocampus, pyriform cortex, caudate nucleus, thalamus and brainstem reticular formation. Daily kindling stimulations were applied until five class V seizures (Racine, R.J., EEG Journal, 32:281, 1972) had been elicited. Spontaneous and evoked ictal and inter-ictal spikes were recorded before, during and after seizure induction, and spike latency and and amplitude were compared. Finally, the animals were sacrificed and in vitro slices of hippocampus were prepared (Dingledine, R. et al., J. Neuroscience Meth, 2:323, 1980). Stimulus evoked population responses were recorded from sub-regions of dentate gyrus and hippocampus during exposure to 3, 6 and 9 mM concentrations of extracellular K^+, and in response to single, double and repetitive (60 Hz) stimulation of afferents.

Our in vivo observations may be summarized as follows: 1) Spontaneous and evoked post-ictal spikes were most readily elicited in the AMY and LOT animals; 2) dominant (in terms of latency and amplitude) spikes (spontaneous and evoked) were most often seen in the amygdala and pyriform cortex, irrespective of stimulation site; 3) the hippocampus tended not to be a dominant site in any of these groups, including HPC.

In vitro, there was little evidence for alteration of hippocampal excitability as measured by the K^+-sensitivity of the evoked responses. There was, however, an increased tendency to generate double-spiked field potentials in 3 mM $[K^+]$.

We conclude that a) of the sites studied, the amygdala and pyriform cortex demonstrate the greatest susceptibility to kindled epileptiform activity, and b) in spite of the low threshold of the hippocampus for evoked epileptiform activity, it appears relatively immune to the long term, spontaneous focal epilepsy generated via kindling stimulation.

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187.5 MECHANISMS OF EPILEPTOGENESIS IN KINDLING-PRONE VS KINDLING-RESISTANT RATS. M. Oshima* and R.J. Racine. (SPON: W. Heron). Dept. of Psychology, McMaster Univ., Hamilton, Ont. L8S 4K1.

The kindling phenomenon is one of several that have proved useful in the study of the mechanisms of epileptogenesis. The epileptiform response, in the kindling preparation, is triggered by electrical brain stimulation, and the strength of the response is increased by repeated activation of stimulated sites. Many studies (e.g. neurochemical analyses) involve the comparison of kindled vs non-kindled animals. One problem with these studies is that observed differences may be produced by, but not be necessary for, kindling.

As another approach to the question of kindling mechanisms, we have begun to consider some of the differences, present prior to kindling, that could account for fast vs slow kindling rates. To do this, we have outbred kindling-prone (KP) and kindling resistant (KR) rat strains (based on amygdala kindling rates). KP rats typically reach the first generalized convulsion within about 7 stimulations with an epileptiform afterdischarge (AD) threshold of about 50 μA, whereas KR rats usually require over 21 stimulations with a threshold of about 100 μA.

KP rats were not different from KR rats in the extent or duration of post-tetanic potentiation or long-term potentiation. They were different, however, in paired pulse effects. There appeared to be a greater paired pulse depression effect in KR vs KP rats. This possibly reflects a stronger recurrent inhibition effect in KR rats (Lømo, T., Exp. Brain Res., 12:46, 1971).

The KP rats also showed increased susceptibility to convulsant drugs that act by suppressing the GABA system (bicuculline, picrotoxin, and isoniazid), but were not different in their response to strychnine (a putative glycine blocker), or in the frequency of occurrence of audiogenic seizures. Higher doses of diazepam were required to prevent seizure responses in previously kindled KP rats. There were no differences, however, in benzodiazepine or muscimol binding in these strains.

We hypothesize that KP rats have lower AD thresholds and faster kindling rates due to a weaker background recurrent inhibition in the affected sites. These inhibitory systems may also be more prone to failure in KP rats.

187.6 EFFECTS OF GABA AGONISTS AND ANTAGONISTS ON WELL-ESTABLISHED AMYGDALA-KINDLED SEIZURES. W.M. Burnham, M.W. Kalichman and K.E. Livingston. Dept. of Pharmacology, University of Toronto, Toronto, Ontario M5S 1A8.

Seven GABA-related drugs were tested against well-established amygdala-kindled seizures. Adult, male Royal Victoria hooded rats served as subjects, each subject being implanted with a single bipolar amygdaloid electrode and "kindled" on a daily schedule to a criterion of 20 (minimum) stage 4-5 seizures. Drugs were administered via the i.p. route in a standard volume of 1 ml/kg body weight. Tests followed at appropriate post-injection intervals. Dose levels ranged from subtherapeutic to toxic.

It was found that 3-Mercaptopropionic acid, bicuculline, and picrotoxin decreased motor seizure latency, but did not otherwise exacerbate kindled seizures. Pentobarbital and gamma-vinyl-GABA both antagonized the generalized component of the seizures, but gamma-vinyl-GABA was active only at high doses which caused severe toxicity. Imidazole acetic acid and the GABA agonist SL 76-002 had no measurable effect.

The results indicate that (except at the ionophore level) manipulation of the GABA system has little effect on kindled seizures. These data, in combination with other results relating to GABA effects on the development of kindled seizures, suggest that kindling is not a GABA-related phenomenon.

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187.7 ANATAGONISM OF MOTOR SEIZURE GENERALIZATION BETWEEN CONCURRENTLY DEVELOPING KINDLED SEIZURE FOCI. J.L. Burchfiel, K. Serpa*, F.H. Duffy. Children's Hospital, Boston MA 02115

The "transfer" effect of kindling is usually thought of in positive terms: kindling of one site accelerates the rate of kindling from other, secondary sites. However, McIntyre & Goddard (EEG Clin. Neurophysiol., 35:533-543, 1973) and Burnham (Can. J. Neurol. Sci., 2:417-428, 1975) have also demonstrated a "negative transfer" effect in which transfer kindling of a secondary site transiently interfered with the kindling of the original, primary site. Recently, we investigated negative transfer effects in more detail (Duchowny & Burchfiel, EEG Clin. Neurophysiol., in press). We attempted to concurrently kindle septum and ipsilateral entorhinal cortex (EC) by stimulating them in alternation. The dominant outcome of this paradigm was antagonism of motor seizure generalization from one of the sites.Only one site kindled; kindling from the other site was suppressed.

The present study extends this investigation by trying concurrent kindling of new pairs of sites in the limbic system of the rat: septum and contralateral EC, amygdala and ipsilateral EC, bilateral amygdalae, and bilateral EC. Each pair was stimulated in alternation on a trial-by-trial basis. Antagonism of motor seizure development was the consistent finding. For all pairs except bilateral amygdalae, one site became dominant and progressed to generalized seizures, while kindling from the other site was suppressed. For pairs of sites with equal rates of primary kindling (e.g., septum and EC), which site became dominant was random. For pairs with unequal kindling rates (e.g., amygdala & EC), the one with the higher rate was always dominant. Kindling antagonism was comparable whether the two sites were in the same or different hemispheres. Concurrent kindling of bilateral amygdalae resulted in mutual antagonism. For most trials, both sites elicited only partial motor seizures; fully generalized convulsions were rare. In all cases, antagonism of kindling was expressed as suppression of motor seizure generalization. The suppressed site consistently elicited ADs, accompanied by arrest of ongoing behavior, automatisms and occasional partial motor seizure activity, but the usual progression to generalized convulsions did not occur.

187.8 LASTING INFLUENCE OF AMYGDALOID KINDLING ON CHOLINERGIC NEUROTRANSMISSION. Noda, Y.*, Uemura, S.*, McGeer, E.G., and Wada, J.A.* Division of Neurological Sciences, University of British Columbia, Vancouver, B.C. Canada V6T 2A1

Eighteen male hooded rats of the Royal Victoria Hospital strain weighing 300-500 g were implanted with nichrome wire electrodes in left amygdala. Nine animals were stimulated once a day with 60 Hz at afterdischarge threshold (100-200 μA) for one second and the other nine animals were handled daily without stimulation and used as control. The animals reached Stage 5 with 11-18 daily stimulations. Stage 5 seizures were induced for additional 18-25 days. Fifteen days following the last stimulation, the animals were killed and various brain areas were dissected from 500 μm thick coronally sectioned brain slices. Choline acetyl transferase (CAT) activity and muscarinic receptor binding were measured with radio chemical assays using acetyl[1-^{14}C] coA and ^{3}HQNB respectively. CAT activities were decreased in whole brain areas examined in kindled animals. Significant changes were detected bilaterally in frontal cortices ($p < 0.05$), hippocampi (same) and amygdalae, right ($p < 0.01$): left, ($p < 0.05$). While no consistent change was found in muscarinic cholinergic receptor binding among various brain areas tested, in the stimulated hemisphere, muscarinic cholinergic binding was decreased in the frontal cortex ($p < 0.01$)., but the amygdala and pyriform cortex showed an increase ($p < 0.01$).

Participation of the muscarinic cholinergic system in the amygdaloid kindling seizure has been suggested by biochemical and pharmacological studies. Furthermore, anatomical study has indicated that the cholinergic projections from the basal nuclei to neocortex and amygdala. Although the possible role of muscarinic cholinergic mechanism in the kindling model of epilepsy has been discussed (Dasheiff, R.M. et al., Brain Research 206: 233-238, 1981), recent study seems to suggest that the change observed shortly after the completion of kindling may not reflect the processes involved in the kindled 'permanent' change. (Peterson et al., Experimental Neurology 71:144-153, 1981). Our results show that the influence of kindling on the cholinergic system is till obvious fifteen days following the last stimulation.

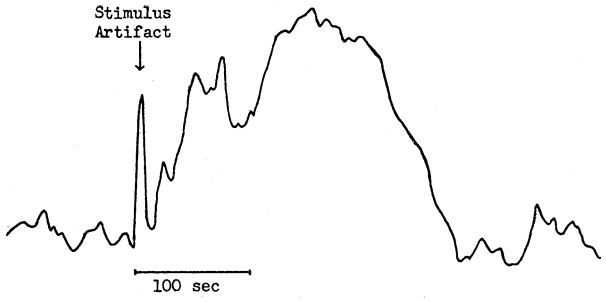
187.9 FACILITATION OF HIPPOCAMPAL KINDLING WITH AMYGDALA LESIONS. D. C. McIntyre* and K. A. Stokes* (SPON: J. Metuzals). Physiological Psychology Lab., Carleton University, Ottawa, Ontario, Canada.

Kindling appears to involve the progressive breakdown of inhibitory influences in the brain (eg., McIntyre, 1980). Noradrenergic systems seem to play an important role in this inhibition, for depletions of forebrain norepinephrine facilitates amygdala kindling (McIntyre, Saari and Pappas, 1979). This effect has also been demonstrated with discrete depletion of norepinephrine in the amygdala (McIntyre, 1980). Thus, it was postulated that the amygdala interacts with the kindling process of other structures of the limbic system. Lesion of the amygdala might have an influence on the kindling rate of the relatively slow-kindling dorsal hippocampus. Electrodes were implanted into the right dorsal hippocampus of Sprague-Dawley rats, and either ipsilateral, contralateral, bilateral or sham direct current lesions (2 mA., 40 seconds) were performed on the amygdala. After a three-week rest period, hippocampal kindling was begun (60 Hz, 100 μA, sine wave, 5 second duration, one stimulation per day). Results indicated that animals with bilateral or ipsilateral lesions of the amygdala demonstrated significantly facilitated hippocampal kindling, as compared to sham and operated controls. Contralateral lesions produced no facilitatory effect on kindling rate. An interesting negative correlation was found between kindling rate and latency to onset of the behavioural seizure. The data support three issues in kindling: 1) kindling involves a breakdown of inhibitory processes; 2) the amygdala influences seizure development of other limbic structures, being inhibitory as far as the dorsal hippocampus is concerned, and 3) the changes involved in kindling are primarily ipsilateral in nature.

187.10 RELATIVE CHANGES IN FOCAL SITE OXYGEN AVAILABILITY MEASURED BY POLAROGRAPHIC MICROELECTRODE IN THE KINDLED EPILEPTIC RAT. T.J. Lynch* and W.J. Jackson* (SPON: G. Doetsch). Dept. of Physiol., Medical College of Georgia, Augusta, Ga. 30912

Following kindling stimuli of 150uA (60 Hz, 3 sec train) applied to the left amygdala of male, Sprague-Dawley rats, focal oxygen availability, aO_2, has been observed to increase to an extent at least 8 times greater than its normal, rhythmic oscillation. A combination aO_2/EEG/stimulation electrode was fabricated, the aO_2 segment of which was a platinum-iridium rod of 120 micron diameter, insulated but for 800 microns at its tip. This working surface was coated with Rhoplex and positioned between the two poles of the electrode's EEG/stimulation aspect, the inter-pole separation of which was 1.5mm. The rest of the aO_2 circuit consisted of a Ag-AgCl reference electrode applying +.6V, and an op. amp. current to voltage converter driving an x-y plotter. The representative trace shown below was made at 100 sec./inch and shows 8.5 min. of pre- to postictal aO_2, the early spike in which is stimulus artifact. Increases in ictal aO_2 are observed in most cases from the first day of stimulation and increase in extent and duration as the afterdischarges take on motor involvement. The increment in aO_2 ranges between 20 and 50% above the pre-ictal aO_2. The downward turn of the ictal aO_2 record toward baseline correlates with the termination of the high frequency-high amplitude segment of the ictal EEG. The postictal aO_2 generally shows a 5 to 10 min. period during which the amplitude of its normal, rhythmic variation is decreased.

(Supported by NIH grant 1-NS6-2340)



187.11 FACILITATION OF KINDLED SEIZURES IN RATS FED CHOLINE-SUPPLEMENTED DIETS. K.J. McCann*, D.P. Cain and D.J. Philbrick* (SPON. J.J. Seguin), Departments of Psychology and Physiology, University of Western Ontario, LONDON, Ontario, CANADA. N6A 5C2.

Repeated electrical or chemical stimulation of the brain at initially subconvulsive levels gradually leads to the development of seizures -- the "kindling" effect. Recent studies have indicated that activation of ACh- containing neurons can facilitate kindling.

In order to study the possible influence of dietary choline on the rate of kindled seizure development, male hooded rats were fed either a choline- deprived diet (ICN Pharmaceuticals choline-deficient diet with 0.8% L-cystine added) or a choline-supplemented diet 556 mg choline/kg diet) beginning at age 40 days. Amygdaloid electrodes were implanted at age 70 days and after-discharge threshold (ADT's) were determined after two weeks The rats were then stimulated at their individual ADT with a 1-second train of biphasic pulses once daily until a generalized seizure developed.

Rats fed the choline-deprived diet had significantly higher ADT's and required significantly more AD's to develop generalized seizures than the choline- supplemented rats (133μA vs 70 μA and 12.4 vs 8.6 AD's respectively). However, the results of the choline-deprived rats are quite comparable to those observed in normal control rats, indicating that the choline-supplemented rats kindled faster than normal. This result is consistent with the finding that dietary choline deprivation does not reduce the level of ACh in the brain, presumably because compensatory mechanisms act to maintain normal levels (Haubrich et al., J. Neurochem. 27: 1305, 1976). It is also consistent with the finding that increased dietary choline intake can affect brain ACh metabolism (Cohen and Wurtman, Science, 191: 561, 1976), and supports the view that cholinergic circuits are important in the development of amygdaloid kindled seizures. Based on these preliminary results, studies are planned to establish a possible dose-response relationship, and to measure brain levels of ACh in the different groups.

Supported by a grant from the Natural Sciences and Engineering Research Council of Canada to D.P.C.

187.12 PENTYLENETETRAZOL SENSITIZATION FACILITATES SUBSEQUENT AMYGDALOID KINDLING IN THE RAT. D.P. Cain. Department of Psychology, Univ. of Western Ontario, London, Ontario, CANADA, N6A 5C2.

The repeated administration of a variety of convulsant agents in initially subconvulsant amounts results in the gradual development of seizures--the kindling effect. We and others have demonstrated that subjects kindled using one convulsant agent can be rendered thereby more susceptible to a second such agent, but the mechanism by which this transfer facilitation occurs is not well understood. In particular, it is not clear whether subconvulsive behavioral sensitization to the initial kindling agent is sufficient for transfer facilitation, or whether full-blown behavioral convulsions also must be evoked. The approach that was taken in the present study was to compare the degree of transfer facilitation that occurred in two intact groups of rats that differed only in terms of their sensitization or seizure history.

Adult male hooded rats were implanted with bipolar electrodes in the amygdala and were randomly placed into sensitized, convulsed, or control groups. The sensitized subjects received 30 bidaily injections (i.p.) of pentylenetetrazol (PTZ) at 20 mg/kg, which resulted in the gradual development of behavioral sensitization (twitching, myoclonic jerks) in all subjects. The convulsed subjects received 25 similar injections, plus a mean of 5.8 injections of PTZ at gradually increased doses until either 2 or 3 generalized seizures had occurred. The control subjects received 30 injections of saline. When the subjects were subsequently electrically kindled through the amygdaloid electrode the sensitized subjects required a mean of 9.5 afterdischarges (ADs), the convulsed subjects required 7.6 ADs, and the control subjects required 13.2 ADs to kindle. Both PTZ-injected groups kindled significantly faster than the control group ($p<.02$ and $p<.01$), but did not differ significantly from each other in their rate of kindling. These results indicate that substantial transfer facilitation of electrical kindling of the amygdala occurs whether sensitization alone or sensitization together with convulsions are induced by prior repeated injection of PTZ. These results, taken together with results obtained earlier in our laboratory, also indicate that transfer facilitation occurs bidirectionally when injection of PTZ and electrical stimulation of the amygdala are the two convulsant agents administered in the rat.

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187.13 ELECTROCONVULSIVE SHOCK INHIBITS AMYGDALA KINDLING

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Amygdala-kindled seizures are associated with the development of long-term alterations in convulsive and behavioral responsivity. In an attempt to alter the development of amygdala kindling, we administered electroconvulsive shock (ECS) either six hours before or immediately after amygdala stimulation in order to distinguish direct and retrograde effects.

Male Sprague-Dawley rats were implanted with 0.25 mm diameter platinum iridium electrodes in the left amygdala. Animals were randomly assigned to three groups to receive either a sham ECS or ECS (120-130 volts for 0.5 seconds) six hours prior to once-daily amygdala kindling. A third group received ECS immediately following termination of the amygdala-kindled after-discharge. Rats were kindled with once-daily stimulation (500-1000 μamps at 50 Hz for 1.0 second). Stimulation current was delivered above each animal's after-discharge threshold or at a maximum current of 1000 μamps. After-discharge duration, seizure stage and duration, and presence of wet shakes were analyzed.

Animals receiving ECS six hours prior to amygdala kindling had markedly inhibited development of major motor seizures in comparison to sham ECS animals ($p < .001$) or those receiving ECS immediately following amygdala kindling ($p < .001$). Only 1 of 9 ECS pretreated rats displayed stage 4 major motor seizures, compared to 7 of 7 sham ECS or 3 of 9 receiving ECS after kindling. The rate of development of after-discharges was also suppressed by ECS six hours prior to amygdala kindling as compared to the other two groups ($p < .001$).

These data demonstrate marked anticonvulsant effects of ECS administered prior to amygdala kindling. ECS also inhibited the rate of growth of amygdala-kindled after-discharges. The findings are of interest in relation to recent reports that anticonvulsants such as carbamazepine (which inhibit amygdala kindling) are useful in the treatment of manic and depressive illness (Ballenger and Post, Am. J. Psychiatry 137: 782-790, 1980). The mechanisms underlying the therapeutic effects of ECS in affective illness are unknown, but the current study raises the possibility that limbic anticonvulsant effects of ECS could contribute to its efficacy.

187.14 ONTOGENY OF ELECTROSHOCK SEIZURE AND RELATED BEHAVIORS IN THE RAT.

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The developmental pattern of electroshock induced seizures was described from birth to 60 days of age over a wide range of shock intensities. Two independent groups were tested. Group 1 was administered electroshock on days 1, 3, 5, and 7, then daily from 8 to 20 days of age and on days 30 and 60. Group 2 was tested only on days 10, 20 and 30 to determine the effects of repeated shock. Electroshocks via scalp electrodes consisted of a 0.2 sec, 60 Hz alternating current at 5, 10, 15, 20, 25, 35, 50, 100, 250 and 500 mA. Animals were randomly assigned a shock intensity each test day. The occurrence of seizure and related behaviors were recorded on a checklist and timed. The thresholds for clonic and tonic seizures were determined from 1 to 60 days of age. The number of pups was 171 in Group 1 and 138 in Group 2. Pup body weights did not differ at any age between Groups 1 and 2. The threshold for clonic seizures, which included an infantile form of seizure behavior and adult-like clonic behaviors, increased to day 5 and then declined on days 10 through 20. The threshold for tonic seizures increased to a maximum on day 7 and then declined to a minimum on day 20. Clonic but not tonic thresholds for Group 1 were increased relative to thresholds for the less frequently shocked Group 2 at 30 days but not at 10 and 20 days of age. On days 13 through 18 and at electroshock intensities from 35 to 250 mA death was often observed following tonic seizures and were not observed at younger or older ages. The behaviors following a seizure-inducing stimulus showed a striking developmental pattern. Locomotion often preceeded clonus up to 20 days of age and typified the locomotor capability of the pup at its developmental stage. Locomotion began at birth with paddling, then circling, and finally to a walk or run. During the first week, subtonic seizure behaviors consisted of paddling with loss of righting or limb clonus and progressed to adult-like clonic behaviors by the second week. In the youngest animals maximum stimulus intensities evoked only forelimb flexion. By two weeks of age maximal tonic responses included the complete adult pattern of fore and hindlimb extension. Opioid-related behaviors were observed during post-seizure depression. Wet-dog-shakes followed clonic convulsions and grooming movements followed tonic seizures. These behaviors were optimally evoked during the second week and were not observed after weaning. These data provide a thorough description of behaviors induced by electroshock during ontogeny of the rat and a basis for assessment of the effects of perinatal toxin or drug exposure on development of the CNS. (Supported by ES-01503 and ES-05145.)

188.1 HIPPOCAMPAL PYRAMIDAL CELL SPINE DENSITY IN SEIZURING AND NON-SEIZURING GERBILS AT SEVERAL AGES. L.A. Paul*, P.T. Duong*, A.B. Forsythe*, A.B. Scheibel. (SPON: R.L. Katz). Depts. of Anatomy and Biomathematics, School of Medicine, UCLA, Los Angeles, CA 90024.

Continuing our investigations of neuronal structural correlates of seizure behavior in an animal model of epilepsy, we have performed analyses on dendrite spine densities of hippocampal CA3 pyramidal cells in gerbils of several ages under two test conditions. Brains of seizing (SS) and nonseizing (SR) gerbils aged 30, 50, 90 and 180 days, following a history of repeated (T) or minimal (NT) seizure testing were stained with a rapid Golgi variant. Dendrite segments from both apical and basilar regions were selected and the spines enumerated according to the procedure previously reported (Science, in press). A maximum likelihood mixed model analysis of variance was used for this nonorthogonal design.

As reported, adult (180 day) SS gerbils had fewer spines per apical dendrite segment than did SR. However, SS animals from the earliest ages sampled (30 and 50 days) showed the opposite pattern: SS had more spines per apical segment than did SR's. In fact, the mean spine density for SS gerbils at age 50 days was higher than that of any other group in the study. Spine counts at ages 50 and 90 days did not differ between the two strains.

The changes in spine density during development in the two strains were also of interest. The seizing gerbil experiences a constant reduction in spine density from the age of 50 days onward. The nonseizing animal, on the other hand, shows no significant decline in spine density with age. Scores for apical and basilar dendrites within each strain during development are approximately parallel. Finally, T-NT effects were noted in the older animals. These effects were more prominent in basilar dendritic systems.

The impact of a group of experimental variables including strain, test condition, and age upon the spine density along two distinct (apical and basilar) dendrite systems deserves interpretation in the light of the unique presynaptic ensembles involved. We speculate that epileptic behavior might be interpreted as the result of a complex interaction among many factors rather than as a single function of dendrite spine density.

(Supported in part by BRSG Grant RR-05756, courtesy of the Neuropsychiatric Institute, UCLA.)

188.2 DISTRIBUTION AND IMMUNOHISTOCHEMICAL LOCALIZATION OF CALCIUM-BINDING PROTEIN IN THE CEREBELLUM AND HIPPOCAMPAL FORMATION OF THE RAT BRAIN. K.G. Baimbridge* and J.J. Miller (SPON: B.R. Sastry). Dept. of Physiology, Univ. British Columbia, Vancouver, Canada. V6T 1W5

The distribution and immunohistochemical localization of calcium-binding protein (CaBP) in the rat brain was examined by radioimmunoassay (RIA), immunoperoxidase and immunofluorescence techniques. RIA indicated a high concentration of CaBP in the cerebellum (15 µg/mg total soluble protein - TSP) with lesser, but significant amounts in many other regions of the brain, including the hippocampus (0.96 µg/mg TSP). Microdissection of the hippocampal formation followed by RIA further indicated a marked differential distribution of CaBP within this structure, notably a concentration in the dentate gyrus of 1.71 µg/mg TSP with lesser amounts in the CA1 and CA3 regions (0.55 and 0.33 µg/mg TSP respectively).

The immunohistochemical localization, using antibodies purified by affinity chromatography, demonstrated that within the cerebellum only the dendrites, somata and axons of Purkinje cells were stained; no other neuronal or neuroglial population was labelled. In the hippocampal formation immunoreactive staining was observed in the perikarya of dentate granule and CA1 pyramidal cells. In both of these regions extensive labelling was also present in the apical dendritic layers. A dense plexus of immunoreactive fibers, corresponding to the mossy fibre system, was observed coursing from the granule cell layer through the hilar zone and terminating in the CA3 molecular layer. The CA3 pyramidal cell bodies and processes were not stained. Both the distribution and relative density of staining observed by these histochemical techniques closely agree with the distribution of CaBP determined by RIA.

We have previously suggested that the function of this protein may be that of an intraneuronal, free cytoplasmic buffering system. In this respect the localization of CaBP within specific neurones, and its absence in others, suggests that the capacity to buffer an influx of calcium may vary from one neurone type to another. The presence of CaBP may therefore have a pronounced effect upon the excitability of neurones which contain it.

(Supported by the B.C. Health Care Research Foundation.)

188.3 LONG TERM ALTERATIONS IN HIPPOCAMPAL CALCIUM-BINDING PROTEIN FOLLOWING KINDLING-INDUCED EPILEPSY. J.J. Miller & K.G. Baimbridge*. Dept. of Physiology, Univ. of British Columbia, Vancouver, Canada V6T 1W5.

Repeated subconvulsive electrical stimulation of various subcortical structures results in progressive changes in neuronal activity eventually culminating in generalized seizure. The physiological and biochemical events which underly this form of kindling-induced epilepsy, and which result in permanent changes in neuronal excitability, remain unclear. Recent studies have suggested that calcium, calmodulin and calcium-binding protein (CaBP) may play a significant role in long-term alterations of synaptic efficacy in the hippocampal formation. The following experiments were undertaken to determine whether alterations of these proteins may be associated with kindling-induced seizure activity.

Rats were chronically implanted with stimulating electrodes in the midline commissural pathway and kindled with once daily stimulation (60 Hz, 100 µA, 1 sec) until at least five consecutive full motor seizures (stage V) were evoked. Animals were sacrificed either 24 h or 7 days following the last seizure response and the hippocampus, cerebellum and frontal cortex dissected free and prepared for the determination of CaBP (using a specific mammalian brain CaBP radioimmunoassay-RIA), calmodulin (using a phosphodiesterase activation method), and total soluble protein (TSP).

In a series of experiments the concentration of CaBP in the hippocampus was consistently reduced for both the 24 h post-seizure group (597±27 ng CaBP/mg TSP) and 7 day post-seizure group (611±63) when compared to weight-matched controls (816±18) and implanted controls (874±47). Calmodulin and TSP remained constant in all groups. Additionally no changes were detected in the CaBP content of other brain regions (cerebellum and frontal cortex) as a result of kindling.

These data indicate that a specific and permanent reduction in the intracellular content of CaBP in the hippocampal formation accompanies kindling-induced epilepsy. In view of the differential distribution of CaBP within hippocampal neuronal elements and its postulated role as a cytosolic buffer for intracellular calcium, any permanent reduction in this buffering capacity may lead to increased excitability and epileptiform discharge.

188.4 KAINIC ACID INCREASES EXCITABILITY AND PROMOTES BURSTING IN HIPPOCAMPAL PYRAMIDAL CELLS Westbrook, G.* and Lothman E., Wash. Univ., Dept. Neurol., St. Louis, MO 63110.

Previously we described the use of kainic acid (KA) in a model of temporal lobe epilepsy that allows comparative in vitro and in vivo studies. We now report the effect of KA on hippocampal monosynaptic excitatory synapses and the cellular correlates of KA-induced interictal spikes.

Slices of rat hippocampus (400 µM) were prepared and maintained according to standard techniques at 37°C in a medium with an ionic content resembling brain extracellular fluid. Recording microelectrodes were positioned in the cell body and apical dendrite layers of CA_1 and semimicroelectrodes placed for Schaffer collateral stimulation. KA (.03-.3 µM) markedly increased the amplitude and number of population spikes without altering the presynaptic volley or population epsp amplitude. Spontaneous paroxysms characterized by a 50-200 msec positive wave with superimposed negative transients over the cell bodies and opposite polarities over the dendrites were seen in 0.1 µM KA. These interictal spikes were triggered in an all-or-none fashion with low intensity stimuli. Rinsing with control media returned responses to normal. In control media intra- and extracellular recordings of CA_3 pyramidal cells showed spontaneous bursts without accompanying field potentials; CA_1 pyramidal cells displayed single action potentials spontaneously or in response to low intensity, low frequency stimuli. With tetanization or high voltage stimuli, afterdepolarizations and/or double action potentials were seen. In KA both CA_1 and CA_3 cells exhibited bursts, either spontaneously or in response to low intensity, low frequency stimuli, which were synchronized with field interictal spikes.

Like others, we conclude that all hippocampal pyramidal cells are intrinsically capable at bursting, but that those in CA_1 only do so under special conditions. The behavior of pyramidal cells made epileptic with KA is like that produced with penicillin. Since penicillin blocks inhibition and KA is believed to be a potent activator of excitatory amino acid receptors, it appears that different mechanisms can lead to bursting of hippocampal neurons. The data indicate that a change in post synaptic "excitability" rather than augmentation of synaptic potentials is a primary mechanism for the convulsant action of KA.

188.5 SEIZURES AND NEURONAL DEGENERATION: RELATIONSHIPS INVESTIGATED BY INTRAHIPPOCAMPAL KAINIC AND IBOTENIC ACID. Caterina Aldinio*, Edward D. French and Robert Schwarcz. Maryland Psychiatric Research Center, Baltimore, MD. 21228.

Both kainic (KA; 2µg) and ibotenic (IBO; 10µg) acid have been shown to cause extensive axon-sparing lesions upon intrahippocampal injection (Schwarcz et.al., Exp.Brain Res. 37:199, 1979). KA and IBO were now employed to examine the question if neuropathological changes may be causally related to preceding seizure activity. The two amino acids were infused into the right hippocampus (HPC) of chronically cannulated unanaesthetized rats and EEG measurements were performed simultaneously in injected and contralateral HPC and bilaterally in the cortices. 4-10 days after injection, the animals were perfused and the brains analyzed histologically by brightfield microscopy.

Application of as little as 500pg KA caused behavioral and concomitant EEG changes. The behavioral syndrome, which became increasingly pronounced at higher doses, included sniffing, enhanced locomotor activity, rearing, grooming, scratching, mouth movements, wet-dog shakes, and, at the highest dose, ipsiversive rotation. No generalized motor seizures were observed in our dose range (up to 250ng KA). EEG changes ranged from a highly rhythmic fast activity (25Hz) and spikes at lower doses to repeated high frequency spiking with increased doses. In quantitative terms 50% of all animals (N=4-6 at each of 7 doses) infused with 5ng KA and every rat treated with>20ng KA developed seizure activity. Latency of onset of the first seizure, which was reliably preceded by EEG irregularities in the ipsilateral HPC, was 10-20 min. Total duration of seizure activity was (in a roughly dose-dependent fashion) 1-3 hr. Notably, after the last seizure, fast electrical activity persisted in the injected HPC for an additional 1-3 hr. Upon morphological examination, only the highest dose employed in this study (250ng KA) could be demonstrated to result in loss of HPC neurons (CA_{3+4} pyramids).

The pattern of EEG changes after IBO was clearly different. Only doses in excess of 15µg reliably resulted in seizure activity. However, seizures were much fewer in number, composed of lower frequencies and characteristic spiking activity was replaced by sustained (2-3 hr) slow wave EEG activity in all leads within 30 min of injection. Behavioral changes, consisting of increased locomotor activity, ataxia, loss of righting reflex, and onset of sleep paralleled EEG changes.

These results show that low doses of KA can elicit seizure activity that is not accompanied by morphological damage. However, excessive neuronal discharge produced by high doses of KA does result in neuropathological changes. Since IBO-induced seizures are markedly less severe than those seen with low doses of KA, it is difficult to relate IBO neurotoxicity to its epileptogenic effects.

188.6 LIMBIC SEIZURES DOWN REGULATE MUSCARINIC CHOLINERGIC RECEPTORS IN HIPPOCAMPAL FORMATION. J. O. McNamara and R. M. Dasheiff. Departments of Medicine (Neurology) and Pharmacology, Duke University; and Epilepsy Center, VA Medical Center; Durham, NC 27705

We have previously reported declines of muscarinic cholinergic receptors (MCR) in the hippocampal formation (HPF) following amygdala kindled seizures. This decline of MCR could be related to the kindling process itself or be a consequence of kindled seizures. The time course of the MCR alterations favor the latter possibility. To test this hypothesis directly, limbic seizures were induced in male Sprague-Dawley rats by making a unilateral, right-sided entorhinal cortex (EC) lesion (see abstract this volume). Controls were sham operated. MCR were measured with a radioligand binding assay using [^{3}H] QNB. Membranes prepared from microdissected HPF showed a bilateral and symmetric decline in MCR number without change in affinity at 3 days post lesion; data is pmol/mg expressed as % control: Dentate Gyrus, Right 69%, Left 66%; Regio Superior, R 85%, L 80%; Regio Inferior, R 84%, L 79%, all statistically significant. The epileptic activity induced by this lesion remits within ten days. The MCR declines parallel this activity, returning towards control values by 10 days and equalling controls by 30 days. Further, if rats are pretreated with phenobarbital 2 days prior and 3 days following the lesion, the MCR declines found at 3 days are reversed in a dose dependent manner, in parallel with blocking of the epileptic activity. The symmetry of the receptor alterations is in striking contrast to the predominantly ipsilateral projections from the EC to HPF. The declines were not increased with bilateral lesions. Together with their time course and reversal with phenobarbital, the data support seizures as the mechanism down regulating MCR in EC lesioned animals. This observation lends credence to the hypothesis that seizures are responsible for the same type of receptor regulation in kindling. We suspect that this biochemical alteration is an endogenous inhibitory response to seizures and the accompanying neuronal depolarization.

188.7 BENZODIAZEPINE RECEPTOR INCREASES FOLLOWING REPEATED SEIZURES: EVIDENCE FOR LOCALIZATION TO DENTATE GRANULE CELLS F. Valdes*, R. M. Dasheiff, F. Birmingham*, K. Crutcher, and J. O. McNamara (SPON: R. Fanelli). Departments of Medicine (Neurology) and Pharmacology, Duke University; and Epilepsy Center, VA Medical Center; Durham, NC 27705

Repeated seizures, whether induced by kindling or electroshock, result in increased numbers of benzodiazepine receptors in hippocampal formation (HPF) membranes (Proc. Natl. Acad. Sci. U.S.A. 77, 3029-3032, 1980). We sought to determine which cells in HPF contain the receptor increases. Kindled seizures were induced by daily administration of low levels of electrical current through electrodes stereotaxically implanted in the right amygdala. Control animals underwent electrode implantation but received no stimulation. Animals were killed 24 hours after the last seizure. In vitro [^{3}H] flunitrazepam binding (mean of fmol/mg protein ± SEM) was performed on membranes prepared from microdissected samples of HPF. Significant increases of benzodiazepine receptors were restricted to the fascia dentata (control 408 ± 24, experimental 576 ± 35, $p < .001$ students' t-test). No significant changes in binding were observed in the adjacent hippocampal gyrus. Scatchard plots of [^{3}H] flunitrazepam binding isotherms indicated the increase to be due to increased numbers of binding sites without alteration in affinity. [^{3}H] flunitrazepam autoradiographs demonstrated significant increases of silver grain density over the granule cell and molecular layers of fascia dentata in seizure treated animals, but not in other regions of hippocampal formation. The discrete lamination of the increased grain density throughout the granule cell and molecular layers is most consistent with a localization to the somata and dendrites of the granule cells. Destruction of granule cells in normal rats by colchicine or neonatal X-irradiation was associated with marked decline of benzodiazepine receptor binding. Together these results provide strong evidence for localization of the receptor increases to the somata and dendritic tree of the granule cells. We suggest that this cellular localization may provide a clue to the network of altered neural circuitry underlying amygdala kindling.

188.8 INTRACELLULAR AND FIELD POTENTIAL RECORDINGS OF EPILEPTIFORM ACTIVITY FROM IMMATURE RAT HIPPOCAMPAL SLICES. John W. Swann. Lab. of Developmental Neurophysiology, Birth Defects Institute, Div. Labs and Research, N.Y.S. Dept. Health, Albany, NY 12201

Hippocampal slices were prepared following methods described previously (Andersen, P., et al., J. Physiol., 302:463, 1980). Slices were taken from immature (1-2 weeks old: 10-40 grams) and mature rats (7-8 weeks old: 200-250 grams). Slices from an immature and a mature rat were placed together in the experimental chamber and comparisons were made between them under identical experimental conditions. After recording from slices in control bathing medium, penicillin (1.7 mM final concentration) was added to the perfusate. Intracellular and field potential recordings were made from the CA3 pyramidal cell body layer. Orthodromic activation was achieved through electrical stimulation of stratum radiatum.

In control bathing media, field potential recordings in both immature and mature slices revealed only isolated spontaneous single unit activity. In both slices, orthodromic activation of the CA3 pyramidal cells resulted in a population spike. Upon exposure to penicillin the mature slices exhibited spontaneous epileptiform bursts of population spikes, which could also be evoked by orthodromic stimulation (Schwartzkroin, P.A. and Prince, D.A., Ann. Neurol., 1:463, 1977). Slices taken from immature rats (9-19 days) generated similar spontaneous epileptiform discharges. However, these discharges were less frequent and often longer in duration. Orthodromic stimulation evoked identical discharges in an all or none fashion. In contrast, these bursts were invariably followed by a large (1-5 mv), slow negative field potential. Riding on the envelop of this slow potential was an afterdischarge often of 15-30 sec duration. Slices from 7 and 8 day old rats did not generate large epileptiform bursts, slow negative field potentials or afterdischarges. The epileptogenic properties of slices from 24 and 25 day old rats appeared similar to those of the mature rat slices.

In control bathing media, orthodromic stimulation resulted intracellularly in an epsp, a resultant action potential, and a subsequent ipsp in both the mature and immature slices . In penicillin the CA3 pyramidal cells from mature slices generated large depolarization shifts (DS's) associated with high frequency bursts of action potentials. The DS's occurred both spontaneously and in response to orthodromic stimulation. Neurons in the immature slices underwent similar DS's which often were longer in duration. The DS's were followed by slow depolarizing afterpotentials and afterdischarges. The afterdischarges consisted of multiple large (20-40 mv) slow (50-100 msec) depolarizing potentials which resulted in one or more action potentials in the neuron.

188.9 ULTRASTRUCTURAL COMPARISON OF HIPPOCAMPAL DAMAGE PRODUCED BY ELECTRICAL STIMULATION OF THE PERFORANT PATH AND THAT INDUCED BY KAINIC ACID, FOLIC ACID OR DIPIPERIDINOETHANE. T. deGubareff*, J. W. Olney and R. S. Sloviter (SPON: E. Robins). Washington Univ., St. Louis, MO and Pennsylvania State Univ., Hershey, PA.

A distinctive laminar pattern of hippocampal damage selectively involving neural elements innervated by putative glutamergic fibers has been described in rats as a consequence of sustained limbic seizures induced by kainic acid (KA), folic acid (FA) or dipiperidinoethane (DPE). Sloviter and Damiano (Fed. Proc. 40, 309, 1981) recently found that sustained intermittent electrical stimulation (x 24h) of the perforant path (PP), the major excitatory input to the hippocampus, duplicates specific electrophysiological effects of KA (decreased recurrent inhibition and increased granule cell activity) while also causing hippocampal damage which, by light microscopy, appears very similar to that induced by KA, FA or DPE. The present study documents, by electron microscopy, that the acute hippocampal pathology resulting from PP stimulation is identical in every essential detail to that induced by KA, FA or DPE. The neuronal changes consist of acute edematous swelling of specific dendritic elements and either swelling or dark cell degeneration of neuronal somata (primarily hilar interneurons and CA4, 3 and 1 pyramids). Massive swelling of specific glia that lie in close proximity to the affected neurons is a conspicuous feature of the acute reaction. Tentatively, we interpret this as a reversible response of glia to high extracellular K^+ concentrations produced by repetitive neuronal depolarization. It is noteworthy that both FA (which is structurally related to KA) and DPE (which is not) reproduce the KA type of sustained limbic seizure activity. The observation that sustained PP stimulation and treatment with KA, FA or DPE result in an identical pattern of hippocampal damage supports the hypothesis that sustained discharge activity *per se* is an important common denominator of this type of neuropathology. Moreover, a cardinal feature of the changes observed (massive dilatation of postsynaptic dendrites with sparing of their presynaptic axonal contacts) is the "excitotoxic" type of reaction that glutamate (Glu) and aspartate (Asp) are known to produce. Since both PP stimulation and KA treatment cause disinhibited excitatory firing of specific hippocampal inputs that putatively use Glu or Asp as transmitter, excessive release of these endogenous excitotoxins at hippocampal synapses may be responsible for the postsynaptic degeneration observed. That a similar mechanism might underlie neuronal degeneration in certain types of human epilepsy warrants consideration. Supported by USPHS grants NS-09156, DA-00259 and RSA MH-38894 (JWO).

188.10 SIMULATIONS OF THE CA3 HIPPOCAMPAL REGION: SINGLE CELLS, ELECTROTONIC INTERACTIONS, SYNCHRONIZATION, SEIZURES. R.D. Traub and R.K.S. Wong. IBM Watson Res. Ctr., Yorktown Heights, NY 10598.

We have developed a detailed model of the CA3 hippocampal pyramidal cell which is capable of generating bursts in either soma or apical dendrites. A burst consists of a series of spikes with growing calcium-mediated afterpotentials terminating in a calcium spike, and depends in part for its generation on a voltage-dependent inactivation of gK. This model is capable of repetitive bursting. Simulations of a pair of cells joined by an electrotonic junction demonstrate spread of bursting with a latency of tens of ms. We have abstracted the essential features of this model to construct a network of 100 pyramidal cells together with a small number of inhibitory interneurons, and have used this network to gain insight into penicillin-induced epileptiform behavior. Synaptic connectivity of the network is consistent with known data: excitatory interconnection of one pyramidal cell to another occurs with probability about 5%. In the penicillin case, recurrent inhibition is assumed severely reduced compared with the non-penicillin case. A shock to a small group of cells leads to a synchronous population burst with latency (experimental and in the model) of about 100 ms. Electrotonic coupling is not required for synchronization, but such coupling may play a modulatory role. With a small tonic drive to some of the cells, the network reproduces periodic synchronous population discharges, mimicking periodic interictal spikes. As excitatory coupling is increased, clonic and tonic seizures develop. Factors influencing the period of the population discharge relative to the period of individual cells are analyzed, as are factors contributing to development of seizures.

188.11 EVIDENCE THAT PENICILLIN-INDUCED SYNCHRONY IN GUINEA PIG HIPPOCAMPAL SLICES IS NOT ACCOMPANIED BY INCREASED ELECTROTONIC COUPLING. J. H. Schneiderman* and P. A. Schwartzkroin (SPON: A. R. Wyler), Department of Neurological Surgery, University of Washington, Seattle, Washington 98195.

Electrotonic coupling of neurons has been proposed as a mechanism for synchronization and spread of epileptiform discharges in mammalian central nervous system (Gutnick, M. J. et al., Science 211:67, 1981; MacVicar, B. A. et al., Brain Res. 196:494, 1980). We present evidence that the synchrony produced by penicillin in the CA3 region of guinea pig hippocampal slices is not associated with an increase in electrotonic coupling.

The field potentials evoked by stimulation of the stratum radiatum were recorded in the CA3 somatic region of guinea pig hippocampal slices *in vitro*. The stimulus intensity was maintained well below that necessary to produce a maximal antidromically-evoked CA3 population spike.

The amplitude of the antidromically-evoked CA3 population spike did not increase after perfusion of the tissue with 3.4 mM sodium penicillin G even though a population burst, riding on a depolarizing envelope, followed the antidromic population spike. If penicillin increased electrotonic coupling, the antidromically-evoked population spike should have increased in amplitude.

188.12 AFTERPOTENTIALS FOLLOWING BURST DISCHARGES IN NEOCORTICAL NEURONS. R.N. Friedman* and D.A. Prince. Dept. Neurology, Stanford Univ. Sch. of Med., Stanford, CA 94305

Epileptogenic burst discharges are followed by slow afterpotentials in neocortical neurons in vivo. We used standard intracellular recording techniques (K-acetate and K-sulfate electrodes) to investigate afterhypolarizations (AHPs) and afterdepolarizations (ADPs) which followed orthodromically evoked depolarization shifts (DSs) and associated spike bursts in neurons of guinea pig neocortical slices treated with penicillin (3.4-10mM) or bicuculline (10^{-5}M). Single neurons usually generated either AHPs or ADPs. Only in rare instances were AHPs replaced by ADPs in a given cell that had a stable resting potential. AHPs and ADPs could be recorded from neighboring neurons in the same slice under identical experimental conditions. Burst afterpotentials were observed in neurons impaled at depths of 160-2000 μm below the pial surface. AHP amplitudes and durations ranging to 8mV and 4 sec were dependent on size of burst, resting potential, $[K^+]_o$, and frequency of triggering. ADPs had durations up to 5 sec and, unlike AHPs, persisted in cases in which spike bursts on the DS failed. AHPs and ADPs were accompanied by conductance increases of 36.9±7.8% (n=8) and 38.2 ± 5.8% (n=7), respectively. AHPs and most ADPs could be reversed by the application of intracellular current. The AHP reversal potential (E_{AHP}) determined with $[K^+]_o$ = 4, 5, and 8 mM was -92.4 ± 6.6 (n=7), -78.3 ± 6.7 (n=6) and -81.0 ± 7.8 (n=6) (mV.±7.8 (n=6) (mV.±S.D.), respectively. The positive shift of the E_{AHP} with increasing $[K^+]_o$ suggests that a K^+ conductance has a significant role in the AHP. Microelectrode impalements of single cells were maintained through changes of perfusing solutions which differed in $[K^+]$ (K^+ substituted for Na^+). Data collected after at least 10 minutes' equilibration in each solution showed that increasing $[K^+]_o$ decreased the E_{AHP} (n=7). E_{ADP} (measured in $[K^+]_o$ = 5mM) was -48.0 ± 13.9 mV (N=10). The amplitude and duration of AHPs and the ADP duration were dependent on the frequency of DS triggering. Rates ≥ 1Hz totally depressed AHPs and markedly decreased ADP duration. AHPs were stable at DS frequencies of ≤.33 Hz. The ADP required as long as 20 sec for complete recovery. AHPs could be recorded in neurons after intracellular application of K-EGTA (.1 - .2M). 14 of 27 EGTA injected cells generated spontaneous or directly evoked bursts suggesting that some neocortical neurons have latent, intrinsic mechanisms for burst generation. The results of these experiments suggest that the AHP is mediated, in large part, by an increase in K^+ conductance. However, the mechanisms for ADP generation are distinctly different, perhaps involving superimposed conductances for Na^+ and $Ca^{++}$and/or contributions of excitatory synaptic events. (Supported by NINCDS Grant NS 06477)

188.13 POWER SPECTRAL ANALYSIS OF SLEEP EEG RESPONSE TO ANTICONVULSANT COMPOUNDS OF DIFFERING THERAPEUTIC EFFECTS IN THE MONKEY. M. B. Sterman and R. A. Kovalesky*. Neuropsychology Lab., VA Medical Center, Sepulveda, CA 91343 and Depts. of Anatomy and Psychiatry, UCLA School of Medicine, Los Angeles, CA 90024.

Four rhesus monkeys were prepared surgically for monitoring of cortical EEG patterns during sleep and for determination of the effects of specific chemical compounds on normal sleep EEG characteristics. Data were quantified using an established application of power spectral analysis which provided standard sleep EEG spectral density profiles during baseline conditions and following the administration of five anticonvulsant drugs, including: pyridoxine, diazepam, phenobarbital, carbamazepine and sodium valproate. Following the completion of these studies, animals were challenged with a convulsant drug, monomethylhydrazine (MMH) and the protective characteristics of these anticonvulsants compared with their effects on sleep EEG spectra.

After recovery from surgery all animals were adapted to sustained chair restraint and EEG polygraphic data collected on paper and analog tape during five consecutive baseline nights. Ten minute samples of continuous nonREM sleep were selected approximately two hours into this period and subjected to power spectral analysis using established techniques. The animals were then administered therapeutic doses of the anticonvulsant drug series in counterbalanced order at two-week intervals. Each drug was administered just prior to the start of recording and data collected as before. Additionally, however, after two hours of recording blood samples were drawn from the femoral vein and evaluated for determination of corresponding blood levels of the compound. On MMH challenge trials these drugs were administered as before but were followed after 10 min. by the convulsant (15mg/kg).

Power spectral profile data based on mean values from the four animals showed significant change following drug administration. Blood levels obtained for these compounds confirmed therapeutic ranges. Normative profiles were stable across baseline nights. Both pyridoxine and diazepam produced a localized and significant decrease in power at 0-3 and 4-7 Hz in rolandic cortex. These drugs were also effective in protecting against MMH induced seizures. The findings to date suggest that a specific EEG "anticonvulsant signature" may be derived for drugs which are clinically effective in various types of seizure conditions.

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188.14 16 CHANNEL SPIKE "RECOGNITION PRINTOUT" SYSTEM FOR EEG. Bradford Merrill* and Reginald G. Bickford. UCSD, La Jolla, CA 92093.

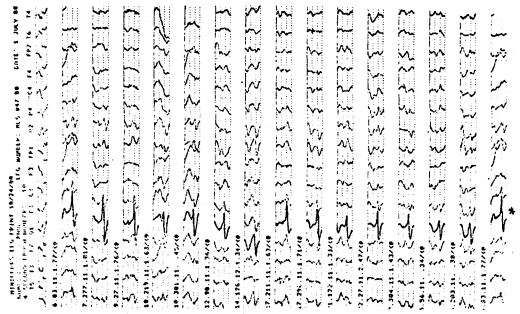
In spite of numerous attempts at spike recognition (Carrie, Frost, Lopez da Silva, Zeterburg, Gose, Luders) clinical EEGers do not trust pure computer approaches.

We have designed a system (using the LSI-11 "MINICEARS"* microprocessor and graphics printer) to select "probable spikes", on line, from 16 channels of EEG, and print them out in two compilations: 1) Spike Pack - locates spikes on a head diagram, and 2) Spike Slice - prints spikes in a 0.2 second EEG background sample across sixteen electrodes. The alignment accuracy, extra time lines and event separation of the matrix ("Digigraphic") spike slice enhances the diagnostic utility (Fig. below).

The algorithm depends on slope, duration, and amplitude parameters and is adjustable, on line, to customize the selective process to the characteristics of the patient's spike. The values of parameters used in selection of a particular spike are printed alongside the spike slice. The recognition performance has been tested on the spike data tape of Gose (1974), and is adequate for preliminary recognition.

Because final validation is done by the eye of an experienced EEGer, the method is considered appropriate for clinical application. The recognition parameters provide significant data in spike categorization, and give important insights to advance the process of spike definition and recognition. By appropriate manipulation of parameters, the system can also recognize "spike wave" and other transients. The performance gives promise of wide clinical utility, including the "anti-convulsant efficacy" estimate of Frost.

* Bik Systems, Inc., La Jolla, CA. Supported by Bik Foundation.



Spike Slice - Epileptic Patient. Focal P3 Spike*

188.15 RELATIONSHIPS BETWEEN THE ANTAGONISM OF ELECTRICALLY-INDUCED MAXIMAL SEIZURES BY PHENYTOIN (PHT) AND CENTRAL NERVOUS SYSTEM (CNS) LEVELS OF ADENOSINE 3',5-MONOPHOSPHATE (cAMP) AND GUANOSINE 3',5-MONOPHOSPHATE (cGMP) IN FROGS AND MICE. S.W. Johnson* and W.K. Riker* (SPON: L. Gronke). Dept. of Pharmacol., Univ. of Oregon Health Sciences Center, Portland, Oregon, 97201.

The discovery that seizures are associated with large increases in CNS levels of cAMP and cGMP has prompted much speculation about relationships between cyclic nucleotides and seizures. Because many anticonvulsant drugs antagonize the rise in cAMP and cGMP levels associated with seizures, it was hypothesized that PHT, a prototypic anticonvulsant drug, may modify the pattern of electrically-induced seizures by this mechanism. To test this hypothesis, we first defined the complete PHT dose-effect curve for the prevention of tonic hindlimb extension (THE), the classic assay endpoint for anticonvulsant efficacy, in frogs (Rana pipiens) and mice (CF #1). We then examined the effects of PHT on cyclic nucleotide levels in the CNS of these animals before and after corneal electroshock. CNS levels of cAMP and cGMP were also measured in quaking mice (qk/qk), a myelin-deficient mutant strain which seize spontaneously. All animals were sacrificed by immersion into liquid N_2, and cAMP was assayed by competitive protein binding while cGMP was assayed by RIA.

In agreement with the results of others, PHT antagonized the electroshock-induced increase in levels of cAMP and cGMP in cerebrum and cerebellum, respecitvely, from CF #1 mice. However, the effective dose-range of PHT for significant reduction of the elevated levels of cAMP and cGMP was 2-5 times higher than that for prevention of THE in 95% of mice. The effective dose-range of PHT for preventing tonic flexion and clonus was nearer to that for alteration of cyclic nucleotide levels, but these endpoints have less relevance to anticonvulsant efficacy than does the classic endpoint, prevention of THE. Also, the greatest reduction of cyclic nucleotide levels occurred at a dose (100 mg/kg, s.c.) which produced toxic signs in mice. In frogs, the electroshock-associated increase in levels of cAMP and cGMP in the CNS was not altered by PHT even when the doses administered were up to twice the ED 95 for prevention of THE. Quaking mice, sacrificed during inter-ictal periods, did not have abnormal levels of cAMP or cGMP in cerebrum or cerebellum, and a dose of PHT (15 mg/kg, s.c.) which abolished all seizure activity did not alter levels of these cyclic nucleotides. Since these data from mice and frogs show that the anticonvulsant effect of PHT is dissociated, by dose, from effects on CNS cyclic nucleotide levels, it is doubtful that the alteration of cyclic nucleotide levels is a mechanism by which PHT exerts its anticonvulsant effect.

188.16 THE SUBSTANTIA NIGRA: SITE OF GABA-MEDIATED ANTICONVULSANT ACTIVITY IN RATS. M.J. Iadarola and K. Gale, Neurology Research Lab, VA Hospital, Durham, N.C. 27705, and Department of Pharmacology, Georgetown University Schools of Medicine and Dentistry, Washington, DC 20007.

Microinjection of the GABA-receptor agonist, muscimol, bilaterally into the substantia nigra (SN) protected against seizures produced by bicuculline (.25 mg/kg i.v.), pentylenetetrazol (PTZ, 40 mg/kg i.v.), and maximal electroshock (MES). Two procedures were used for microinjection: 1) Muscimol (25, 50 and 75 ng in 0.4 ul saline over 5 min) was infused while rats were under ether anesthesia, and the rats were tested with MES or PTZ at 2.5, 5 and 8 hr after surgery, 2) muscimol (10ng in 0.2ul saline) was infused via chronic indwelling cannulas, and the rats were tested with PTZ or bicuculline 20 min later. Complete protection against tonic hindlimb extension in the MES test was obtained up to 5hr after 75ng, and at 2.5hr after 50ng; partial protection was obtained with 25ng at 2.5hr and 50ng at 5hr. Complete protection against PTZ-induced major clonic seizures and tonic forelimb extension was obtained 20 min after 10ng muscimol; protection against clonic and tonic components of bicuculline-induced seizures was obtained 20 min after 10ng muscimol. All of the above results were significant in comparison to saline-microinjected control rats. At 8 hr after microinjection of muscimol, no significant protection was obtained in any seizure model. At all doses tested, muscimol caused hyperactivity, accompanied by stereotyped sniffing and gnawing; the duration of this behavior was dose-dependent, lasting 2hr after 10ng and 6hr after 75ng.

The irreversible inhibitor of GABA-transaminase, gamma-vinyl-GABA (GVG), was microinjected bilaterally into SN (5ug in 0.5ul saline) and rats were tested with MES, PTZ and bicuculline at 6 and 24 hr after GVG. At both times GVG completely protected against tonic hindlimb extension induced by MES and tonic forelimb extension induced by bicuculline; partial but significant protection was obtained against PTZ. Injections of GVG into forebrain areas (thalamus, striatum, cortex), into superior colliculus, or into hindbrain areas (pontine tegmentum), were without anticonvulsant effects, despite marked elevation of GABA (3-5X) in the vicinity (3mm radius) of the injections. Anticonvulsant effects of intranigral GVG were correlated with a 2-3X increase in GABA in SN; by 4 days, seizure activity and GABA content returned to control levels. No changes in spontaneous motor activity or reflexes accompanied the GVG microinjections.

Our data suggest that GABAergic synapses in the vicinity of the SN may be critically involved in the control of seizure propagation.

188.17 IN VITRO AUTORADIOGRAPHIC LOCALIZATION OF MUSCARINIC CHOLINERGIC BINDING SITES IN RAT HIPPOCAMPAL FORMATION: EFFECTS OF AMYGDALA KINDLING. D. D. Savage and J. O. McNamara, Departments of Medicine (Neurology) and Pharmacology, Duke University; and Epilepsy Center, VA Medical Center; Durham, NC 27705

Kindling has been proposed as a useful model for the study of limbic epilepsy. We have found that kindling induced by electrical stimulation of the amygdala results in a decrease of approximately 25% of the total number of ^{3}H-quinuclidinylbenzylate (QNB) binding sites in membranes of hippocampal formation (HPF). This reduction was most striking in the dentate gyrus of HPF. We have implemented the in vitro autoradiographic technique of Young and Kuhar (Brain Res. 179:255, 1979) to characterize the regional distribution of muscarinic cholinergic receptors in rat HPF and to determine the anatomical localization of the kindling-induced reduction in ^{3}H-QNB binding sites.

Slide mounted 8 μm coronal sections of whole brain containing dorsal HPF in cross section were used in these experiments. Slides were incubated with 0.75 nM ^{3}H-QNB for one hour at 25° C in the absence or presence of of 1 μM atropine and rinsed ten minutes in ice cold buffer (120 mM sucrose, 50 mM Na^+-K^+ phosphate, pH 7.4). Preliminary experiments indicated that the specific binding reaction was saturable, reaches equilibrium within 45 minutes and has an apparent affinity constant (K_d) of 0.05 nM ^{3}H-QNB. Specific binding was 95% of total ^{3}H-QNB binding.

Autoradiographic analysis of ^{3}H-QNB binding to HPF in normal rats demonstrated the presence of dense bands of silver grains over the dendritic fields. Specifically, the highest grain densities were observed over the stratum (s.) moleculare of both the superior and inferior blades of the dentate gyrus and the s. radiatum and s. oriens of the CA1 region of hippocampal gyrus. Grain densities 40 to 80% lower than those measured above were observed in s. granulosum and hilus of dentate and across the entire CA3 field of hippocampal gyrus. These results are similar to the in vivo autoradiographic decription of ^{3}H QNB binding in HPF by Kuhar and Yamamura (Brain Res. 110:229, 1976).

Preliminary autroadiographic analysis of HPF from an amygdala kindled rat indicates a significant reduction (25%) in grain density in the s. moleculare of dentate (30.9 ± 1.6 grains/1600 μm^2) compared to the electrode implanted unstimulated control (39.8 ± 2.2 grains/1600 μm^2). Experiments are in progress to determine which cell constituents contain the ^{3}H-QNB binding site reduction in s. moleculare.

189.1 MEMBRANE PROPERTIES OF RAT LOCUS COERULEUS NEURONS. S.A. Shefner, R.A. North and S. Nishi.* Neurophysiology Laboratory, Department of Pharmacology, Loyola University of Chicago, Maywood, IL 60153.

Intracellular recordings were made from 40 neurons in the locus coeruleus (LC) in slices (about 300 μm thick) cut from rat pons. Resting membrane potentials ranged from -45 to -60 mV and were stable for up to several hours. Neuronal input resistances varied widely (222 MΩ ± 32 MΩ, mean ± S.E. mean, n = 13). Electrotonic potentials were close to exponential with time constants of up to 80 ms. Action potentials evoked by passing depolarizing current pulses had an amplitude of 64 ± 1.5 mV (n = 11), maximum rate of rise of 168 ± 12 V/s and rate of fall of 89 ± 10 V/s (n = 9). Spike duration at half-amplitude was 1.0 + 0.6 ms (n = 10). The action potential was followed by a hyperpolarization of amplitude 12.8 ± 1.3 mV (n = 11) and duration of 100 - 500 ms. This after-hyperpolarization reversed in polarity at a membrane potential of about - 100 mV. Increasing the intensity of the depolarizing current caused an almost linear increase in the frequency of action potential discharge until this became asymptotic at 60 - 100 Hz. Focal stimulation of the slice at a distance of up to 500 μm from the recording electrode evoked graded depolarizing synaptic potentials in some LC neurons. In most cells hyperpolarization increased and depolarization decreased the synaptic potential amplitude. These properties of LC neurons were in sharp contrast to those of adjacent cells in the mesencephalic nucleus of the trigeminal nerve, which had ten times lower input resistances, much shorter duration action potentials and could not be activated synaptically.

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189.2 EXCITATORY AND INHIBITORY ACTIONS OF PUTATIVE NEUROTRANSMITTERS ON CULTURED CEREBELLAR NEURONS. Donna L. Gruol. Arthur V. Davis Center for Behavioral Neurobiology, The Salk Institute, La Jolla, CA 92037.

Organotypic cultures of cerebellar cortex were prepared from 20 day rat embryos. After 1 month in culture, medium sized neurons (25 μ) with 1 or more thick, branching process, thought to be Purkinje neurons, were selected for electrophysiological studies. Intracellular recordings were made using conventional electrophysiological techniques. The recording media consisted of modified Hank's BSS, pH 7.4. All experiments were done at room temperature. Putative neurotransmitters were applied from large tip (5 μ) micropipettes by pressure or by superfusion. Pressure pipettes contained 50 μM solutions of GABA, glycine or glutamate or 0.05 to 5 mM norepinephrine (NE). Intracellular recordings were obtained from over 160 neurons in 27 cultures. Because of the small size of the neurons, membrane potential (Vm) varied over a large range (-30 to -60 mV) depending on the degree of injury during electrode penetration. Under optimal conditions Vm ranged from -50 to -60 mV and could be maintained for several hours. Virtually all neurons studied displayed spontaneous activity included epsps, ipsps and action potentials.

The cultured neurons were sensitive to several putative neurotransmitters thought to mediate synaptic transmission in the cerebellum. In all cells tested, glutamate (Glu) was excitatory (n=31) while GABA was inhibitory (n=68). Glycine was ineffective (n=8). The GABA and Glu responses were associated with Vm changes and an increase in membrane conductance. The mean reversal potentials for these responses were -20 mV for Glu (n=3), -51 mV for GABA when K+-acetate recording electrodes were used and -33 mV when KCl electrodes were used. These data suggest that the Glu response in cerebellar neurons is at least partially mediated by an increase in Na+ conductance while the GABA response is mediated by an increased Cl- conductance. The reversal potential for the spontaneous ipsps was similar to that for GABA (n=2). Both the GABA response and the ipsps were depressed by 1 μM bicuculline. NE applied by pressure or superfusion was relatively ineffective at concentrations below 1 mM (n=41). When tested in the mM range (n=16), NE was inhibitory in the majority of neurons (n=10), reducing the spontaneous activity (epsps, ipsps, spikes) in a dose-dependent, reversible manner. The depression of the ipsps by NE did not appear to involve a depression of postsynaptic receptor sensitivity since the GABA response was unaltered (n=4). These data support the hypothesis that Glu is an excitatory transmitter in the cerebellum while GABA and NE are inhibitory transmitters.

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189.3 ELECTROPHYSIOLOGICAL PROPERTIES OF NEOCORTICAL NEURONS MAINTAINED IN VITRO. B.W. Connors, M.J. Gutnick* & D.A. Prince. Dept. Neurology, Stanford Univ. Sch. of Med., Stanford, CA 94305

We investigated membrane and synaptic properties of neurons in coronal slices (350-500 μm thick) of guinea pig sensory-motor cortex maintained in vitro (37°C). Neurons impaled at all subpial depths between 150-1700 μm had high resting potentials (-75 ± 8mV, mean ± S.D., n = 40), input resistances (R_Ns) of 24 ± 13 MΩ, and time constants of 8 ± 4.5 msec (n = 35). Sixty percent of neurons showed apparent increases in R_N during depolarization (anomalous rectification, AR). In the remainder R_N was ohmic or showed apparent decreases during depolarization (delayed rectification). AR was greatly enhanced by reduction of K^+ conductances (intracellular CS^+ injection, extracellular Ba^{2+}), but was abolished by blocking Na^+ conductance (gNa) (extracellular TTX, intracellular QX-314, removal of external Na^+). Ca^{2+} current blockade (extracellular Mn^{2+} or Co^{2+}) had relatively little effect on AR. The data indicate the presence of a voltage-sensitive, noninactivating gNa^+ in most neocortical neurons. Action potentials (amplitude = 92 ± 9 mV; duration at base = 1.7 ± 0.4 msec; n = 20) could be elicited from all neurons. Single spikes displayed a slowly decaying depolarizing afterpotential (DAP), with no hyperpolarizing undershoot. Prolonged depolarizing pulses generated repetitive firing and variable degrees of progressive spike broadening. Repetitive action potentials could elicit long, Mn^{2+}-sensitive afterhyperpolarizations in some cells. Spikes were invariably blocked by TTX, intracellular QX-314 or perfusion with a Na^+-free medium. However, intracellular injection of Cs^+ or TEA led to the generation of broadened spikes with prominent DAPs. Slow spikes in these neurons persisted when gNa^+ was blocked, were inhibited by external Mn^{2+} or Co^{2+}, and probably represent calcium-dependent electrogenesis. A small number of untreated cells generated intrinsic, all-or-none burst potentials when depolarized with current. These neurons were always located between the subpial depths of 900-1300 μm (deep layer IV, upper V), although the more characteristic, nonbursting cell types were also seen at these depths. Most neurons exhibited spontaneous, exclusively depolarizing synaptic events. Focal cortical stimulations of moderate intensity evoked depolarizing PSPs in all cells. Membrane polarization uncovered EPSP and IPSP components of these responses. Repetitive stimulation at rates as low as 0.5 Hz caused a rapid, reversible depression of the longer latency IPSPs.

Neocortical neurons in vitro have properties very similar to those which have been recorded in vivo. Our studies have characterized some of the ionic mechanisms underlying these properties.

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189.4 MEMBRANE PROPERTIES OF NEOCORTICAL NEURONS IN THE IN VITRO SLICE PREPARATION. C.E. Stafstrom, P.C. Schwindt*, & W.E. Crill, Dept. of Physiol. & Biophys., and Medicine, Univ. Washington Sch. Med., Seattle, WA 98195.

Neurons in layer V of motor cortex project to segmental motor systems of the spinal cord, and are believed to represent the final pathway for cortical neuronal activity leading to the initiation of movement. These pyramidal tract (PT) neurons have been classified as "fast" and "slow" according to their axonal conduction velocities, but relatively little information is available regarding the passive and active properties of these cells. In order to investigate their membrane characteristics in more detail, we have developed an in vitro slice preparation of cat motor cortex.

After craniotomy, 400μ thick slices from area 4γ (Hassler & Muhs-Clement, J. Hirnforsch. 6: 423-436, 1964) are maintained in an oxygenated chamber by standard techniques (eg, see Schwartzkroin, Brain Research 85: 423-436, 1975). Intracellular recordings obtained from layer V have revealed that cells in the slice retain the electrical and firing properties reported in intact animals. Pial or white matter stimulation evokes orthodromic and antidromic impulses, respectively. Input resistances (4-10 MΩ) and time constants (6-12 msec) agree closely with reported values. The cells demonstrate marked anomalous rectification and membrane potential overshoots to subthreshold current pulses. Spike potentials characteristic of fast and slow PT cells are seen. Repetitive firing can be elicited by prolonged depolarizing pulses. The cells show no accomodation to ramp currents as long as 1 second. When exposed to penicillin in epileptogenic doses (5 mM), "paroxysmal depolarization shifts" can be evoked by orthodromic stimulation and occur spontaneously as well.

These results indicate that the cat cortical slice can be used to investigate passive and active properties of both normal and epileptic neurons. The preparation also allows comparison between cell types in different layers, and may permit elucidation of cortical circuitry. In addition, the largest cells will facilitate anticipated double-electrode voltage clamp experiments.

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189.5 CALCIUM IONS: SLOW DIFFUSION IN THE HIPPOCAMPUS. M.E. Morris[1] and K. Krnjević[2], [1]Depts. Anaesthesia & Pharmacology, Univ. of Toronto, Toronto, Ont., and [2]Depts. Anaesthesia Research & Physiology, McGill Univ., Montréal, PQ, Canada.

The time course of the alterations in extracellular ionic levels which are evoked in synaptic regions of the CNS by neuronal activity must depend on the diffusion characteristics of the particular ions. In the present study, microelectrodes were used to measure changes in extracellular Ca^{2+} concentration $[Ca^{2+}]_o$ produced by iontophoretic release of Ca^{2+} in the CA1 pyramidal cell layer of the hippocampus of the rat, anaesthetized with urethane. Inter-tip separations of electrodes were 7-50 µm. The effective transport number (n) for the release of Ca^{2+} from electrodes *in situ* (calculated from $m = In/zF$, with m being the estimated flux, I the applied current, z ionic valency, and F the Faraday constant) was only 6.82 (S.E. ± 1.54) x 10^{-3}, which is 1/60th that for Ca^{2+} movement in a simple solution of 0.1 M $CaCl_2$. This probably reflects Ca^{2+} binding at the tip of microelectrode and may explain the difficulties frequently experienced in releasing Ca^{2+} in tissue. Ejection currents of 30-200 nA produced increases of $[Ca^{2+}]_o$ of 0.05-3.0 mM above tissue resting levels of 1.3-1.5 mM. A very slow time course for both rise and decay of $[Ca^{2+}]_o$ were consistently observed with different electrodes and different animals. When curves, calculated from the diffusion equation for a continuous point source, were fitted to experimental data points - especially for the decay phases - with a computer graphic system, a mean diffusion coefficient (D) for Ca^{2+} of 0.077 (S.E. ± 0.0101) x 10^{-6} cm^2/s was obtained. This is ≈ 1/100th the values of D for self-diffusion of Ca^{2+}, or the apparent D observed following release of Ca^{2+} from microelectrodes in saline or agar. It contrasts sharply with the tissue diffusion rates for tetraethylammonium (with solely extracellular location) and for K^+ (with both intracellular and extracellular components of diffusion) which are about 1/5th of diffusion rates in aqueous solutions (Lux & Neher, Exp. Brain Res., 17:190, 1973; Krnjević & Morris, Can. J. Physiol. Pharmacol., 52:852, 1974; Nicholson et al., Brain Res., 169:580, 1979) and may reflect the reversible binding of Ca^{2+} to cell surfaces and/or intercellular macromolecules. Even brief Ca-inward currents arising from neuronal activity will therefore produce relatively prolonged depressions of $[Ca^{2+}]_o$, with a more profound influence on synaptic function. Such a possibility is in keeping with the time course of the $[Ca^{2+}]_o$ falls and associated events which are evoked in the hippocampus by repetitive stimulation (Krnjević et al., Can. J. Physiol. Pharmacol., 58: 579, 1980).

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189.6 ELECTROPHYSIOLOGICAL STUDIES OF THE INPUT FROM THE AMYGDALOID COMPLEX TO THE DENTATE GYRUS OF THE RAT. S.R. Thomas*, S.Y. Assaf*, T.V.P. Bliss* and S.D. Iversen*. (SPON: M.S. Eison). National Institute for Medical Research, Mill Hill, London NW7 1AA and The Psychological Laboratory, Downing St., Cambridge CB2 3EB.

We have studied electrophysiologically a disynaptic input from the lateral nucleus of the amygdaloid complex to the dentate gyrus, which corresponds to that recently described anatomically (Krettek, J.E. and Price, J.L., J. Comp. Neurol., 178:255, 1978).

In rats anaesthetized with urethane (1.5 g/kg, i.p.) single-pulse stimulation of the lateral nucleus of the amygdaloid complex (AL) evoked long-latency (up to 22 msec) positive-going synaptic wave responses in the granule cell-body layer of the dentate. The AL-evoked responses resembled the effects of low-intensity stimulation of the monosynaptic perforant path (PP) input to the dentate from the entorhinal cortex, but were of longer latency (up to 22 msec compared with up to 7 msec for the PP) and exhibited relatively large variations of latency and amplitude. When population spikes were evoked by AL stimulation they were potentiated by repetitive activation. Paired-pulse experiments revealed a potent facilitation (up to 200% of control) of PP-evoked population spikes in the dentate by a preceding pulse to the AL; the optimal interval for this effect was 50 to 70 msec.

Analysis of the depth profiles indicated that the AL input is restricted to the outer zone of the main PP termination on the dendrites of the granule cells, a zone which receives input mainly from the lateral portion of the entorhinal cortex (Steward, O., J. Comp. Neurol., 167:285, 1976). Micro-infusion of procaine solution (0.5 ul of 30ug/ul over 1 min) directly into the PP reversibly eliminated the DG responses evoked by PP and AL stimulation on the EC side of the infusion, while the response to a PP electrode on the DG side of the infusion site was unaffected, indicating that the AL input travels via the PP.

The results show that the lateral nucleus of the amygdaloid complex has a potent input to the dentate gyrus, which is probably a routed via synapses in the lateral entorhinal cortex. We are currently investigating in greater depth the possibility that this apparently disynaptic input will support long-term potentiation in the dentate.

190.1 AMINO ACID RELEASE FROM CEREBELLAR CULTURES: Brian R. Pearce* and Gary R. Dutton, Department of Pharmacology, University of Iowa, Iowa City, Iowa 52242.

Our dissociated cell cultures of early postnatal rat cerebellum have been characterized for cell type and proportion (Brain Res. 183: 241-246, 1980; Brain Res. 199: 473-481, 1980), thus enabling us to correlate biochemical results with specific cell type. With the use of a novel perfusion apparatus we have determined that these cultures contain a population of neurons (∿ 7% of total cells) which accumulate [^{3}H] GABA and then release it in a Ca^{2+}-dependent manner in response to K^+-stimulation (J. Neurosci. Methods 3: 255-259, 1981; Brain Res. 206: 485-489, 1981). We have tentatively identified these cells as stellate and basket neurons. However, the most numerous neurons in the cultures are thought to be granule cells (∿ 85%). The neurotransmitter of this cell type, while still in doubt, is thought to be glutamate.

Our autoradiographic studies show that granule cells in these cultures do not accumulate exogenous [^{3}H] glutamate, uptake being confined to the glial cells. Consequently we investigated the Ca^{2+}-dependent, K^+-stimulated release of endogenous glutamate and GABA along with several other amino acids (aspartate, alanine, leucine, taurine and glutamine). Data indicated that only GABA and glutamate were released under these conditions.

With these findings and the fact that these results were not observed in experiments using glia-enriched cultures, we conclude that GABA and glutamate are probably utilized by cerebellar neurons as neurotransmitters--the latter being the transmitter of the granule cell.

This work was supported by USPHS grant NS 16518 and by Biomedical Research Grant RR 05372 from the Biomedical Res. Support Br., Div. of Res. Facilities, NIH.

190.2 PASSIVE ELECTRICAL PROPERTIES USED TO DISTINGUISH SOMATIC FROM DENDRITIC RECORDING SITES IN CULTURED NEURONS. J.K. Engelhardt, K. Ishikawa*, and D. Katase*. Dept. of Neurology, USC School of Medicine, Los Angeles, California 90033.

The identification of the structure from which one is recording is a major problem for electrophysiologists working in the mammalian CNS. This problem has been partially solved with the recent introduction of dye injection techniques. Unfortunately, these techniques involve time consuming histological processing before electrophysiology can be correlated with morphology. We report here the preliminary results of work designed to establish electrophysiological criteria for the intracellular location of the recording electrode.

Experiments were performed on the NG 108-15 neuroblastoma-glioma cell hybrid. This preparation was selected because electrophysiological and biochemical studies indicate that this cell line may serve as a useful model for alpha motoneurons (Hamprecht, B., Int. Rev. Cytol., 49:99, 1977). Cells were seeded at a low density on 60 mm plastic tissue culture dishes and examined with KCl filled intracellular microelectrodes following the development of neurites (3 to 7 days after plating). Microelectrodes were inserted into the soma or neurite of a cell under direct visual control, and the voltage responses to long pulses of hyperpolarizing current were recorded. This data was analyzed using a theoretical model developed by W. Rall (Exptl. Neurol., 2:503, 1960) in which a lumped RC model for the cell body is connected to one end of an infinitely long cable. The membrane time constant (τ_m) was determined from the data and Rall's equation 9 was solved in order to determine the coupling constant (ρ) at $t=\tau_m$. This analysis revealed that when the electrode was in the soma of a cell, ρ was small (3.5 or less) indicating a dominance of the lumped RC terms. When the electrode was in a neurite, ρ was indeterminantly large, indicating a dominance of the infinite cable terms. The key to the success of this technique appears to have been that the electrical properties were determined in a high conductance region of the cell current-voltage relation. When the membrane conductance was high, the space constant was short and the geometry of the cell near the electrode determined the response.

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190.3 EFFECT OF LASER MICROBEAM SURGERY UPON SPONTANEOUS ACTIVITY OF INTERCONNECTED MICROFRAGMENTS FROM CULTURED MOUSE SPINAL TISSUE. J.H. Lucas*, B. Horwitz, and G.W. Gross (SPON: J. Hines). Departments of Biology and Physics, Texas Woman's University, Denton, TX 76204.

An ultraviolet laser microbeam system (337.1 nm wavelength) has been used to isolate selected 'circuits' of small interconnected neuronal clumps in tissue culture from their surroundings by transection of large multi-process cables as well as of single processes. These interconnected spinal tissue microfragments (30-100 μm in diameter) from cultures of mouse spinal cord were examined as an intermediate stage in our on-going efforts to develop ordered two-dimensional cellular arrays. Standard techniques of extracellular recording demonstrated continued spontaneous electrical activity following laser surgery. Fluctuations in activity may have reflected mechanical disturbances accompanying surgery as well as interruptions of interfragment communication. Use of UV laser irradiation as a direct stimulus of electrical activity within clumps produced variable results including occasional intense bursts of activity. Indirect laser stimulation via small shock waves from substrate vaporization, however, consistently elicited immediate and sustained increases in frequency of spontaneous firing as well as the appearance of larger spikes. It is concluded that the laser is a useful tool for 'tailoring' systems of interconnected neuronal tissue microfragments. Further characterization of microfragments is being conducted through the use of histological techniques, electron microscopy and administration of various pharmacological agents to block inhibitory or excitatory components.

The high accuracy of UV laser microbeam methods also allows microsurgery on single neurons. Studies have been initiated to investigate changes in intracellular electrical activity during and following laser transection of neurites.

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190.4 OXYGEN CONSUMPTION OF HYPOTHALAMIC TISSUE SLICES AFTER VARYING INCUBATION PERIODS. S.R. Kelso*, M.A. Lessler* and J.A. Boulant. Dept. of Physiology, Ohio State Univ., Columbus, Ohio 43210.

The in vitro brain slice preparation has emerged as a powerful tool in pharmacological and electrophysiological studies. During the first few hours of incubation, however, electrophysiological recordings are rarely attempted, since most investigators report that spontaneous neuronal activity is often absent. The current study examines the oxygen consumption of hypothalamic tissue slices to determine if this electrophysiological phenomenon correlates with metabolic activity.

The preoptic area and anterior hypothalamus (PO/AH) of male Sprague-Dawley rats (250-300 gm) was blocked and cut into 300μm slices. Eight PO/AH tissue slices were obtained from each rat. In each experiment, oxygen uptake was immediately measured in two of the slices. The remaining slices were placed in an incubation/recording chamber, continuously perfused with oxygenated (95% O_2; 5% CO_2) glucose medium (Yamamoto, Exp. Brain Res. 14: 423, 1972), and incubated at 36 ± 1°C for up to four hours. At periodic intervals, a slice was removed from the chamber for measurement of O_2 consumption. O_2 consumption was measured by modified YSI Model 53 Biological O_2 Monitors. Single slice O_2 uptake was determined at 35°C in a glucose medium equilibrated with room air. The slope of the oxygen uptake curve during the initial eight minutes after transfer from the incubation chamber to the O_2 monitor was used to calculate O_2 uptake. Slice samples were then removed quantitatively, dried to constant weight, and weighed with a semi-microbalance.

O_2 consumption of fresh PO/AH tissue was 6.12 ± 0.97 (μl O_2/hr)/mg dry weight. Similar oxidative levels were maintained throughout the entire 4 hour incubation. During the early incubation periods, however, the O_2 consumption of the slices showed greater variability, when compared with the slices having longer incubation periods. For example, the O_2 uptake (μl/hr/mg) at 90 minutes was 4.6 ± 1.2 (S.E.), compared to a 3-hour value of 5.6 ± 0.3 (S.E.). In a control experiment, slices incubated at high temperatures (45°C) for 30 minutes, showed no oxidative capacity. In addition, there was no consistent difference between slices taken from 1.5 mm rostral to 1.0 mm caudal to the anterior commissure/optic chiasm plane of section.

These experiments support the electrophysiological findings which indicate that the tissue viability does not diminish during these incubation periods. Furthermore, the O_2 uptake variability during early incubation may correlate with the previously reported variability of electrophysiological recordings.

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190.5 RAT SYMPATHETIC NEURONS FORM ELECTROTONIC SYNAPSES WHEN THEY ARE MAINTAINED IN A SERUM-FREE, DEFINED MEDIUM IN VITRO. D. Higgins*, H. Burton and R.P. Bunge. Dept. Anatomy & Neurobiology, Washington University, Sch. Med., St. Louis, MO 63110.

Principal neurons from the superior cervical ganglia of rat fetuses (21 day) were maintained for more than 12 weeks in dissociated cell cultures in a serum-free, chemically defined medium (Bottenstein and Sato, PNAS 76:514-517, 1979) supplemented with 2.5S NGF (100 ng/ml). Non-neuronal cells were killed by the periodic addition of fluorodeoxyuridine (10 μM) to the medium. Under these conditions, neurons extended processes within 24 hours; by the end of the second week in vitro, these extremely fine processes, which course alone or in small bundles, formed a dense network over the tissue culture dish.

Intracellular recordings, obtained at various times between the 17th and 80th day, showed that these neurons could generate substantial action potentials (up to 90 mV; duration ∿4 msec) in response to depolarizing current injections. These responses were dependent on tetrodotoxin-sensitive Na^+ channels, cobalt-sensitive Ca^{++} channels, and tetraethylammonium (TEA)-sensitive K^+ channels. Prominent hyperpolarizing afterpotentials (> 10 mV and >100 msec) were present even when the Na^+ action potential was blocked. Iontophoretic application of acetylcholine close to the soma also evoked action potentials.

Simultaneous intracellular recordings were obtained from pairs (n >50) of neighboring neurons. In more than 50% of the cases stimulation of one neuron elicited very short latency (< 1 msec) postsynaptic potentials in the second neuron; under optimum conditions (pH 7.7, TEA= 5 mM) an action potential in one cell usually caused an action potential in the recipient cell. These synaptic interactions were not blocked by hexamethonium (100 μM), atropine (3 μM), phentolamine (5 μM), chlorpromazine (1 μM) or propranolol (0.3 μM), or by elevation of the Mg^{++}/Ca^{++} ratio; thus the excitatory synaptic interactions between cells grown in serum-free medium do not appear to be chemically mediated. Furthermore, in about 40% of the pairs, injection of hyperpolarizing (depolarizing) current into one neuron caused the second neuron to hyperpolarize (depolarize). These experiments indicate that rat sympathetic neurons, as late as the 21st embryonic day, can form electrotonic synapses when they are maintained in a serum-free culture medium. Because these neurons acquire the ability to synthesize catecholamines during the 2nd week of gestation and retain this capacity in defined media (Iacovitti, et al., this volume), we conclude that an initial expression of the adrenergic phenotype does not preclude an electrotonic mode of synaptic transmission. (Supported by NIH Grants NS 14416 and NS 09809).

190.6 TOXIC EFFECTS OF FOOD DYES ON NGF-STIMULATED NEURITES IN CULTURE Bibie M. Chronwall and Stephen J. Morris (SPON: Richard L. Irwin) Surgical Neurology Branch and Neurotoxicology Section, NINCDS, NIH, Bethesda, MD 20205.

Erythrosin B (EB, USFD&C Red 3, tetraiodofluorescein) and other halogenated fluorescein derivatives have widespread use as food and cosmetic dyes, as well as histological stains and mordant dyes for wool, silk and cotton. Some of these dyes also inhibit uptake of neurotransmitters into synaptosomes, inhibit brain Na^+-K^+ ATPase and block ouabain binding. There is evidence for EB interaction with a "receptor-like" recognition site which is associated with, but distinct from, the cardiac glycoside binding and ATP catalytic sites (cf. abstract by Silbergeld, Morris and Anderson, these proceedings). In addition brominated and iodinated dyes are potent type II photo-oxidizing agents which have been shown to destroy the functions of several enzymes and to crosslink membrane proteins. These effects can be distinguished by the requirement for exposure to light in the presence of oxygen.

We have investigated the effects of low concentrations of ouabain and these dyes on the nerve growth factor (NGF)-promoted growth and differentiation of chick dorsal root ganglia explants employing an improved culture technique. The ganglia are embedded in gels of polymerized native collagen producing neurites of > 3 mm length. Ouabain inhibits neurite outgrowth with an IC_{50} of ~ 5 μM which is comparable to the toxicity of the cardiac glycosides reported for other cells in culture.

The iodinated and brominated dyes also inhibit outgrowth with an IC_{50} of ~ 10 μM and in addition promote early retraction of growing neurites. Some of these dye effects are due, at least in part, to photo-oxidation of the NGF. Preincubation of dye + NGF in light (60 watt incandescent bulb 80 cm from sample for 24 hours at 4°C) inactivates the growth factor. NGF incubated with dye in the dark or in light in an argon atmosphere is fully active.

However, dye-treated NGF-stimulated cultures grown in complete darkness still show reductions in neurite length when compared to non-treated controls. This suggests that the dyes may also have a direct (ouabain-like) toxic effect on the cultures.

In addition, this improved culture system should provide a rapid and inexpensive in vitro test for screening potential neorotoxins which would effect neurite growth and development.

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190.7 CYCLIC AMP INDUCES REPETITIVE FIRING IN CULTURED EMBRYONIC RAT MUSCLE. James Blosser, James McManaman* and Michael Merickel. Dept. Neurology, Baylor College of Medicine, Houston, TX 77030.

Although there is ample evidence suggesting the importance of cyclic nucleotides in neural membrane events, their role in muscle activity is not well characterized. We have utilized primary cultures of rat embryonic skeletal muscle to study the effect of cAMP on electrical membrane properties. Treatment of these cells with cAMP results in the development of a repetitive spontaneous firing pattern characterized by a delayed onset and diminished afterhyperpolarization.

Hind limb muscle from newborn rats was dissociated and cultured for 7 days in Delbecco's modified essential medium containing 10% horse serum and 0.5% chick embryo extract. Intracellular cAMP levels were elevated by exposing cells to cholera toxin or membrane permeable analogs of cAMP.

Treatment of myotubes with cholera toxin did not have an immediate discernable effect on fiber twitch or membrane electrical properties. However, between 2 to 4 hours after treatment a gradual hyperpolarization (approximately 10 mV) of the resting membrane potential (RMP) and decrease in action potential (A.P.) afterhyperpolarization amplitude was noted. Within 24 hrs., the number of myotubes exhibiting spontaneous twitching increased and was correlated with increased A.P. spontaneous firing. At later times (24-30 hrs.) A.P.'s exhibited no afterhyperpolarization and virtually all cells were fibrillating, having lost a one-to-one relationship between A.P. firing and observable twitch. Repetitive firing of A.P.'s occurred at frequencies up to 10 Hz. A.P. frequency in those control cells which were spontaneously active did not exceed 1 Hz.

These effects were specific for cAMP. Cholera toxin, 8-bromo cAMP(10^{-3}M), dibutyryl cAMP(10^{-3}M), PCPT cAMP(10^{-4}M) and a phosphodiesterase inhibitor, RO201724, all elicited identical effects while 8-bromo-cGMP and dibutyryl cGMP(10^{-3}M) were ineffective. Further, cholera toxin increased intracellular cAMP levels up to 10-fold above basal levels within 1.5 hrs., well before any change in membrane electrical properties. If cells were pretreated with cycloheximide (1 μg/ml), the development of repetitive firing in response to cAMP was completely abolished. Cycloheximide decreased ^{35}S-methionine incorporation into protein by 90% and did not alter RMP, A.P. properties, or normal spontaneous firing. These results demonstrate that cAMP can alter electrical properties of embryonic muscle through a process which appears to require protein synthesis. (Supported by the Muscular Dystrophy Association, Kleberg Foundation, and NSF Grant BNS 7914115)

190.8 EVIDENCE FOR SYNAPTIC TRANSMISSION BETWEEN CILIARY GANGLION NEURONS IN CELL CULTURE. Joseph F. Margiotta* and Darwin K. Berg, Dept. of Biology, Univ. of Calif., S.D.; La Jolla, CA. 92093.

Chick ciliary ganglion neurons are parasympathetic cholinergic neurons that innervate smooth and striated muscle in the eye, and receive cholinergic input from preganglionic neurons in the accessory oculomotor nucleus. In cell culture the neurons develop high levels of choline acetyltransferase activity, and when grown with muscle, form functional cholinergic synapses on the myotubes. We now present physiological evidence that the neurons can innervate each other under these conditions as well.

Ciliary ganglion neurons from 8-day chick embryos were grown with skeletal myotubes at a density of 1-2x10^4 neurons/35 mm dish for 5-14 days in culture medium supplemented with 5% chick embryo extract. Intracellular recording from the neurons revealed discrete depolarizing potentials occurring in the absence of stimulation. The potentials varied from barely detectable amplitudes (< 1 mV) to events greater than 10 mV and were clearly excitable, sometimes triggering impulses. The potentials resembled ACh-mediated excitatory postsynaptic potentials (EPSPs) having rapid rates of rise, slow exponential decays, and sensitivity to blockade by cholinergic antagonists. The potentials were completely abolished by perfusing the cultures for 10 min with medium containing either 100 μM d-tubocurarine (d-TC) and 1.4 mM hexamethonium, or 25 μM d-TC alone. Partial recovery of EPSPs was observed after 10 min of washout for neurons that were continuously monitored throughout the drug treatment and perfusion changes. After 30 min of washout, other neurons tested in the same cultures showed EPSPs at frequencies comparable to control levels. Drug treatment did not prevent impulses in response to direct electrical stimulation, and did not affect the resting potential which averaged -53 ± 1 mV (mean ± SE; n=45) for all neurons.

When grown with muscle, 35 neurons out of 39 tested (10 cultures, 3 platings) displayed spontaneous EPSPs. The frequency of EPSPs ranged from 2/min to 180/min. Neurons grown without muscle in culture medium supplemented with 3% embryonic eye extract, also displayed EPSPs but much less frequently. Only 4 neurons out of 14 tested (5 cultures, 3 platings) showed spontaneous EPSPs; their rates varied from 2/min to 90/min.

These results indicate that ciliary ganglion neurons form functional excitatory cholinergic synapses with each other in cell culture. The formation of such synapses in culture may reflect the absence of the normal preganglionic input as has recently been found for frog parasympathetic neurons in vivo (Sargent and Dennis, 1981). (Supported by NIH grant NS 12601.)

190.9 EFFECTS OF THYROXINE ON MEMBRANE POTENTIALS OF DEVELOPING RAT SKELETAL MUSCLE CELLS IN CULTURE. S. R. Sampson and Rena R. Bannett*. Life Sci. Dept., Bar-Ilan Univ., Ramat-Gan 52 100, Israel.

Skeletal muscle cells in culture are a convenient model for the study of the development of biochemical and electrophysiological properties of this tissue. Despite the recognized importance of thyroxine (T_4) to normal growth and maturation, there are no reported studies on the influence of T_4 on these phenomena under conditions that preclude interaction with other hormones or the nervous system. Accordingly we have studied effects of T_4 on transmembrane resting potentials (Em) of myotubes obtained from 19-21 day-old rat embryos and maintained in culture for 5 to 21 days. At various times after fusion, T_4 was added to the growth medium to a final concentration of 10^{-7} to 10^{-6}M. Transmembrane potentials were recorded with 2.8M KCl-filled glass fiber microelectrodes. Em values increase with age from about 50mV at 3 days in culture to a plateau at about 72-75mV by the 7th day. Approximately 24 hrs after addition of the hormone, the mean Em recorded from T_4-treated cells was significantly higher than those in diluent-treated sister cultures (mean difference of 4-14mV), the effect beginning 4-5 days after plating. Possible explanations of this effect include an increase in membrane permeability to K^+, or activation of an electrogenic pump. To test the former possibility, we compared the relation between Em and external K^+ concentration in control and T_4-treated cells. Whereas the T_4-induced increase in Em persisted at each external K^+ concentration, there was no difference between the two groups in the slope of the line relating Em to $[K^+]_o$. To examine the role of an electrogenic pump, we studied the effects of ouabain and dinitrophenol (DNP). Ouabain (10^{-3}M) reduced the Em of both control and T_4-treated cells to approximately the same level within 30-40 min after addition to the culture. DNP (10^{-3}M) produced a similar effect. The onset of the ouabain effect occurred earlier in T_4-treated cells than in control cells and coincided with that of the T_4-induced increase in Em obtained after 4-5 days in culture. The results demonstrate the presence of an electrogenic sodium pump in rat skeletal muscle cells in culture, and further suggest that T_4 causes early appearance of this pump and stimulates its activity, leading to an increase in transmembrane potential.

190.10 SEROTONERGIC TRANSMISSION BETWEEN ISOLATED LEECH NEURONES IN CULTURE. Leslie P. Henderson. Dept. of Neurobiology, Stanford Univ. Med. School, Stanford, California. 94305

For studying synaptic transmission and synaptic neurochemistry, isolated leech neurones grown in culture provide distinct advantages. These cells maintain many of the electrical and chemical characteristics of their counterparts in the animal, and yet the simplicity of the culture conditions provides access to cell surface receptors free of glia and to synaptic terminals which, in the animal, occur at a great distance from the soma in the neuropil.

In the present experiments, the Retzius to Pressure sensory cell synapse was analysed. The presynaptic Retzius cell was found to synthesise ^{3}H-5-HT (serotonin) from its immediate precursor, ^{3}H-5-hydroxytryptophan. The presence of 5-HT in cultured Retzius cells was confirmed by staining with neutral red and by fluorescence induced by glyoxylic acid. The Retzius cells also released ^{3}H-5-HT into medium in the presence of elevated potassium (58 mM). This release was found to be calcium dependent and was blocked by elevated magnesium (25 mM).

The regional sensitivity of the post-synaptic P sensory cell to 5-HT (50 mM, pH 7.4) was studied by pressure ejection from a micropipette, Terminals of sprouts, especially those with growth cone-like structures, were found to have a higher sensitivity than either cell somata or non-terminal areas of processes. The responses elicited by ejected 5-HT were compared to those seen on P sensory cells in situ and to the synaptic response observed in cultured P cells upon stimulation of presynaptic Retzius cells. P cells in culture and in situ showed an initial hyperpolarising response that rapidly desensitised, but whose time course was similar to the synaptic potential seen between cultured Retzius and P cells. This initial response was often masked, both in cells in culture and in situ, by a depolarising response that did not desensitise.

While these results implicate 5-HT as a transmitter from Retzius to P sensory cells in culture, it remains to be determined what mechanisms underlie the slow synaptic potentials and whether additional transmitters are involved.

190.11 RECORDING OF SPONTANEOUS ACTIVITY FROM CULTURED MOUSE SPINAL NEURONS WITH LASER DE-INSULATED PHOTOETCHED MULTIMICROELECTRODE SURFACES. G.W. Gross, A.N. Williams*, Department of Biology, Texas Woman's University, Denton, TX 76204 and Stephen Popik*, Central Research Labs, Texas Instruments, Dallas, TX.

A matrix of gold conductors photoetched onto the floor of a tissue culture chamber has been used to record spontaneous activity from dissociated mouse spinal cord tissue (12-14 day embryos) growing on the multimicroelectrode surface. The two-dimensional electrode arrays (36-50 gold conductors 10-12 µm wide in a 0.5 mm by 1.0 mm area) were insulated with a polysiloxane resin and de-insulated with a laser shot at an energy density of 4.0 µJ/cm^2 and a wavelength of 337.1 nm. The hydrophobic insulation layer was oxidized with a butane flame to produce a hydrophilic surface for better cell adhesion. Recording was begun at nine days postseeding. Consistent spontaneous activity was recorded from microfragments of tissue measuring 50 to 100 µm in diameter. Maximum signal to noise ratios of 8:1 were achieved without the use of platinum black to reduce impedances which usually measured between 4 to 8 megohms. At 35°C, most microfragments revealed vigorous multiunit activity with high frequency bursts from several large units repeated at intervals of 1-5 seconds. Large units ceased firing at 28°C and all activity stopped at 25°C. Activity recovered to normal levels within two minutes after raising the temperature to 28°C. Careful changes of 50% of the medium during recording normally resulted in short activity increases followed by almost total cessation of activity which often lasted over one hour. This reveals that even with stationary, integrated electrodes small mechanical and pH fluctuations have a surprisingly severe effect on the spontaneous activity. Microfragments below 50 µm in diameter have not consistently shown spontaneous activity. Simultaneous recordings from directly interconnected tissue fragments are being initiated. We consider the microfragment an intermediate step between explants and totally defined two-dimensional networks. Microfragment activity has been monitored thus far for a maximum of three days; future experiments will follow activity for longer periods.

Supported by NIH grant 1RO1 NS 15167

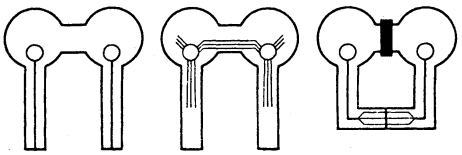
190.12

WITHDRAWN

190.13 CODING FOR A CENTRAL CONNECTION IS ALSO PRESENT IN THE PERIPHERY. Robert D. Hadley, Richard G. Wong* and Stanley B. Kater, Dept. of Zoology, Univ. of Iowa, Iowa City, IA 52242.

Identified neurons 5R and 5L of the snail, Helisoma are not electrically coupled in normal adult buccal ganglia. In response to proximal peripheral nerve crushes which sever the axons of these neurons, the short axonal stumps are resorbed. Upon culture, neurons 5R and 5L sprout and grow central neurites; forming a specific novel electrotonic connection between the neurons (Bulloch and Kater, 1981, Science 212:79-81).

Latent recognition between homologous neurons may be localized to narrowly defined neuronal regions, as are many synaptic contacts, or could be uniformly distributed over the cell surface. The problem of the distribution of recognition capability over the neuronal surface can be approached by in vitro culture of ganglia with manipulations to allow neurites to contact one another in the periphery.



When ganglia are cultured with closely apposed, cut peripheral nerve trunks and tightly ligated or severed commissures, 5R-5L neuronal interactions occur only in peripheral regions. In such ganglia specific 5R-5L electrotonic connections occur with a fidelity like that in central interactions. These data imply that: 1) axonal resorption per se is not necessary for the formation of this novel adult neuronal connection; 2) no special properties of the central neuropile are required for the connection; and 3) coding for such neuronal recognition is distributed over the peripheral elements of these neurons.

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190.14 ELECTRICAL AND CHEMICAL SYNAPTIC TRANSMISSION BETWEEN APLYSIA NEURONS IN DISSOCIATED CELL CULTURE. E. Proshansky*, S. M. Schacher*, and J. S. Camardo* (SPON: K. Weiss). Center for Neurobiology & Behavior, Depts. of Anatomy, Physiology, and Psychiatry, Columbia University, P & S, New York, N. Y. 10032.

The application of dissociated cell culture techniques to the invertebrate nervous system will permit increased access to the synaptic structures of identified neurons. Recent work has shown that mature, isolated neurons of the leech regenerate processes and form electrical and chemical synapses in culture (Ready and Nicholls, 1979). Aplysia neurons in culture also regenerate processes and form electrical synapses (Dagan and Levitan, 1980). We have established Aplysia neurons in dissociated cell culture and have found that: 1) in addition to electrical synapses, chemical synapses also routinely form between cultured neurons and 2) neuronal growth is increased in medium containing Aplysia hemolymph.

Neurons isolated by enzymatic and mechanical dissociation of ganglia from small adult Aplysia californica were plated in Leibovitz medium that contained 5% fetal bovine serum and was adjusted to the osmolarity of seawater. Within 36 hr following plating the cells sprouted 1-10 processes up to 8 μm in diameter. Electron microscopy showed that these processes, which in the light microscope appeared to be single structures, were in fact fascicles of several tightly apposed fine neurites. The addition of cell-free Aplysia hemolymph to the culture medium resulted in: 1) more rapid initiation of neurite outgrowth (by 12 hr); 2) a doubling of the rate of outgrowth and 3) richer neuritic arborizations.

Intracellular recordings made in artificial seawater showed that the resting and action potentials of cultured cells were comparable to those of cells in vivo. DC current injected into either member of a cell pair connected by neurites or with apposed cell bodies showed that as many as one-third of these cell pairs were electrically coupled. Coupling coefficients ranged from .1-.8. Chemical synaptic transmission was evident as early as 3 days in culture and occurred only between cells whose soma appeared to be in contact. Both EPSPs and IPSPs of up to 10 mV, with latencies of 8-60 msec, were observed. These synaptic potentials were reversibly blocked by high Mg^{++} solutions and their amplitude and polarity were sensitive to changes in postsynaptic membrane potential. As previously shown in vivo in Aplysia (Shapiro et al., 1980) we found that transmitter release was sensitive to changes in the presynaptic membrane potential. Release increased when the presynaptic cell was depolarized and decreased when it was hyperpolarized, suggesting that, in culture, synaptic release sites are electrically relatively close to the cell body. This ability to experimentally control the synaptic processes of cells in culture will prove useful in the analysis of transmitter release and its modulation, particularly when applied to neurons of Aplysia with known plastic properties.

190.15 **PROLIFERATING NEURON-LIKE CELLS IN MOUSE HIPPOCAMPAL CULTURES.** J.H. Peacock and C.R. Walker Division of Neurology, Univ. of Nevada Medical School, Reno, NV 89557

Immature neurons in cell cultures prepared from many regions of the central nervous system do not appear to undergo more than 1 or 2 cell divisions after initial plating. However, hippocampal cell cultures contain neuron-like cells which proliferate in standard growth media with 10% serum and without potassium augmentation. An increasing number of such cells are observed under phase contrast microscopy over several weeks in culture and the process of active cell division has been confirmed by ^{3}H-thymidine autoradiography.

Proliferating neuron-like cells (PNLCs) can be readily identified by their small size (soma diameter less than 10 μM), lack of neurites, and their tendency to occur in large groups of similar cells. Phase-dark and phase bright cells are intermixed. Some of these cells appear to develop long branching processes in month old cultures in areas where they attach directly to the surface of the culture dish.

The following electrophysiologic properties have been obtained from PNLCs in separate culture series including those with ^{3}H-thymidine label. Resting membrane potentials are high, 87.8±1.7 mV (mean ± S.E., n=23). Depolarizing current injection characteristically elicits graded peak-shaped potentials which lack inflection points on the rising slope and which are sometimes followed by a 2nd positive potential. Small anode break potentials are occasionally seen. Depolarizing activity persists in media containing 4 mM Na+ and either 8mM Ca++ or 8 mM Mn++ substituted for Ca++. After the depolarizing response, there is a prominent afterhyperpolarization (AHP) which can be reversed by steady negative currents. Depolarizing potentials and AHPs are blocked by Ni++ but not by TEA, 4AP, or chloride-free media.

PNLCs are responsive to GABA iontophoresis. These cells do not take up ^{3}H-GABA (29 nM) but do selectively take up ^{3}H-glutamate (22 nM) on the basis of autoradiographic studies.

Finally, PNCLs do not have immunohistologic staining for glial fibrillary acidic protein (courtesy of L. Eng and D. Bagley, Palo Alto VA Hosp.), a result which argues against the possibility that these cells are astrocytes. Thus on the basis of evidence for active membrane properties, GABA sensitivity, selective glutamate uptake, and absence of evidence for astrocytes we propose that PNLCs are neurons. It would not be suprising if PNLCs are granule cells which in the intact mouse hippocampus have their greatest proliferative activity for 3 weeks after birth.

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190.16 CELL CULTURE AND CHEMICALLY INDUCED FUSION OF DISSOCIATED NEURONS OF DROSOPHILA. C.-F. Wu, N. Suzuki* and M.-m. Poo. Dept. of Zoology, Univ. of Iowa, Iowa City, IA 52242 and Dept. of Physiology, Univ. of California, Irvine, CA 92717.

We have obtained primary cultures of dissociated cells from brains and ganglia of third instar larvae of Drosophila. Morphology of the cultured cells have been studied with phase contrast and Nomarski optics and scanning electron microscopy. Within a few hours after plating, cells initiated axon-like processes with growth cones. More than 50% of cells showed features of neurons. Cells cultured for 24 hours exhibited one or more long processes with branches and formed contacts with other cells These differentiated cells have been maintained in culture up to one week.

Synthesis and accumulation of acetylcholine in the cultured cells were detected after one hour incubation with ^{3}H-choline. The amount of acetylcholine accumulated and the efficiency of conversion from choline to acetylcholine were comparable to those determined by using intact brains and ganglia.

Fusion of cultured cells was induced with polyethylene glycol (PEG). This treatment produced large multinucleated cells of the size up to 100 μm, in contrast to the sizes of untreated cells ranging from 1-10 μm. PEG treated cells had smooth and continuous surface as revealed by scanning electron microscopy. Continuity of the cell surface was further evidenced by very large bubbles of cell membrane induced by osmotic swelling of the fused cells. In contrast, aggregates of untreated single cells under the same condition formed small individual bubbles. Fused cells have been grown in culture medium for several days. One day after PEG treatment, fused cells showed processes with growth cone-like structures of large sizes.

Neurons obtained from adult lethal mutants and mutants with altered membrane excitability have also been cultured. This nerve cell culture system of Drosophila can provide easy access to electrophysiological and molecular genetic analyses of the development and function of neurons.

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191.1 LOCALIZATION OF THE RECEPTOR SITE FOR RABIES VIRUS ON THE MOUSE DIAPHRAGM AND ON CULTURED CHICK MYOTUBES. Thomas L. Lentz, Thomas G. Burrage, Joan Crick* and Gregory H. Tignor*. Section of Cell Biology and Department of Epidemiology and Public Health, Yale University School of Medicine, New Haven, CT 06510.

The entry of rabies and other neurotropic viruses into the central nervous system has been explained by uptake of virus at nerve terminals and centripetal transport to the neuronal cell body. However, the receptor sites for virus binding and the mechanisms governing uptake or restriction of uptake of virus at nerve terminals have not been defined. In order to determine the site of rabies virus binding, we have investigated the localization of labeled rabies antibody following exposure of mouse diaphragm and cultured myotubes to rabies virus.

The diaphragm and its attached phrenic nerve was removed from several strains of mice. The preparation was immersed in oxygenated Tyrode's solution containing rabies virus (10^5 infectious units) for varying periods of time, after which the whole mount was stained directly for rabies antigen with FITC-conjugated rabies antibody followed by counterstaining for acetycholinesterase. Rabies antigen was found by immunofluorescence to co-localize with acetylcholinesterase-positive sites as early as 30 min. after immersion. During the early time periods from 30 min. to 1 hour, antigen was limited to junctional sites. However, at 4 hours, antigen was present at both junctional and extra-junctional sites. In addition, by 2 and 4 hours, antigen was found in nerves. Electrical stimulation of the phrenic nerve intermittently for the first 30 min. reduced the number of rabies antigen-positive sites detected.

Cultures of chick myotubes were readily infected by rabies virus, and a neurotropic variant of Sindbis virus (Ar86), but not by the non-neurotropic prototype strain of Sindbis, nor by vesicular stomatitis virus (Indiana strain). The rabies virus antigen in infected myotubes occurred in patches and resembled the distribution of high-density acetylcholine receptor sites as revealed by staining with rhodamine-conjugated α-bungarotoxin (α-BTX). Pre-incubation of myotubes for 2 hours with α-BTX (10^{-6}M) prevented rabies virus and Sindbis Ar86 virus infection of myotubes and reduced the amount of radio-labeled virus which attached to these cells. α-BTX (10^{-3}M) did not inactivate virus nor did addition of α-BTX to cultures after virus had been adsorbed prevent infection. Cultures treated with α-BTX were susceptible to infection 3 days after the toxin was removed.

These results suggest that the receptor site for rabies and possibly other neurotropic viruses is located at sites of high acetylcholine receptor density. (Supported by NIH grants AI12541 and AI11132, and NSF grant BNS 80-18520).

191.2 NITROIMIDAZOLE NEUROPATHY: A QUANTITIATIVE ULTRASTRUCTURAL STUDY OF AXONAL MITOCHONDRIA. Michael A. Casey and Robert D. Yates*, Dept. of Anatomy, Tulane Univ. Sch. Med., New Orleans, La. 70112.

Misonidazole (MIS; a 2-nitroimidazole) is being evaluated clinically as a radiosensitizer of hypoxic tumor cells at several radiotherapy centers throughout the world. However, multiple doses of MIS cause peripheral neuropathy in humans, (Dische et al., Br. J. Cancer, 35:567, 1977) and MIS-induced pathological changes resembling Wallerian degeneration have been produced in the peripheral nerves of rodents (Griffin et al., Neurotoxicol., 1(3): 653, 1980; Casey et al., Anat. Rec., 199(3): 47A, 1981). In addition, we have also observed that the myelinated axons of MIS-treated mice contain more mitochondrial profiles than untreated axons when viewed in cross-section. Axonal mitochondrial profiles have been reported to increase focally in the early stages of Wallerian degeneration (Webster, J. Cell Biol., 12:361, 1962). These data prompted us to conduct a quantitative ultrastructural analysis of axonal mitochondria on thin sections of peripheral nerves from mice treated with either MIS (0.5 mg/g) or a control series injected with saline (0.85%).

Following 5 weeks of daily IP injections the medial plantar nerves of mice were prepared for transmission electron microscopy. The volume density (V_d) of mitochondria per unit volume of axoplasm (of myelinated axons) was determined by differential point counting. The mitochondrial V_d (mean ± SEM) was significantly ($p < 0.05$) higher in the axoplasm of MIS-treated mice (5.04% ± 0.24) compared to the saline controls (2.74% ± 0.25). The mean mitochondrial diameter did not differ significantly between the two groups (MIS = 0.170 μm ± 0.004; saline = 0.172 μm ± 0.002), indicating that the increase in V_d was not due to mitochondrial swelling in the MIS-treated axons.

A similar study was performed with desmethylmisonidazole (DMM), a 2-nitroimidazole recently introduced into clinical trials which is expected to be less neurotoxic than MIS. Following 5 weeks of daily IP injections (0.5 mg/g) of DMM, no pathological changes were observed in the peripheral nerves. The axonal mitochondrial V_d was determined to be 2.53% ± 0.22. This value was not significantly different from the controls, but was significantly ($p < 0.05$) lower than MIS-treated. Thus, at equal doses DMM appears to be less neurotoxic to peripheral nerves than MIS.

These results indicate that axonal mitochondrial profiles increase significantly in experimental MIS neuropathy. The difference in mitochondrial profiles may represent an early structural change in mouse peripheral nerves similar to that previously described in Wallerian degeneration. More importantly, the quantitation of axonal mitochondria may prove to be a reliable morphological assay for use in screening nitroimidazoles (or other compounds) for peripheral nerve toxicity.

191.3 LONG-TERM STUDIES OF INDIVIDUAL CEREBRAL AXONS Harvey A. Swadlow. Dept. Psychol. (U-20), Univ. Conn., Storrs, Conn.06268

Little is known about the long-term stability of conduction properties in central axons. In the present study chronic extracellular microelectrode techniques were combined with antidromic identification of efferent neurons of the corpus callosum in order to begin to examine this question in cerebral axons. In adult Dutch rabbits, banks of stimulating electrodes were implanted in the splenium of the corpus callosum and, in some cases, in the contralateral hemisphere. Most microelectrodes consisted of 25 μm Pt-Ir wire insulated with Teflon and cut flush. Microelectrodes were implanted into superficial cortical layers near the border of visual area I and II. Thirty-one neurons have been studied for 10 days or longer (mean= 26 days). One cell has been studied for 101 days and at the time of this writing is still under observation. Initial antidromic latencies of the above cells ranged from 5.5-31.3 ms (median= 16.1 ms) and conduction velocities were approximately 0.4-2.2 m/s.

Some of the above neurons demonstrated a progressive increase (10 cells) or decrease (5 cells) totaling more than 4% of the initial antidromic latency. Increases in antidromic latency were of greater magnitude than were decreases in latency and in some cases occurred at a steady rate of nearly 1%/day for 6-10 weeks. The conditions leading to such variations in conduction velocity are under study. For the remaining 16 neurons which demonstrated stable antidromic latencies, 4 additional measures obtained on successive days were analyzed: (a) minimum inter-stimulus interval yielding two conducted impulses; (b) minimum inter-spike interval (the least interval between two conducted impulses); (c) maximum decrease in antidromic latency following a single prior impulse (the supernormal period); and (d) maximum increase in antidromic latency following tetanic stimulation with 10 or 20 prior pulses (the subnormal period). Minimum inter-spike interval was the most stable of these measures, with a total range of variations rarely exceeding 12% of the mean value. Measures of the supernormal and subnormal periods were also very stable. For each of these measures, the variations observed within a cell on successive days were much less than those seen between different cells of the same approximate axonal conduction velocity. In contrast, successive measures of minimum inter-stimulus interval (the traditional measure of the refractory period) were quite variable.

191.4 NERVE TERMINAL STRUCTURE AND QUANTAL OUTPUT IN DYSTROPHIC CHICKEN MUSCLE. J.S. Gunther* and M.S. Letinsky. UCLA School of Med., Dept. of Physiol., Ahmanson Lab. of Neurobiology, and Jerry Lewis Neuromuscular Research Center, Los Angeles, CA. 90024.

The relationship between nerve terminal quantal output and motor endplate structure was studied at identified endplates in the EDII muscle of normal and dystrophic chickens (lines 412 and 413) ranging from 8 to 20 wks ex ovo. The quantal content was determined in curarized muscle by the coefficient of variation method. Subsequently, the pre- and postsynaptic components of these same neuromuscular junctions were stained using combined tetranitroblue tetrazolium and AChE staining. Nerve terminal area was measured from camera lucida drawings and quantal output per square micron of nerve terminal area was calculated. Also, the structure of identified terminals was compared to large numbers of randomly selected terminal drawings to determine where the identified terminals fell within their respective distributions.

The structure of the dystrophic nerve terminals differed characteristically from normal. They were less compact, smaller in total area, and their synaptic boutons were smaller and connected by long, fine branching processes rarely observed in the normal terminals. Growing sprouts were frequently present within the dystrophic terminals. Nerve-evoked release from these dystrophic terminals varied greatly in quantal output. In some cases EPP quantal content was less than at the normal terminals, but in many cases it was comparable to normal levels, so that release per square micron of dystrophic terminal was actually greater. A few terminals within the dystrophic muscles appeared structurally and physiologically normal, with no difference in total quantal output or in release per square micron of terminal compared to normal. Instances of empty postsynaptic gutters were observed in dystrophic muscle (AChE staining in the absence of nerve terminal staining); and this morphological evidence of denervation was confirmed by failure to evoke an EPP. Morphological evidence of multiple innervation of individual dystrophic muscle fibers was confirmed electrophysiologically by grading stimulus strength to the nerve. Histological evidence of multiple innervation was abundant in dystrophic muscles, and rarely observed in normal muscle.

These experiments provide evidence for: (i) great variability in quantal output from dystrophic terminals showing evidence of structural pathology; (ii) the occurrence of denervation of dystrophic muscle fibers--confirmed histologically and physiologically; (iii) greater incidence of multiple innervation in dystrophic muscle.

This work was supported by USPHS NS14417 and The Muscular Dystrophy Association of America.

191.5 PROPERTIES OF SPINAL MOTONEURONES IN A NEUROPATHY CAUSED BY THE TOXIN FROM TULLIDORA (Karwinskia humboldtiana). A. Hernández-Cruz* and E.J. Muñoz-Martínez. Dept. Physiol. & Biophys., Centro de Investigación y de Estudios Avanzados del I.P.N. México 14, D.F.

The neurotoxin from Tullidora (Karwinskia humboldtiana) produces a peripheral neuropathy characterized by segmental demyelination, nerve conduction block and functional denervation of hind limb muscles (Muñoz-Martínez & Chávez, 1979). This alterations are more intense in the distal portions of the hind limbs nerves, but it cannot be excluded that the motoneurones cell bodies are primarily affected. In the present experiments the properties of spinal motoneurones in cats treated with Tullidora were studied with conventional electrophysiological techniques. A single dose of Tullidora extracts were orally given to cats. Three to five weeks later treated animals showed hind limb flaccid paralysis. After barbiturate anesthesia, the soleus and the medial gastrocnemious muscles were activated through the nerve and through direct stimulation of the muscle bellies. The nerve elicited twitches were 50-95% smaller than the twitches obtained by direct muscle activation. In the same animals, membrane and action potentials of spinal (triceps surae) motoneurones were not different from normal values (88.67±11.6 mV and 68.11±3.4 mV, respectively); input impedance was slightly increased in these neurones.The afterhyperpolarization which follows the action potential remained unchanged in the case of medial gastrocnemious motoneurones, but it was consistently shortened in the soleus motoneurones. In some motoneurones from these treated cats, the amplitude of monosynaptic e.p.s.p. was normal (≈8.8 mV) but in other motoneurones from the same pool, the e.p.s.p. amplitude was significantly decreased.

We conclude that the neurotoxin from Tullidora does not primarily affect the cell bodies of spinal motoneurones. The observed changes are similar to those found after axotomy (Kuno, Miyata & Muñoz-Martínez, 1974), or partial denervation of the muscle (Huizar, Kuno, Kudo & Miyata, 1977).

191.6 PREVENTION OF DELAYED ORGANOPHOSPHORUS NEUROPATHY BY PHENYLMETHYLSULFONYL FLUORIDE (PMSF). A.B. Drakontides, T. Baker and W.F. Riker, Jr. Dept. of Anatomy, New York Medical College, Valhalla, N.Y. 10595 and Dept. of Pharmacology, Cornell University Medical College, New York, N.Y. 10021

Organophosphates like diisopropylfluorophosphate (DFP) are well known for producing a "dying-back" neuropathy in both humans and animals. A single 2 mg/kg intraarterial dose of DFP into the cat femoral artery results in uncoordinated muscle movements and diminished responsiveness to noxious stimuli in the injected limb. These deficits are at a peak 21-28 days after DFP administration. At this time functional and morphological changes occur in soleus α-motor nerve terminals. The stimulus-bound repetitive neural discharges generated by these terminals after tetanic conditioning (400 Hz for 10 sec) are severely impaired or lost, as is the obligatory post-tetanic potentiation of soleus muscle contractile response. From a random sampling of approximately 200 motor end plate regions of soleus muscle from treated legs of 8 DFP cats, only 5% of the motor nerve terminals were characterized as normal in their morphologic characteristics. All other motor nerve terminals had extensive structural alterations. These included the presence of lamellar whorls that filled the terminal and obscured or compressed synaptic vesicles close to the presynaptic membrane (73% of sample), and terminals that were fragmented and retracted from the junctional cleft, the cleft being filled by Schwann cell elements (22% of sample). Intramuscular nerves also contained lamellar whorls, and disrupted axoplasm.

PMSF is a protective inhibitor of the neurotoxic esterase which is associated with the development of the delayed organophosphorus neuropathy. Treatment with PMSF (30 mg/kg i.p.) 24 hours before the DFP administration prevented the delayed neuropathic changes. All motor nerve terminals examined from 3 cats treated with PMSF and DFP were normal in appearance. The morphological integrity of nerve terminals was corroborated by the functional capacity, in that stimulus-bound repetition of soleus motor nerve terminals was still evident and its incidence was greater than that seen in the delayed neuropathic cat. (Supported by NINCDS 01447.)

191.7 AGONIST-INDUCED RELEASE OF LACTATE DEHYDROGENASE FROM SKELETAL MUSCLE: AN ASSAY OF A MYOPATHY. J. P. Leonard. Section of Neurobiology and Behavior, Cornell Univ., Ithaca, NY 14853.

The release of cytoplasmic enzymes has often been used as an assay of cell damage. Increases in serum enzyme levels are diagnostic for certain diseases of heart and skeletal muscle (see L. P. Rowland, Arch. Neurol. 33:315). We have previously reported damage in mouse extensor digitorum longus (EDL) muscles in the vicinity of the neuromuscular junction (nmj) after prolonged treatment with the stable agonist carbamylcholine (carb) (Leonard and Salpeter, J. Cell Biol. 82:811). This damage was assessed on the basis of finestructural changes including: vesiculation of the junctional sarcoplasm, dilation of the sarcoplasmic reticulum, swelling of mitochondria and dissolution of Z-disks. The morphological damage is fully prevented by pretreatment with α-bungarotoxin (α-BGT) or use of a Ca^{++}-free Krebs bath (+5mM EGTA), indicating that the muscle damage is mediated by Ca^{++} and requires the activation of the nicotinic acetylcholine receptor at the nmj. In this study, we determined whether lactate dehydrogenase (LDH) release was a concomitant of carb-induced finestructural damage.

After 3 hrs. in Krebs alone (no carb) ∿1 unit (nanomole substrate/min) LDH is found in the medium per mg muscle wet weight. When 10^{-4} M carb is added for the first 20 min of the total 3 hour incubation time, 3 units are found in the medium, and when carb is present throughout the 3 hr incubation, 29 units are found. Ca^{++}-free (+5mM EGTA) medium or α-BGT pretreatment results in a return to control (no carb) values.

These results suggest that LDH release is controlled in parallel with the previously described morphological damage by prolonged Ca^{++} influx through the agonist-activated nmj. Agonist induced finestructural damage which occurs after anticholinesterase treatment (Ariens, Experientia 25:57) may also extend to the release of LDH from the sarcoplasm. Cytoplasmic enzyme release provides a rapid assay for studying damage induced by prolonged transmitter or agonist treatment of neuromuscular junctions.

(Supported by NIH grant NS09315.)

191.8 MICROTUBULE BINDING AND EPILEPTOGENESIS MAY UNDERLIE THE NEUROTOXICITY OF COLCHICINE FOR DENTATE GRANULE CELLS E. Lothman, D. Stein* and G. Wooten, (Spon. J. Trotter), Dept. Neurol., Wash. Univ., St. Louis, MO 63110.

Recently Goldschmidt and Steward (PNAS 77:3047, '80) reported that injections of colchicine into the hippocampal formation destroys granule cells of the dentate gyrus while sparing hippocampal pyramidal cells. To better understand the mechanisms of this selective neurotoxicity we studied electrophysiologic, histologic and metabolic effects of microinjections of colchicine and related compounds into the rat hippocampus. Colchicine (0.6-6 nmol in 0.2-0.3μl of normal saline, pH 7.2-7.4) was delivered via a 32 gauge cannula into the dorsal and ventral hippocampus. Three to thirty days later both Nissl and Timm's stained sections of brains revealed the preferential degeneration of granule cells and mossy fibers described by Goldschmidt and Steward. In some rats studied less than 2 weeks after injection, especially with higher doses of colchicine (4-6 nmol), there was a pallor of hippocampal pyramidal cells. However, these cells appeared normal after 2 weeks with doses of 2-4nmol colchicine. Vinblastine (0.4-1.2 nmol), a vinca alkaloid that also binds to tubulin subunits, produced neuropathology like colchicine. However, injections of lumicolchicine (4-6 nmol), a derivative of colchicine devoid of tubulin binding activity, did not. EEG recordings at the site of colchicine injection showed interictal and ictal discharges beginning 15 minutes after colchicine and lasting for several hours. Limbic seizures characterized by automatisms, staring spells and wet dog shakes were associated with the EEG paroxysms. ^{14}C-2-deoxyglucose autoradiography demonstrated localized changes of glucose utilization in the first few hours after colchicine administration. There was a profound increase in the hilum of the dentate gyrus ("CA_4") and less marked changes over the apical dendrites of CA_3. Regional glucose utilization was normal 7 days after colchicine injection except for a moderate reduction in the atrophic dentate gyrus.

These studies confirm that a highly selective lesion of dentate granule cells can be made with intrahippocampal injections of colchicine. That vinblastine but not lumicolchicine mimics the neuropathological action of colchicine suggests that the mechanism for the neurotoxicity may be a consequence of disruption of microtubules. This action may have a variety of adverse effects on neuronal function (e.g., blockade of transmitter release or axonal transport). Further, the neurotoxicity appears to be associated with electrical seizures. The precise relationships among neurotoxicity, epileptogenicity and tubulin disruption remain to be elucidated.

191.9 SITES OF 2,5-HEXANEDIONE BINDING IN MAMMALIAN PERIPHERAL NERVE M.I. Sabri and P.S. Spencer. Institute of Neurotoxicology, Albert Einstein College of Medicine, Bronx, NY 10461.

2,5-Hexanedione (2,5-HD) is a primary neurotoxic agent that causes axonal degeneration when mammalian nerves are exposed locally or systemically. Affected regions of nerve fibers display giant axonal swellings containing accumulations of slow- and fast-transported organelles. The sites of 2,5-HD binding and the mechanism underlying transport blockade are unknown. We have approached this problem by studying the subcellular distribution of radioactively labeled 2,5-HD following administration to normal peripheral nerves in vitro and in vivo. In the first group of experiments, (1,6-^{14}C)-2,5-HD (3.7mCi/mMole) was intraneurally injected into the sciatic nerve of deeply anesthetized adult Sprague-Dawley rats. Animals were sacrificed after 20h by perfusion through the aortic arch with cold normal saline. Sciatic nerves were removed, pooled, weighed and cut into small pieces with a razor blade. Nerve segments were homogenized in cold 0.25M sucrose and subcellular fractions prepared by differential centrifugation. Total radioactivity was determined by solubilizing aliquots of fractions in 0.5 ml of Soluene at 50°C and adding 10 ml of liquid scintillation cocktail. High-affinity toxin binding was determined by washing aliquots of each fraction on a Millipore filter of 0.22um diameter with 3ml of cold 0.25M sucrose. Radioactivity was present in nuclear, mitochondrial, myelin, microsomal, and soluble fractions, and in a floating lipid fraction. The largest amount of 2,5-HD binding (about 38%) was present in the crude mitochondrial fraction: less was found in the crude myelin pellet (about 29%). However, when the fractions were washed with sucrose on a Millipore filter, most of the label in the crude mitochondrial fraction was removed. By contrast, most of the ^{14}C label in the crude myelin fraction remained bound to high-affinity binding sites. High-affinity binding (expressed as a percentage of total binding) to subcellular fractions was as follows: myelin fraction, 23.6%; mitochondrial fraction, 8.5%; soluble fraction, 8.0%; nuclear fraction, 6.5%; microsomal fraction, 2.8%; and floating fraction, 2.8%. A similar pattern of differential high-affinity binding was found in sciatic nerves incubated for 30 min in oxygenated Krebs Ringer containing labeled 2,5-HD. Pretreatment of the sciatic nerve with unlabeled 2,4-HD (a non-neurotoxic analogue) before in vitro incubation with labeled 2,5-HD resulted in a slight increase in total binding, particularly in the crude myelin fraction. Otherwise, 2,4-HD failed to alter the distribution of high-affinity binding. In summary, these data are consistent with the report of prolonged retention of 2,5-HD in sciatic nerve. Further experiments are needed to reveal the specific sites involved in the neurotoxic action of this compound. (Support: OH 00535 and OH 00851.)

191.10 The peripheral nerve in motor end-plate disease ("med" mutation) in the mouse.

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"Motor end-plate disease" is an hereditary neuromuscular disease of the mouse. Two allelic forms of the "med" mutation exist, both leading to death of the affected med/med animals, although med^J/med^J mice seem to be less clinically affected than med/med mice. Swelling and sprouting of the nerve terminals have been observed in all skeletal muscles studied (silver nitrate staining and light microscopy). Enlargement of the non-myelinated gap at the nodes of Ranvier in the peripheral nerves is frequently observed (ultrathin sections, electron microscopy); their morphology range from normal to clearly pathological. Several myelin abnormalities can be observed, such aberrant myelinated bodies, increased interlamellar distances or long Schwann cell processes, extending along bare axolemmal regions. This abnormality of the nodes of Ranvier and myelin may be observed at early stages of the disease (12-14 days after birth) and is sometimes accompanied by abnormalities of the axon, suchas protusions and excrescences of the axoplasm. In motor end-plate disease , as in some other animal or human neuropathies, the myelin and the axon seem to be both affected and could represent primary or secondary targets of the mutation or even express a more general cell (membrane ?) defect.

(Supported by CNRS and Muscular Dystrophy Association of America)

191.11 NICOTINE IMPAIRS CYTOARCHITECTONIC AND SYNAPTOLOGICAL MATURATION IN DEVELOPING NEOCORTEX. J.V. Waldman* and D.A. Kristt. Dept. of Pathology, The Johns Hopkins Univ. Sch. of Medicine, Baltimore, MD 21205.

Despite evidence suggesting that nicotine exposure during brain development impairs brain function, morphological abnormalities have never been demonstrated. In this study, newborn Sprague-Dawley rat pups received two daily doses (1 mg/kg) of nicotine subcutaneously. The treatment period extended from the day of birth to six days of age. At the latter time, the animals were anesthetized and perfused transcardially with an aldehyde fixative. Following treatment, pups exhibited transient hyperactivity, but there was no cyanosis throughout this episode which began within five minutes of the injection and lasted for several minutes thereafter. All animals appeared to nurse normally and gained weight as usual. At 6 dpn, brain weights showed a tendency to be lower in the nicotine-treated group: mean, 0.51 grams; range, 0.45-0.57 grams as compared to control animals: mean, 0.57 grams; range, 0.54-0.60 grams (n=5 pups/group). Body weight was comparable in both groups: range, 12-16 grams. Synaptological analysis of superficial somatosensory cortex, which contains relatively large numbers of synapses at 6 dpn, was also undertaken. The number of synapses in the marginal zone (layer I) per 875 μm of inspected tissue is 50-60 in control animals (n=30) [Kristt, Brain. Res., 1979] and 16-25 in treated animals (n=2). In view of small brain size in nicotine-treated pups, the difference between treated and control animals may even be greater than experimentally observed. It should be noted that this synaptic density is approximately that of the newborn infant [Kristt, 1979], so that nicotine-treated animals appear to exhibit an almost complete retardation of synaptogenesis during the first postnatal week. Histological evaluation of cytoarchitectonics showed that, at 6 dpn, somatosensory cortex of treated animals had barely matured past the 3-dpn level. Between 3 and 6 days in normal rats, the unilaminar cortical plate develops a distinct trilaminar appearance. In contrast, nicotine-treated 6-dpn rats exhibit a cytoarchitectonic pattern more similar to that found between 3 and 4 days, i.e., a unilaminar cortical plate with only a slight suggestion of subdivision. Consequently, there also appears to be a retardation in cytoarchitectonic maturation, albeit less than that seen for synaptogenesis. These data suggest that nicotine may have an effect on the earliest phases of synaptogenesis and cortical development.

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191.12 CARPAL TUNNEL PRESSURE INCREASES IN MONKEYS CHANGE ELECTROCUTANEOUSLY EVOKED AVERAGED RESPONSES IN THE MEDIAN NERVE. A. Lee Dellon*, R. Burke*, R A. Cowley*, and Richard J. Schneider. Johns Hopkins University and The Maryland Institute for Emergency Medical Services Systems, Baltimore, Maryland 21201.

Macaca mulatta monkeys were anesthetized with pentobarbital administered i.v. and supplemented as necessary. Ring stimulating electrodes were attached to the index finger and delivered isolated, constant current pulses (10/sec., 3-5 mA) of 100 microsecond duration. Evoked responses (potentials) were recorded from a surface electrode on the skin over the median nerve between biceps and triceps muscles in the elbow. A differential amplifer (ear reference) fed the responses to a signal averager which summed 765 sweeps of 25 msec. duration. A wick catheter attached to a pressure transducer was placed in the carpal tunnel. An intracath was used to increase pressure by dripping saline or ringers into the tunnel. Evoked potentials (EPs) were taken before and after insertion of the cannula and intracath as well as before and after pressure was increased and after the pressure increase was terminated.

Pressure effects were measured as the percent of control EPs A-beta and A-delta waves were, at given elapsed times. EPs provided a rapid, non-invasive method of monitoring these changes. Wave form changes were a function of time and pressure. A-beta waves at low pressures (10-30 mmHg) first increased in amplitude then decreased over a period of 5-6 hours. In this time a pressure of 20-50 mmHg was the minimum required to abolish the waves. Rapid abolition was seen with pressures in excess of 50 mmHg. A-delta waves increased in amplitude while A-beta waves were decreasing, but with higher pressures, A-delta waves were also eventually abolished. The time it took to return to normal conditions was inversely related to the amplitude of the pressure applied.

These conclusions were drawn: 1) Low pressures abolished EPs as effectively as high pressures but over a longer time course; 2) Initial facilitation of nerve conduction amplitude occurred with lower pressures; 3) A-delta waves were potentiated by initial pressure increases; 4) Higher pressures exerted for shorter durations depressed sensory waves more quickly but produced a shorter time course of recovery following termination of elevated pressure; 5) EPs should be an effective method of monitoring critical pressure increases in emergency situations.

Supported by a Grant from the Raymond M. Curtis Research Foundation of Union Memorial Hospital.

195 SYMPOSIUM

SYNAPTIC REGULATION OF VOLTAGE-GATED CHANNELS

P.R. Adams (Chairman; Univ. of Texas), L.K. Kaczmarek (Caltech), M. Klein* (Columbia Univ.), H.C. Hartzell* (Emory Univ.), R.L. MacDonald (Univ. of Michigan).

The frog neuromuscular junction and the squid giant axon are the classical prototypes for chemical synaptic transmission and action potential propagation respectively. However many more recently studied electrical events in nerve or muscle cells share certain features of both and yet resemble neither. For example potassium currents in neuronal somata are often triggered by a chemical internal transmitter, calcium. Voltage sensitive channels that are controlled by more conventional external transmiters are the subject of this symposium. These transmitters mediate "slow" synaptic actions lasting seconds or more. In one case the transmitter opens channels that are normally closed, and which show rather weak voltage sensitivity (heart cells, to be discussed appropriately, by Hartzell). In the other cases various transmitters or synaptic actions turn off channels that may already be open, or at least openable. These channels open mostly at potentials positive to rest, though some of them may contribute to the resting potential. They influence all parameters such as threshold and spike shape and number. When turned off by appropriate transmitters, their absence makes itself felt as a change in the behavior of the cell when responding to more conventional inputs. Specific examples to be covered include neurons in Aplysia, bullfrogs, mice, rats and guinea pigs, in tissue culture, isolated ganglia or brain slices.

196 SYMPOSIUM

REPRESENTATION OF SPACE WITHIN SENSORY SYSTEMS. M. M. Merzenich* (Cochairman, UCSF), E. I. Knudsen (Cochairman, Stanford), G. F. Poggio (Johns Hopkins Univ.) and D. C. Van Essen (Caltech).

Recent physiological, anatomical and behavioral studies have provided new insight into how perceptual spatial constructs relate to and might be accounted for by neural constructs. Eric Knudsen shall describe results of studies within the homologue of the inferior colliculus (MLD) and within the optic tectum of the barn owl, revealing the nature of evident topographic representations of sound location within each structure. In the optic tectum, the relationship of overlapping auditory and visual field maps will be described, along with evidence for plastic changes of these emergent maps (created within the central nervous system by convergence of auditory inputs) resulting from deprivation.

Gian Poggio shall consider how the three dimensions of vision are represented within visual cortex. He shall emphasize the results of studies of the depth-selective response properties of neurons in Area 17 in alert adult primates. The representation of visual depth constitutes another instance (like sound location) in which a psychophysical dimension is derived from convergence of information from different sources within a sensory projection system.

There are numerous "visual field representations" within extrastriate cortex in primates, and investigators have recorded significant differences in the internal organization, fidelity of topography and completeness of visual field representation within them. David Van Essen shall outline the nature of these differences, considered as clues to their role in the processing of visual information and their contribution to visual perception.

Finally, Michael Merzenich shall describe results of studies of the somatosensory projection system that have revealed the topographic details of significant dimensional plasticity of "maps" of the skin surface within that system in adult primates. Some of the implications of those results shall be discussed, with consideration of how tactile form might be represented within this system.

Taken together, these reviews should provide a synopsis of the implications of recent investigations of these three sensory systems for the representation of space within them.

197.1 REGULATION OF SUBSTANCE P IN SYMPATHETIC NEURONS. J.A.Kessler, J. Adler,* M. Bohn, and I.B. Black. Division of Developmental Neurology, Cornell University Medical College, New York, New York 10021.

Mechanisms regulating the putative neurotransmitter substance P (SP), were examined in the adult rat superior cervical ganglion. Surgical section of the preganglionic nerves to the SCG significantly increased ganglion content of SP. Treatment with chlorisondamine, which blocks ganglionic transmission, also increased SP, whereas treatment with phenoxybenzamine, which reflexly increases sympathetic activity, reduced ganglion content of SP. These observations suggest that impulse activity of preganglionic nerves decreases ganglion SP through a trans-synaptic process.

To further characterize this process, neonatal ganglia were studied in vitro. Ganglion SP content increased 6-fold within 12 hours, and 30-fold within 24 hours in culture.The increases were completely prevented by cycloheximide, partially blocked by camptothecin and actinomycin D, and unaffected by cytosine arabinoside. Thus, protein and RNA synthesis, but not DNA synthesis are necessary for the increase in SP. Treatment with veratridine also completely prevented the rise in SP in vitro, and tetrodotoxin blocked the effects of veratridine. This suggests that sodium ion influx and membrane depolarization prevent the increase in SP. Immunohistochemical examination of cultured ganglia demonstrated that SP is localized within the perikarya and nerve processes of the principal sympathetic neurons. Our observations suggest that trans-synaptic impulses, through the mediation of post-synaptic sodium flux, decrease SP in sympathetic neurons.

(This work was supported by grants from the NIH and the Dysautonomia Foundation Inc.)

197.2 DEVELOPMENT OF SUBSTANCE P AND SOMATOSTATIN IN SENSORY GANGLIA IN TISSUE CULTURE. J.E. Adler*, J.A. Kessler and I.B. Black (SPON: E. Bloom). Division of Developmental Neurology, Cornell University Medical College, New York, New York, 10021.

Substance P (SP) and somatostatin (SS), putative neurotransmitters, have been localized to small neurons in the dorsal root ganglion (DRG). SP appears to mediate somatic pain, and exerts an excitatory influence on neurons in the dorsal horn of the spinal cord. The role of SS is as yet undetermined but its post-synaptic action is inhibitory. We have compared development of these two peptides in the DRG in vitro.

Neonatal rat ganglia were placed in organ culture and SP, SS and total protein content measured. Ganglion SP content more than doubled during the first 24 hours, and increased 3-fold by 72 hours. In contrast, ganglion SS decreased significantly, while total protein was unchanged.

The rise in SP was further analyzed using metabolic inhibitors. Cycloheximide, an inhibitor of protein synthesis, completely blocked the rise in SP, whereas Actinomycin D, an RNA synthesis inhibitor, had no effect.

Our studies suggest that the contents of SP and SS vary independently in the DRG in culture. These observations are consistent with the contrasting physiologic roles of the two peptides and suggest that their relevant regulatory mechanisms differ.

197.3 SUBSTANCE P AND ENKEPHALIN CONTAINING PATHWAYS IN SPINAL CORD. V.M. Pickel, K.K. Sumal*, J. Chan*, D.J. Reis and R.J.Miller. Dept. of Neurology, Lab. of Neurobiology, Cornell Univ. Med. College, N.Y. and Dept. of Pharmacology and Physiological Sciences, The Univ. of Chicago, Chicago, Ill.

Substance P (SP) containing axon terminals in the substantia gelatinosa and other parts of the dorsal and ventral gray of the spinal cord are reportedly derived primarily from sensory afferents in the dorsal root ganglia and from descending bulbospinal pathways. These immunocytochemical and transport studies are limited by difficulties in detection of SP immunoreactivity in perikarya and processes of neurons in the normal adult nervous system. Using two complimentary experimental approaches to enhance detectable levels of the peptide in axons, we sought to determine by light microscopic immunocytochemistry (1) whether SP is present in as yet unidentified ascending or descending pathways in the rat spinal cord and (2) whether similiar methodology could be used to demonstrate pathways of another neuropeptide, enkephalin (Enk). The two experimental procedures are based upon either the enhanced visualization of neuropeptides in outgrowing axons of the fetal nervous system or the accumulation of immunoreactivity (pile up) in axonal segments located behind nerve transections. Frozen sections through the spinal cord and lower medulla of fetal rats at embryonic (E) days 16-21 or of adult rats sacrificed 24-72h after hemisection of the spinal cord at cervical segment (C) 2 were labeled for SP or Enk using the peroxidase-antiperoxidase method. In the fetal spinal cord, SP is localized to longitudinally oriented axons in (1) the dorsal funiculus corresponding to the tracts of N. gracilis and cuneatus, (2) the ventrolateral funiculus and (3) a restricted region below the central canal. The position of growth cones on the rostral portions of the processes suggested that axons in the dorsal funciculus belonged to ascending sensory neurons and that the axons in the ventrolateral funiculus and ventral to the central canal were descending pathways. The location and directionality of the SP containing pathways were confirmed in the lesioned adult spinal cord by the accumulation of reaction product in axons distal to the lesion in the posterior funiculus and rostral to the lesion in the ventral tracts. In contrast to SP, the results of both the developmental and lesion studies demonstrated that Enk was detectable only in a descending pathway located in the ventrolateral funiculus. This pathway may correspond to the previously described enkephalinergic bulbospinal projection. (Hokfelt, Neurosci, Let. 14, 1979)(Antiserum to SP generously supplied by Dr. S.E. Leeman. Supported by NIH grants MH24285, HL18974 and MH 00078)

197.4 COEXISTENCE AND CO-RELEASE OF ACETYLCHOLINE AND THE LHRH-LIKE PEPTIDE FROM THE SAME PREGANGLIONIC FIBERS IN FROG SYMPATHETIC GANGLIA. Yuh Nung Jan and Lily Yeh Jan* (SPON: G. Reiness). Dept. of Physiology, Univ. of California, San Francisco,CA 94143.

Stimulation of preganglionic C fibers (arising from the 7th and 8th spinal nerves) initiates two responses in C cells in lumbar sympathetic ganglia. One response, the fast epsp, lasts tens of msec and is mediated by acetylcholine. The other one, the late slow epsp, lasts for minutes and is mediated by a LHRH-like peptide (Jan et al. Proc. Natl. Acad. Sci. 76, 1501-1505; 77, 5008-5012; Nature 288, 380-382). Here we report electrophysiological and anatomical experiments which suggest that acetylcholine and the LHRH-like peptide are contained within, and released from, the same preganglionic C fibers.

(1) Intracellular recording demonstrated that a C cell often receives 3-5 different cholinergic inputs which have different thresholds. By stimulating the preganglionic nerves at different strengths, we found that each time a cholinergic fiber was recruited, initiating an additional fast epsp, a peptidergic fiber was recruited as well, causing the late slow epsp to grow larger. Thus the thresholds of cholinergic fibers correlate well with the thresholds of peptidergic fibers, suggesting that the same preganglionic fibers for C cells supply both cholinergic and peptidergic inputs.

(2) We marked all terminals on C cells either by filling preganglionic fibers with horseradish peroxidase or by using a monoclonal antibody specific for synaptic vesicles (W.D. Matthew, L. Tsavaler, and L.F. Reichardt, Cold Spring Harbor Reports in the Neurosciences, vol. 2), and then stained the same sections using antibodies specific for LHRH. At least 90% of all preganglionic terminals on C cells contained the LHRH-like immunoreactivity, suggesting that at least some terminals contain both the LHRH-like peptide and acetylcholine. Therefore, it appears that most, if not all, preganglionic fibers for C cells contain and release both acetylcholine and the LHRH-like peptide.

197.5 THE ORIGIN OF THE CHOLECYSTOKININ-CONTAINING EFFERENTS OF THE CAUDATOPUTAMEN IN THE RAT. M.C. Beinfeld*, D.K. Meyer*, W.H. Oertel and M.J. Brownstein* (SPON: C. Helke) Lab. Clinical Science, NIMH, Bethesda, MD 20205.

Large amounts of cholecystokinin octapeptide (CCK) are present in the caudatoputamen (cp) of the rat. By immunocytochemistry, the peptide is found in axons and nerve endings but not in perikarya in the cp. Since some perikarya in the substantia nigra stain for both CCK and dopamine, it has been suggested that this structure may provide some of its CCK-containing neuronal processes (T. Hökfelt, et al., Neuroscience, 5, 2093, 1980). The origin of CCK in the cp was investigated in this present study using immunocytochemistry to visual CCK containing projections to the cp, and a CCK radioimmunoassay to evaluate the effect of selective knife cuts on the CCK content of the cp.

The cp receives afferents from the amygdala, ventral tegmental areas, substantia nigra, dorsal raphe nucleus, several cortical areas, and the thalamus. Apart from the thalamus, all of these structures contain perikarya which stain for CCK. A surgical lesion of the nigral-striatal pathway completely reduced the dopamine concentration in the cp but did not decrease its CCK content. This result indicates that neurons in the substantia nigra contribute little CCK to the cp. A knife cut severing the afferents from the amygdala reduced CCK content of the cp by about 30%. Removal of those parts of the frontal, parietal, and occipital cortex which are accessible after a dorsal craniotomy had no effect on cp CCK content. A knife cut severing the insular cortex from the cp likewise had no effect on cp CCK content.

Immunocytochemical staining showed heavy staining for CCK in the claustrum and adjacent piriform cortex, revealing CCK containing perikarya in both areas, in addition to nerve terminals. This suggested to us that the cp might receive CCK-containing afferents from these areas. A knife-cut severing the claustrum and the piriform cortex from the cp decreased CCK content in the cp by about 70%. However, the close proximity of the CCK staining perikarya in the piriform cortex to the claustrum prevented selective destruction of the piriform cortex which might have enabled us to decide whether the cp receives its CCK-containing afferents from the piriform cortex and/or the claustrum.

The claustrum receives afferents from various cortical areas while the piriform cortex is part of the olfactory system. Thus, CCK might be the transmitter of claustral and/or piriformal neurons which mediate impulses from various cortical areas and/or the olfactory system to the cp.

197.6 VASOPRESSIN-REVERSIBLE CHANGES IN ANGIOTENSIN-CONVERTING ENZYME ACTIVITY IN INTERMEDIATE AND POSTERIOR PITUITARY LOBES AND HYPOTHALAMIC NUCLEI OF RATS LACKING VASOPRESSIN. J.M. Saavedra, C. Chevillard* and J. Fernandez-Pardal*. Laboratory of Clinical Science, Section on Pharmacology, NIMH, Bethesda, MD 20205.

Changes in angiotensin-converting enzyme (ACE) activity were noted in the intermediate, posterior and anterior lobes of the pituitary gland and in the nuclei paraventricularis and supraopticus of rats with inherited diabetes insipidus, which lack vasopressin (Brattleboro rats; di/di:homozygous for the trait; and di/+:heterozygous) when compared to homozygous (+/+) control Long Evans (LE) rats.

In the posterior lobe, ACE activity was higher in di/+ rats with respect to LE rats (+43%), and even higher in di/di rats (+170%). The same phenomenon, but more pronounced, was observed in the intermediate lobe (+288% and +828% for di/+ and di/di compared to LE rats). In both posterior and intermediate lobes, the changes in ACE activity were reversed by vasopressin treatment (3 U per rat twice daily for seven days).

In the anterior lobe of the pituitary, ACE activity was lower in di/+ with respect to LE controls (-44%) and even lower in di/di rats (-69%), and these changes were not significantly reversed by vasopressin treatment.

In the supraoptic nuclei, increased ACE activity was observed in di/+ rats (+57%) and in di/di rats (+90%). In the nucleus paraventricularis, there was no significant change in di/+ rats with respect to LE controls (+11%) but a decreased enzyme activity in di/di rats (-32%). In both supraoptic and paraventricular nuclei, vasopressin administration reversed the changes in ACE activity. No changes in ACE activity were observed in the subfornical organ.

Our results suggest the possibility of a regulation by vasopressin of the activity of ACE, and therefore of the renin-angiotensin system, at the neuropituitary level and in discrete magnocellular hypothalamic nuclei.

197.7 $ACTH_{1-24}$ ANTAGONIZES OPIOID-INDUCED ANALGESIA IN THE RAT SPINAL CORD. H.L. Fields, G. Belcher* and T. Smock*. Depts. Neurology and Physiology, Univ. Calif. San Francisco, San Francisco, CA 94143.

ACTH and the potent opioid peptide β-endorphin (βE) are derived from a common precursor. ACTH antagonizes morphine suppression of spinal evoked responses (Zimmerman & Krivoy, Prog. Br. Res. 39:383). βE produces behavioral analgesia when injected into the rat 4th ventricle and we have shown that this effect is reversed by $ACTH_{1-24}$ (Smock & Fields, Brain Res. 212: 202). The spinal cord dorsal horn is rich in opiate receptor and analgesia is produced by direct opiate injection into the lumbar spinal intrathecal space. We examined the interaction of $ACTH_{1-24}$ and opioid agonists on a behavioral measure of analgesia at the level of the spinal cord.

Drug and control injections were made into the lumbar subarachnoid space. Injections were made via PE-10 cannulae placed through a slit in the atlanto-occipital membrane in barbiturate anesthetized rats. Drugs were diluted in balanced ion solution. Tailflick (TF) latency to noxious heat (baseline 4-5 sec, cut-off 15 sec) was measured. No significant change in TF latency was produced by up to 15 μg ACTH (n=12).

In studies on chronically implanted, unanesthetized rats, ACTH (2-10 μg) produced a slight increase in TF latency in 15 of 21 trials (cf. Walker, et al, Science 210:1247). Higher doses of ACTH (35-40 μg) increased TF latency in 4 of 8 rats tested. However, in no case did ACTH cause TF latency to go to cut-off. Morphine sulphate (MS) (10-30 μg) increased TF latency in 17 of 23 trials (13 to cut-off) and βE increased it in 6 of 8 trials (4 to cut-off). Ten min following administration the mean increase in TF latency for rats receiving ACTH was 0.5 ± 0.2 sec compared to 4.5 ± 0.9 sec for rats receiving MS.

In contrast to its weak agonist action, intrathecal ACTH (3-10 μg) reversed MS analgesia in all 15 trials (10 rats) and reversed βE analgesia in 3 of 4 rats. Reversal was complete by 15 min in most cases. Injection of equal volumes of balanced ion solution and mannitol (Cortrosyn vehicle, Organon) produced no latency changes.

These studies extend previous demonstrations of opposing actions of $ACTH_{1-24}$ and opioid agonists on systems relevant to analgesia.

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197.8 IN VIVO STUDIES OF THE RESTING AND EVOKED RELEASE OF CHOLECYSTOKININ (CCK) AND VASOACTIVE INTESTINAL PEPTIDE (VIP) FROM CAT CEREBRAL CORTEX AND VENTRICLES. J.-Y. Wang, K. Jhamandas, T.L. Yaksh and V.L.W. Go, Depts. of Pharmacology, Neurosurgical Res. and Gastroenterology, Mayo Foundation, Rochester, MN 55905.

The differential distribution of VIP and CCK in the CNS has been demonstrated by immunohistochemical studies and radioimmunoassays (RIA). Subcellular fractionation studies have shown that these peptides are high in brain synaptosomal fractions. Mapping with RIA, we found that these peptides are present in high levels in the cortex and periventricular tissue in the cat. To study the release of these peptides, we employed two in vivo superfusion preparations using chloralose-urethanized cats: a) cortical cups (two 1 cm plastic cylinders were agar-sealed on the surface after bilateral craniotomy and reflection of the dura); and b) ventriculocisternal perfusions (a 22 ga inflow cannula inserted into the lateral ventricle and an outflow cannula placed in the cisternal magna). In both preparations, animals were perfused at 150 μl/min with artificial CSF containing albumin/bacitracin. Changes in pupil size and blood pressure were recorded for each pharmacological manipulation. Levels of VIP and CCK were determined simultaneously using RIA in the same sample of lyophilized perfusate. The levels of VIP and CCK obtained from cortical or ventricular perfusion and the effects of adding picrotoxin in each preparation to the CSF are shown below for 3-5 cats.

	CORTEX		VENTRICLE	
	Basal Level[1]	Picrotoxin[2] (↑%)	Basal Level	Picrotoxin (↑%)
CCK	5.4 ± 1.1	113.2 ± 39.5	9.9 ± 1.24	350.8 ± 140.1
VIP	1.9 ± 0.4	180.7 ± 81.2	2.8 ± 0.8	260.2 ± 81.7

[1]μ ± S.E.; fmol/ml/10 min; [2]100 μg/ml.

Two depolarizing agents (K^+ and veratridine) were also observed to increase the release of CCK and VIP. Using a Sephadex G-50 superfine gel filtration column, the VIP-like immunoreactivity obtained from tissue extracts and the cortical and ventricular release samples revealed that all activity traveled in a single peak which co-migrated with authentic VIP-28. For CCK, the immunoreactive species in tissue co-migrated with 8, 33, and 4 amino acid peptide fragments. However, in the release sample, the activity migrated with the peak corresponding primarily to CCK-8 (with only a trace of CCK-4). These studies suggest that in vivo tissue stores of VIP and CCK can be released by pharmacological agents and their simultaneous release can be studied. (Supported by NS 16541 (TLY) and AM 20973 (VLWG).)

197.9 SIMULTANEOUS MEASUREMENT OF VIP AND CCK RELEASED FROM RAT AND CAT CORTICAL SLICES. P. Micevych, V.L.W. Go[1] and T. Yaksh, Depts. of Neurosurgery and [1]Gastroenterology, Mayo Fdn., Rochester, MN 55905.

Vasoactive intestinal polypeptide (VIP) and cholecystokinin (CCK) are polypeptides which were originally isolated from the porcine gut and subsequently have been shown to have a central nervous system distribution. The highest concentrations of CCK and VIP have been shown to be in the cerebral cortex. We examined the release of CCK and VIP, measured by radioimmunoassay (RIA) in the same sample, to compare the release of these peptides in response to a depolarizing stimuli. Both rat and cat cerebral cortical slices were studied to determine whether any species differences could be observed. In both preparations approximately 20 mg of cortical tissue was superfused with a modified Krebs-Ringer bicarbonated buffer at 37°C. The buffer continued 0.3 g/L bacitracin, 0.15 g/L albumin and 2 g/L dextrose. The buffer was continually oxygenated and superfused at a rate of 300 µL/min. Samples were collected every 10 min for a total of 50 min. The tissues were stimulated with potassium and veratridine to evoke CCK and VIP release. In the rat, spontaneous release of VIP was determined to be 2.25 fmoles/min/ml/mg while CCK simultaneously measured was 9.4 fmoles/min/ml/mg tissue. High potassium, 50 mM, elevated the release of VIP 2.4 fold to 9 fmole/min/ml/mg tissue and CCK rose 5.6 fold to 52.7 fmol/min/ml/mg tissue. The molar ratios (CCK:VIP) for both the spontaneous and evoked release were similar (basal was 4.2 and evoked was 5.8). The profile of CCK and VIP release from the cat cortex paralleled the rat release data. Removal of calcium from the superfusion buffer abolished the K^+ evoked release of VIP and CCK but had no effect on spontaneous efflux. Gel filtration column chromatography (Sephadex G-50 superfine) of rat tissue showed that the VIP immunoreactivity co-migrated with authentic VIP-28 while the CCK immunoreactivity is isographic primarily with CCK-8 though some activity appeared in the eluate containing the CCK-4 standard. These results are consistent with gel filtration chromatography of cortical tissue extracts which suggest that the primary molecular species in rat cortical tissue are the VIP-28 and CCK-8 peptides. These results are consistent with the notion that VIP and CCK may be cortical transmitters in cat and rat. Interestingly, although the tissue levels of VIP are high in rat and cat cortex the amount of released VIP measured in superfusates is significantly lower than released CCK in the same superfusates. These data may indicate that CCK although in lower concentrations in the cortex is more easily releasable in response to a depolarizing stimulus and thus may have an equally significant effect on cerebral cortical transmission as does VIP. (Supported by NS 16541 (TY) and the Mayo Foundation.)

197.10 ACTH INTERACTION WITH OPIATES AND ENDORPHINS IN VITRO. T. Smock*, H.L. Fields, M. Grossman*, S. Kansky* and J. Ramachandran*. Depts. Physiology and Neurology, and Hormone Research Lab., Univ. of California, San Francisco, San Francisco, CA 94143.

ACTH (one microgram intraventricularly) is capable of blocking analgesia produced by morphine or β-endorphin (Smock and Fields, Brain Res., in press). Similarly, small amounts of ACTH (1-24) oppose opiate effects on spinal nociceptive reflexes and on nociceptive neurons in the brainstem. Since ACTH binds weakly to the aggregate of opiate receptors in the brain (Akil et al, Eur. J. Pharmac. 64:1-8, 1980) the apparent antagonism could be mediated by direct competition for some subset of binding sites. In an initial test of this possibility, we examined ACTH effects on a number of model opiate receptor preparations.

Morphine effects on the guinea pig ileum (GPI, µ-receptor, ID50 41-44 nM) were not significantly affected by ACTH (1-24) at concentrations of up to 1 µM (ID50 in presence of ACTH 40-70 nM, n=5). ACTH had no independent effect on GPI twitch tension. Likewise, β-endorphin effects on the mouse vas deferens (δ-receptor) and rat vas deferens (ε-receptor) were unaffected by ACTH in a wide range of concentrations.

In the rat vas deferens, morphine enhances twitch tension in contrast to β-endorphin which is inhibitory (Huidobro et al, Br. J. Pharmac. 70:519-25, 1980). In our hands, the excitatory effect of morphine on the rat vas deferens was variable, obtainable only at high concentrations (ED50 48-106 µM) and was markedly attenuated by ACTH when the two were applied in molar ratios similar to those used in the rat brain to prevent opiate analgesia. Examination of ACTH effects alone revealed that the apparent antagonism resulted from opposing actions rather than competition. ACTH (1-24) inhibited the neuromuscular preparation independently (maximum effect: 32% inhibition, dose at half-maximum inhibition: 7-11 nM). The effect was shared by ACTH (1-39), α-MSH and synthetic fragments that included the ACTH sequence (4-10), but was not produced by ACTH (7-38) or by poly-lysine. Neither the morphine effects nor the ACTH effects were naloxone-reversible.

It was concluded that ACTH/opiate antagonism in the rat brain is probably not due to direct competition for an opiate receptor but may instead be due to independent interactions similar to those obtained for morphine and ACTH in the rat vas deferens.

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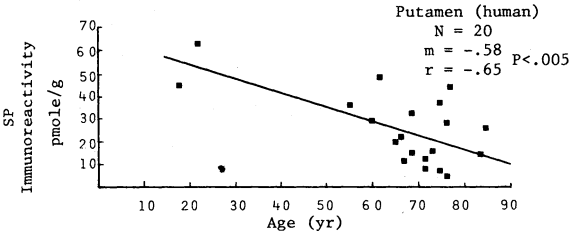
197.11 SUBSTANCE P LEVELS IN THE MAMMALIAN CNS IN AGING. S.H. Buck, H. I. Yamamura, T.F. Burks, and M.N. Rossor*. Dept. of Pharmacology, University of Arizona College of Medicine, Tucson, AZ 85724 and MRC Neurochemical Pharmacology Unit, Cambridge, England CB2 2QD.

Neurochemical changes that occur in the CNS of laboratory animals in aging include reductions in levels of certain neurotransmitters, reductions in activity of synthetic enzymes for these neurotransmitters, and declines in number of receptors for some transmitters. Similar decreases in neurotransmitter levels and in synthetic enzyme activities have also been observed in the human CNS in aging. Age-related changes in CNS neuropeptides have not been investigated in animals or in humans. We have obtained preliminary evidence of an age-related decline in human basal ganglia levels of substance P (SP) that does not occur in rodents.

SP levels were determined in acid extracts of tissues by RIA using an antiserum with cross-reactivity of 1% with physalaemin, 0.01% with eledoisin, and less than 0.00001% with each of 15 other neuropeptides. Extraction of SP was quantitative and assay of varying dilutions of samples revealed curves that were parallel to the standard curve.

In Fischer 344 rats 3, 10, or 27 months old (from NIA colony), there were no differences among the age groups in SP levels in frontal cortex, limbic system, corpus striatum, substantia nigra, hypothalamus, brainstem, or cerebellum. In post-mortem tissues from humans, there was no correlation of SP levels with age in frontal cortex, thalamus, or hypothalamus. In putamen, however, there was a significant age-related decrease in SP levels of about 6 pmole/g tissue/decade. Conclusive determination has not yet been made in human caudate or nigra.

These results suggest that there may be differences between rats and humans in aging in basal ganglia and that reductions in SP levels may contribute to human basal ganglia dysfunction in aging. (Supported by USPHS grants and by an RSDA to H.I.Y.)



197.12 POLYPEPTIDES AS STOCHASTIC LIGANDS. A. J. Mandell, P. V. Russo* and S. Knapp. Dept. of Psychiatry, University of California at San Diego, La Jolla, CA 92093

The presence of polypeptides with neurochemical, physiological, and behavioral activity in biogenic amine neurons is well established. Their role, thought to be regulatory, has not been mechanistically explicated.

Recent in vitro studies of time-dependent fluctuations in the activities of rat striatal tyrosine hydroxylase and rat raphe tryptophan hydroxylase, as well as in crude striatal membrane ^{3}H-spiroperidol binding, have demonstrated stochastic properties that are sensitive to polypeptide hormones as reflected by such ligand-sensitive indicators as moments, autocorrelation length, probability and spectral densities, and characteristic exponents.

TRH and its methyl analogues, somatostatin, and other polypeptide ligands appear to change the stability (sensitivity) of enzymatic and binding processes. Such statistical mechanical phenomena as randomization, coupling, and bifurcation among populations of dissipative, nonlinear protein oscillators may partially determine the responsivity of the system to other regulatory influences. The more diffusely circulating polypeptides may be long-range regulators, as more local neurotransmitters and ions are short-range regulators.

This research is supported by DA-00265-09.

197.13 PARTIAL CHARACTERIZATION OF SOMATOSTATIN-LIKE MATERIAL IN THE BOVINE ADRENAL MEDULLA. A. Baird*, R. Benoit*, N. Ling*, P. Böhlen* and R. Guillemin, Laboratories for Neuroendocrinology, The Salk Institute, La Jolla, CA. 92037.

Immunohistochemical methods have demonstrated the presence of somatostatin-like immunoreactivity (SLI) in cells derived from the embryonic neural crest. In this report we present the detection of SLI in extracts of bovine adrenal medulla (BAM) and its partial characterization by gel permeation and reverse-phase high-performance liquid chromatography (HPLC).

350 BAM (950 g) were extracted in acid, defatted and pumped through a 7 x 25 cm column of octadecasilylsilica (ODS, Böhlen et al., 1981), the material retained on the ODS column was eluted with 60% propanol in 0.36 M pyridine formate, pH.3, lyophilized and 3.0 g applied to a Sephadex G-50 column. The amount of SLI in the ODS-retained fraction and the column effluent was monitored using an antibody generated against somatostatin (SS-14) which reads SS-14 and N-terminally extended somatostatin (SS-28) in an equimolar ratio. The ODS-retained material contained $\geq$5 ng of SLI per BAM, which, after G-50 chromatography, was resolved into three distinct forms. The greater part of the SLI (75%) eluted in a zone compatible with a peptide of 1.2k Daltons, while the remaining SLI was divided between a zone compatible with 2.5k Daltons and another located in the void volume (MW>10k). The fractions corresponding to the 1.2k and 2.5k Dalton zones of SLI were pooled and further analyzed by HPLC. No SLI was found in a similar extract of bovine adrenal cortex.

Our data suggest that there are three forms of SLI in the bovine adrenal medulla, but none in the adrenal cortex. Gel filtration indicates that these forms are consistent with the existence of SS-14- and SS-28-like peptides, as well as a putative precursor of higher molecular weight. Chemical and biological characterization of the two smaller SLI activities is now in progress.

This work was supported by grants from the NIH (grant numbers AM-18811-006 and HD-09690-006), the Kleberg Foundation, the M.R.C. and the C.R.S.Q.

198.1 MORPHINE DIFFERENTIALLY AFFECTS ICSS INTERACTIONS BETWEEN THE LOCUS COERULEUS AND THE MEDIAL FOREBRAIN BUNDLE. F. Gimino*, F. Villegas*, R. Alvarez*, J. Abreu*, M. Sano*, S. Steiner, S. Ellman*. Behavioral Physiology Lab., City College of New York, New York, N.Y. 10031.

The C-T monophasic pulse pair technique can be employed to produce behavioral interactions between brain loci. In this technique response rates elicited with simultaneous or near simultaneous stimulation of two ICSS sites can be shown to be significantly greater than the sum of the response rates elicited from each site stimulated individually.

12 albino Sprague-Dawley rats were implanted with electrodes aimed at Locus Coeruleus (LC) and medial forebrain bundle (MFB). Animals were shaped to respond for monophasic rectangular pulse pairs at a variety of current intensities.

In one condition, the C pulse was delivered to the LC at its within site intensity, and the T pulse was delivered to the MFB. In the reverse condition, the C pulse was delivered to the MFB at its within site intensity and the T pulse was delivered to the LC.

After completion of this procedure, animals were then administered subcutaneous injections of saline for 3 days followed by 6 days of 1.25mg/kg of morphine sulfate according to a counterbalanced design. The effects of these injections were observed on the response rates of animals in their within site condition. All subjects then received one injection day of naloxone HCL at 1.0mg/kg and morphine sulphate at 1.25mg/kg. Animals then received 3 more injection days of saline followed by a four day sequence of D and L amphetamine.

Subjects were then returned to baseline control rates of responding during a six day injection series of saline during which pulses were again "split" between electrode loci. The effects of morphine sulfate and D and L amphetamine were observed in this split pulse condition.

Two patterns of drug responsivity have been delineated on these test interactions. Animals demonstrating bidirectional interactions between the LC and MFB were also more responsive to d-amphetamine and evidenced morphine induced rate facilitations in both directions. Unidirectional animals displayed either an L or D-amphetamine sensitivity dependent upon the location of the C-pulse in either the MFB or the LC. These Ss were more responsive to morphine and when the interaction was D-responsive. Thus, the C-T "split-pulse" technique allows for the differentiation of interacting ICSS networks.

198.2 MORPHINE AND LOCUS COERULEUS LESION EFFECTS ON ICSS ARE SITE SPECIFIC. A. Tempel, S.J. Ellman*, C. Pavlides*, D. Ocheret*, S. Berman* and S.S. Steiner. Behavioral Physiology Lab., Dept. Psych., The City College of New York, New York, N.Y. 10031.

ICSS has been discretely localized within the CNS. Neuroanatomical and immunohistochemical studies have demonstrated that both opiate receptor sites and opioid peptides are discretely localized within the CNS. Therefore, it seemed likely that morphine would act at some discrete brain loci and not others. The following study was aimed at determining if morphine acts differentially at specific neuroanatomical loci to facilitate ICSS.

Rats were stereotaxically implanted with 2 pairs of bipolar stainless steel electrodes, aimed at various combinations of the following sites: fields of forel (ff), crus cerebri (cc), medial forebrain bundle (mfb), mammillothalamic tract (MT), zona incerta (ZI), and anteroventral thalamus (Thl). Stimulation consisted of monophasic rectangular pulse pairs. Animals received 7 days saline, 7 days morphine, 1 day morphine + naloxone, and 6 days saline. Acute, unilateral locus coeruleus (LC) lesions were then made in all animals. Following the lesion, animals were run for 21 days of saline to allow for complete degeneration. ICSS drug paradigms post-lesion were identical to the pre-lesion paradigm.

Electrodes localized in the MT, FF, MFB, CC and ZI showed an ICSS response rate facilitation under morphine administration. These facilitations were naloxone reversible. Although the thalamic electrodes supported ICSS behavior, morphine did not produce any alteration in ICSS response rates.

ICSS from electrodes located in the mammillothalamic tract and thalamus were unaffected by the LC lesion.

These results suggest that it is possible to use the lesion technique and pharmacological tools to delineate anatomical loci of reward.

198.3 ENDOGENOUS OPIOIDS & PLEASUREABLE BEHAVIOR

Angello de la Sierra, University of Puerto Rico Medical Sciences and Cayey Campus, Puerto Rico

Some years ago we observed that rats tolerated pain while self stimulating the medial forebrain bundle (MFB) pleasure area. Subsequently we measured the thresholds to foot shocks in both MFB and rats evidencing aversion after stimulation to the reticular area (RET). The results led us to assume that pleasurable behavior (increased rate of MFB stimulation) and analgesia (increased threshold to foot shock) shared the same neuronal mechanisms. Their opposites, ie, aversion with pain, shared also same neuronal mechanisms. When we injected Naloxone (2 mg/kg,IP) to MFB and RET groups, the former were no longer protected from pain while the latter's threshold to foot current was lowered. Unexpectedly, MFB-naloxone group explored their environment more actively and increased their H_2O and food intake. When ethanol was substituted for H_2O, consumatory behavior increased in proportion to Naloxone levels. MFB-naloxone groups learned faster to obtain concealed food but preferred many food-seeking trips than a prolonged single feeding. Data suggests that (1) high endorphin levels in obesity may be coincidental (2) ethanol may substitute for morphine and viceversa in the therapy of withdrawal symptoms of either one, (3) Naloxone may be useful in the management of amnesia, apathy or decreased learning performance. Data also suggests that (1) consumatory behavior may be more related to metabolic needs than to pleasure, (2) learning may be unrelated to pleasurable reinforcements.

198.4 ATTENUATION OF INTRAVENOUS HEROIN REWARD BY VENTRAL TEGMENTAL OPIATE RECEPTOR BLOCKADE. M.D. Britt* and R. A. Wise. Center for Research on Drug Dependence, Department of Psychology, Concordia University, Montreal, P.Q., Canada H3G 1M8.

Rats self-administering opiates will normally maintain a relatively constant rate of responding, but will increase their response rate if the dose per infusion is decreased or if opiate antagonists are administered in low doses (J.R. Weeks and R.J. Collins, Psychopharmacologia, 6, 267, 1964). This compensatory increase in self-administration is indicative of a functional decrease in the reward value of each infusion. The present experiment was designed to localize the site of rewarding opiate action by microinjecting opiate antagonists into various regions of rats involved in intravenous heroin self-administration. The hydrophilic opiate antagonist diallyl-normorphinium bromide (DNMB) was used for microinjections because the more commonly used antagonists are highly lipophilic and spread rapidly throughout the brain. In pilot studies microinjections of naloxone (5 µg) into any brain region tested resulted in compensatory increases in responding for heroin.

Rats were prepared with chronic jugular catheters and bilateral intracranial cannulae into either the nucleus accumbens (NAS), the corpus striatum (CS) or the ventral tegmental area (VTA). Following recovery the rats were allowed to lever press for heroin (50 µg/kg/infusion) for 4h daily. Microinjections of DNMB (2.5, 1.25 0.63 µg/0.5 µl saline) were given in a descending and ascending sequence so that each dose was tested twice in each animal. The injections of DNMB were given every other day at 1h into the session. On alternate days sham or vehicle injections were given for control comparisons. Systemic naloxone was also tested (0.1 mg/kg, i.p.) before and after the microinjection series.

All rats showed increased response rates for the hour following i.p. naloxone. Intracranial DNMB caused response accelerations only when injected at the higher dose and only when injected into the ventral tegmental area. Thus the reward-attenuating effects of systemic naloxone are mimicked by central DNMB when injected into the opiate receptor field of the VTA, but not when injected into the receptor fields of NAS or CS. These data fit well with reports of morphine self-administration into the ventral tegmental area but not other regions (M.A. Bozarth and R.A. Wise, Soc. Neurosci. Abstr., 6, 309, 1980) and suggest the VTA as the site of rewarding opiate action in the brain.

This work was supported by a NIDA postdoctoral fellowship to M.D.B. and by the Medical Research Council of Canada.

198.5 DIFFERENTIAL AVERSIVE EFFECTS OF HEROIN AND MORPHINE. L. Switzman*, T. Hunt*, M. Sossanpour* and Z. Amit. Center for Research on Drug Dependence, Concordia University, Montreal, Canada H3G 1M8.

In the conditioned taste aversion (CTA) paradigm ingestion of a novel flavor followed by morphine results in subsequent avoidance of the novel flavor by rats, indicating aversive stimulus properties of the drug. In a CTA experiment (single pairing, one-bottle test), six doses of heroin (.5 - 12.0 mg/kg, ip) and the Ringer's vehicle were tested for aversive effects. Heroin did not induce a significant CTA at any of the doses tested. In a second CTA experiment, three doses of morphine (4 - 12 mg/kg, ip) and the Ringer's vehicle were tested in an identical paradigm to that previously implemented. Morphine induced a significant CTA at two of the doses tested (8 and 12 mg/kg). A third experiment was carried out to determine the relative analgesic effectiveness of non-aversive heroin doses and aversive morphine doses. Using the hot-plate procedure drug- or Ringer's-injected rats were subjected to 4 repeated test trials. The dependent measure was paw lick latency. The heroin doses tested (2 and 4 mg/kg) resulted in analgesia equal to or greater than the morphine doses tested (8 and 12 mg/kg). Therefore, although heroin is more potent than morphine as an analgesic, heroin is less potent than morphine as a CTA-inducing agent. In a fourth experiment we attempted to maximize conditions to enable heroin to induce a CTA. Three doses of heroin (3, 6 and 9 mg/kg, ip) were paired with a novel saccharin solution. Five such pairings occurred with two water days intervening between each pairing day. On the third day following the final pairing the rats were presented with a choice of water or saccharin. Three additional heroin groups were run with the same procedure but each group received 3 injections spaced 20 min apart (0, 1, 2 and 3 mg/kg/injection) on each pairing day. Heroin still did not induce a CTA. The inefficiency of heroin as an aversive agent is discussed in terms of differing stimulus properties from those of morphine.

198.6 EFFECT OF SYSTEMIC MORPHINE ON CORTICALLY-EVOKED UNIT ACTIVITY IN THE CAUDATE NUCLEUS. T.C. Napier, J.H. Pirch and S.L. Peterson. Department of Pharmacology and Therapeutics, Texas Tech University Health Sciences Center, Lubbock, TX 79430.

The caudate nucleus is well endowed with opiate receptors and is also known to receive extensive cortical inputs. The ability of morphine to alter cortically-evoked caudate unit activity was investigated in this study.

Sixteen gauge guide cannulas were permanently implanted directly above the caudate nucleus, and three bipolar stimulating electrodes were embedded in the frontal and motor cortex in male Sprague-Dawley rats. After recovery from surgery the animals were lightly anesthetized with sodium phenobarbital and replaced in the stereotaxic instrument, with the earbars inserted into implant earbar holders to circumvent pain produced by insertion into the auditory canal. A tungsten microelectrode was lowered through the guide cannula and a spontaneously active caudate neuron was located. The spontaneous activity was monitored throughout the experimental period. Monophasic square wave pulses (.25-5 mA; .3 msec; 1 Hz) were used for cortical stimulation. The optimal cortical site and threshold current necessary for driving the unit were determined. Under these experimental conditions, cortical stimulation typically evoked a response of approximately 10 msec latency. However, the latency varied according to the cortical site being stimulated. In those cells with sufficiently high rates of activity this evoked response was often followed by an inhibitory phase, usually lasting approximately 100-250 msec before returning to the control spontaneous rate. Morphine sulfate, in cummulative doses of 1,5,10,20 and 30 mg/kg, was injected at ten minute intervals via a tail vein cannula. Naloxone (1 mg/kg iv) was given at the end of the experiment.

Less than 50% of the units demonstrated a morphine-induced depression of firing rate which was antagonized by naloxone. The majority of cortically-evoked responses were also unaffected by the doses of morphine used in this study. If the corticostriatal pathway plays a role in the actions of systemic morphine, then only a small portion of caudate cells which respond to cortical stimulation are involved. (Supported by USPHS BRSG RR05773 and MH29653 and by the Tarbox Parkinson's Disease Institute at Texas Tech University Health Sciences Center.)

198.7 EFFECTS OF MORPHINE AND NALOXONE ON FETAL RAT SPONTANEOUS ACTIVITY AFTER CHRONIC OPIATE EXPOSURE. M. L. Kirby and S. G. Holtzman*. Dept of Anatomy, Med. Col. of Georgia, Augusta GA 30912 and Dept. of Pharmacology, Emory Univ., Atlanta GA 30332.

Chronic maternal morphine administration during gestation causes changes in the offspring, of postnatal behavior and responsiveness to opiates. Acute opiate administration during late gestation causes a dose-related decrease in fetal spontaneous activity; however, it is not known what effect chronic opiate administration has on fetal response to morphine and naloxone. In the present study, pregnant Wistar rats were injected s.c. 4 times daily with 5 mg/kg/injection of morphine or 0.8 mg/kg/injection of levorphanol or dextrorphan (an equipotent dose) or 2 times daily with 10 mg/kg/injection of morphine. Control animals were injected with saline. A final group of pregnant animals was pairfed with the morphine-injected animals. Injections and pair feeding were initiated on day 12 of gestation. On gestation days 18-20 the effects of varying doses of morphine and naloxone on fetal activity in the pretreated animals were assessed by visual observation and quantification as reported previously. When challenged with varying doses of morphine, fetuses exposed twice daily to morphine showed a depression in spontaneous activity similar to animals pretreated with saline or dextrorphan or pairfed. Animals injected 4 times daily with morphine showed tolerance to the depressant effect of morphine. Levorphanol-treated animals were not tolerant to the depressant effect of morphine. All of the animals exposed chronically to morphine or levorphanol were hyperactive after challenge with naloxone while animals injected with saline or dextrorphan or pairfed were not responsive to naloxone. Even though these changes in responsiveness to morphine and naloxone were present in utero following chronic exposure to morphine, 30-day neonates which had been treated identically prenatally did not show changes in the analgetic response to morphine in the hotplate test. Supported by NIH Grant DA 02060.

198.8 DEXAMETHASONE EFFECT ON COLD-INDUCED AND MORPHINE-INDUCED HYPOTHERMIA IN RESTRAINED RATS. P.R. Marques* and J.N. McDougal (SPON: G.W. Van Hoesen) Dept. Pharmacology, Univ. Arizona Health Sciences Center, Tucson, AZ 85724.

The effect of morphine on thermoregulation in the rat is influenced by dose and restraint. Low doses (1-5 mg/kg) elevate the rectal temperature (Tr) of restrained and unrestrained rats whereas 25 mg/kg and above decrease the Tr of restrained rats. Unrestrained rats show less hypothermia at these higher doses. An hypothesis that restraint stress potentiates or entirely accounts for the morphine hypothermia at high doses was supported by studies in which dexamethasone (Dxm) pretreatment before 30 mg/kg morphine reversed the hypothermia. The assumption was that Dxm, via negative feedback inhibition of corticotropin and endorphin release from the pituitary prevented the stress hypothermia. We hypothesized that Dxm reversal or attenuation of morphine hypothermia is a consequence of the direct action of Dxm and related corticosteroids to support metabolic heat production. The Tr of Sprague-Dawley derived rats was monitored at 15 min intervals for 1 hr before and up to 4 hr after treatment. Rats pretreated with Dxm, (400 µg/kg at 24 hr and 200 µg/kg at 2 hr prior to 30 mg/kg morphine) and installed in a metal restrainer, returned Tr toward basal 1.25 hr earlier than saline pretreated rats given 30 mg/kg morphine (S-M). Control groups receiving saline instead of morphine (D-S, S-S) did not differ significantly from each other or from basal. Further studies in which the Dxm dose was doubled and the morphine dose halved enhanced separation of the curves. In this case, group D-M reached a Tr minimum of 3.68 $\pm$ 0.51 C below basal at 2 hr. Group S-M rats did not reverse the Tr decline until 4.80 $\pm$ 0.21 C. Significant group differences persisted throughout the 2.5-4.0 hr period of measurement. Exposure of non-morphinized rats to a low Ta (6 C) resulted in a similar rate of heat loss for both Dxm (400 + 200 µg/kg) pretreated and saline pretreated rats during the first hr. Dxm rats' Tr stopped the decline at 1.25 hr and at 1.75 C below basal; control rats lost heat for 2.5 hr to a 2.33 $\pm$ 1.0 C drop from basal. Sustained significant differences between groups emerged at 3 hours. These data are consistent with our hypothesis that Dxm attenuation of morphine hypothermia is secondary to increased substrate availability for thermogenesis. Enhanced morphine hypothermia in restrained vs unrestrained rats is more parsimoniously explained by postural factors such as increased body surface exposure and inability to adjust fur loft for insulation in restrained animals. Supported by USPHS grant NS15420.

198.9 PUPILLARY DILATION AFTER MICROINJECTIONS OF MORPHINE, NORMORPHINE AND CLONIDINE INTO THE EDINGER-WESTPHAL NUCLEUS (EW) IN THE CAT. Lawrence G. Sharpe and Wallace B. Pickworth. NIDA Addiction Research Center, Lexington, Kentucky 40583.

When administered peripherally, morphine and clonidine produce pupillary dilation in the cat but constriction in the dog. In the dog, these drugs appear to produce miosis through a central mechanism which activates parasympathetic input to the iris (Lee and Wang, JPET 192, 415-431, 1975; Sharpe, Pickworth and Martin SN, #967, 1977). In the cat, different sites have been proposed for the mydriatic actions of morphine and clonidine. Morphine may act peripherally to increase sympathetic tone (Wallenstein and Wang, Am. J. Physiol. 236, R292-R296, 1979), whereas clonidine may act centrally to inhibit parasympathetic tone to the iris (Koss, Eur. J. Pharmacol. 55, 305-310, 1979). Our purpose was to further assess the central site of these mydriasis-producing drugs in the cat. Morphine, normorphine, and clonidine (0.5 µl solutions) were microinjected via chronic indwelling cannulae into a region of the EW that yielded pupilloconstriction to electrical stimulation. Pupillary diameter was measured photographically in the awake cat acclimated to a sling restraint. All three drugs (3-100 nmol) produced dose-dependent pupillary dilation which began within 5 min and lasted throughout the 1-hr test period. The order of potency was clonidine > normorphine > morphine. Mydriasis did not occur if these drugs were microinjected in adjacent EW sites not yielding pupilloconstriction to electrical stimulation. We conclude from this data that the parasympathetic preganglionic cell groups controlling pupillary diameter may be the region from which peripherally administered morphine and clonidine act to produce mydriasis in the cat. These drugs may inhibit, either directly or indirectly, parasympathetic tone to the iris. Since the dog becomes miotic after microinjections of opioids and clonidine in the EW (Sharpe et al. SN, 1977) we further conclude that the neurochemical and/or neuroanatomical nature of this region may differ in the dog and cat.

198.10 THE OPIATE ANTAGONIST NALOXONE REVERSES THE ISCHEMIC NEUROLOGIC DEFICIT PRODUCED BY UNILATERAL CAROTID LIGATION IN GERBILS. Yoshio Hosobuchi,* David S. Baskin,* Sidney Woo* and Horace H. Loh* (SPON: N. M. Lee). Departments of Neurological Surgery and Pharmacology, School of Medicine, University of California, San Francisco, CA 94143.

Because of anatomic anomalies commonly found in the circle of Willis, homolateral ischemic brain damage and a consequent neurologic deficit (stroke) are produced in 30 to 50% of gerbils that undergo unilateral common carotid ligation. Under pentobarbital anesthesia (40 mg/kg), the right common carotid artery was occluded in 70 adult gerbils. Within 4 hours, 23 of them developed stroke (2 died within 4 hours) (Group A); 47 gerbils that were ligated did not develop stroke (Group B). In 14 of 15 Group A gerbils, 1 mg/kg of naloxone injected i.p. reversed the neurologic deficit within 2 to 5 minutes; 1 gerbil required an additional 1 mg/kg of naloxone to obtain reversal. Typically, reversal lasted 20 to 30 minutes, after which the neurologic deficit returned. However, reversal could be obtained repeatedly by injection of naloxone. Eight of 11 Group B gerbils injected i.p. with 15 mg/kg of morphine developed naloxone-reversible stroke within 10 minutes. Naloxone-reversible stroke was produced in the other 3 gerbils by i.p. injection of an additional 15 mg/kg of morphine. The stereoisomeric opiate agonist levorphanol (5 mg/kg i.p.) was given to 10 Group B gerbils, 8 of which developed mild stroke symptoms that were reversed by naloxone. Dextrophan (15 mg/kg), the enantiomer of levorphanol, was given i.p. to 10 Group B gerbils, none of which developed stroke. The delta receptor-specific enkephalin analogue Sandoz #FK33824 was given i.p. to 10 Group B gerbils at a dose of 15 mg/kg, which is 80 times the mouse ED_{50} analgesic dose. None developed stroke, although all were totally analgesic over 24 hours.

Naloxone reversal of stroke induced by cerebral ischemia is a novel observation. Our results strongly suggest that the action of opiates and naloxone on this neurologic deficit are stereospecific and mu receptor-specific. Recent findings of naloxone reversal of neurologic deficit caused by cerebral ischemia in a small group of patients are in accord with these findings in gerbils.

198.11 INTERACTIONS OF MIDBRAIN AND BULBAR OPIATE ANALGESIA SUBSTRATES IN RAPHE MAGNUS. J. Peter Rosenfeld, M. Heinricher* and C. McWilliams*. Depts. of Neurobiology & Physiology, and Psychology Cresap Neuroscience Laboratory, Northwestern University, Evanston, Ill 60201.

Morphine was microcannulated into periaqueductal gray (PAG: .7µg) and nucleus reticularis paragigantocellularis (PGC: .35µg in separate doses as well as in combination. Effects of combined injections of PAG and PGC doubled the baseline (behavioral) facial thermal-nociceptive threshold. The single injections raised the threshold to 140% of baseline. Combined injections of left and right PGCs had a similar effect.

The electrophysiological substrate of these interactions was studied by obtaining effects of morphine and fentanyl, microinjected into PAG and PGC, on single neuronal firing rates recorded in Nucleus Raphe Magnus (NRM) of urethene-anaesthetized rats. As of the date of submission of this abstract, **19** cells in NRM (baseline firing rates = 5-50 Hz) have been studied with the following results: 17 responded to nociceptive pinch with increased firing rate, 1 with decrease. In 13, .35µg (n=2) to 1µg (n=10) of morphine sulphate in PGC produced either excitation (n=2), inhibition (n=7), or no effect (n=4). In 6 other cells, PAG (.7µg) as well as PGC (.35µg) were both injected with morphine. Results: There were 3 cells in which conjoint effects were suggested: In 1 cell PGC injection led to inhibition, whereas PAG injection later led to excitation. In another cell, PGC injection produced excitation followed by PAG-produced inhibition. In the 3rd, PAG injection produced a mild inhibition followed by a profound PGC-produced inhibition, totally reversible with IP naloxone, 1 mg/kg. Of the 3 remaining cells, 2 were first PGC-injected with no effect, followed by PAG-produced excitation in one case, and no PAG effect in the other. Finally 1 cell was PAG-injected with no effect, then depressed by PGC.

198.12 EVIDENCE THAT THE ANALGESIC EFFECT OF MORPHINE MICROINJECTED INTO THE 4TH VENTRICLE INVOLVES AN ENDOGENOUS OPIOID LINK AT THE LEVEL OF THE SPINAL CORD. Steven R. Lane, Jon D. Levine*, Newton C. Gordon* and Howard L. Fields. UCSF, Depts. Neurol. and Oral Surgery, San Francisco, CA 94143.

Microinjection studies demonstrate that morphine can produce profound analgesia when injected at discrete sites within the CNS, including the periaqueductal gray, the ventromedial medulla and the spinal cord. These results can be explained by postulating that morphine activates an endogenous opioid mediated analgesia system (EOMAS). Evidence has accumulated that EOMAS exists and acts in part via enkephalinergic neurons with terminals in the superficial layers of the dorsal horn (Basbaum & Fields, Ann Neurol, 4:451, '78). The following studies were designed to study the dynamic interactions between spinal and supraspinal opiate sensitive sites in the production of morphine analgesia.

Fifty, 250-350g, male Sprague Dawley rats were chronically implanted with PE-10 tubing in the lumbar subarachnoid space. In addition, 4th ventricular guide cannulae were placed on the midline, 2mm posterior to the interaural line (15° head-tilt). Morphine, naloxone or vehicle were isovolumetrically injected either intracerebroventricularly (ICV) or through the spinal (SP) cannula. Analgesia was assessed in unanesthetized rats by measuring both the tail flick (TF) latency (3.5" baseline, 20" cut-off) and the threshold for "pain"-induced vocalization (PIV) produced by electrical stimulation of the base of the tail (1msec pulses, 100Hz for 200msec).

Results were comparable using either method of assessing analgesia. Microinjection of 15 µg of morphine either SP or ICV produced only partial analgesia. ICV injection consistently produced greater analgesia than SP. Simultaneous injection at both sites produced analgesia as strong as 10mg/kg systemic morphine (100% MPE on the TF). Ten µg SP naloxone partially reversed an established analgesic effect of 15 µg ICV morphine. The same dose of SP naloxone injected 15' prior to ICV morphine completely blocked the development of analgesia (n=6). Thus SP naloxone is much more effective in preventing the effect of ICV morphine than in reversing it once it is established.

Thus, ICV morphine produces more profound analgesia than SP morphine at a dose of 15 µg; ICV morphine can be reversed by SP naloxone; and SP naloxone is more effective in pre-blocking than in reversing ICV morphine effects.

These results are consistent with an interaction between spinal and supraspinal opiate sensitive sites in the analgesia produced by systemic morphine and supports the hypothesis that supraspinal morphine activates a descending system with an opioid link at the spinal cord level.

199.1 NON-OVERLAPPING PROJECTIONS OF MUSCLE AND CUTANEOUS AFFERENTS IN THE BRACHIAL SPINAL CORD OF ADULT BULLFROGS. S. Jhaveri* and E. Frank. Department of Neurobiology, Harvard Medical School, Boston, MA 02115.

The peripheral ends of primary sensory axons in the 2nd. spinal nerve of bullfrogs innervate the skin and muscle of the forelimb. The central projections of these sensory axons, as revealed by labelling the entire 2nd. nerve with horseradish peroxidase, terminate in two distinct areas in the gray matter of the brachial spinal cord: the dorsal and ventral neuropil regions. The contribution of muscle or cutaneous sensory fibers to these two neuropils was investigated by selective labelling of a single muscle or cutaneous nerve branch with horseradish peroxidase.

Individual branches of the 2nd. spinal nerve, innervating forelimb muscles or skin, were placed in a cuff containing horseradish peroxidase and lysolecithin. Following a 2-week survival period, the spinal cord was fixed using an aldehyde fixative, and processed for the visualization of the horseradish peroxidase reaction product, with 3,3',5,5'-tetramethyl benzidine as a chromogen. The muscle nerves studied incluced the medial, internal and external branches of the triceps nerve. Sensory afferents from these nerves projected exclusively to the ventral neuropil region. On the other hand, when the nerves backfilled were cutaneous nerves, such as the cutaneous branches of the ulnar or radial nerves, the labelled sensory afferents were observed to terminate only in the dorsal neuropil region of the spinal cord.

Thus, primary sensory afferents carrying information about two different classes of sensory modalities project to discrete areas of the spinal cord gray matter.

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199.2 Transganglionic transport of WGA/HRP conjugate in the upper cervical cord of the cat. V.C. Abrahams, Dawne Downey*, Oriette Morris*, and Frances J. Richmond, Department of Physiology, Queen's University, Kingston, Ontario. K7L 3N6

We have used Wheat germ agglutinin/HRP (WGA/HRP) conjugates to trace the spinal and medullary destination of primary afferents from large dorsal neck muscles in the cat. These experiments have shed some light on the pathways followed by neck muscle afferents, but also raise questions about the axonal transport of WGA/HRP conjugates. The central cut end of C2 and C3 cat neck muscle nerves were exposed for 90-240 minutes to a concentrated solution of WGA/HRP freshly made from lyophilized powder (supplied by E-Y laboratories). After 2-4 days, the animals were anaesthetized and perfused with modified Karnovsky fixative. Sections were then cut through the C1 to C3 spinal segments and the medulla. An intense band of HRP staining was consistently present in the ipsilateral dorso-lateral part of the dorsal horn. Reaction product was concentrated in lamina II, with lighter and more scattered staining in lamina III, and some scattered staining in lamina IV. No staining could be seen in lamina I. Some light staining was also apparent at obex levels in a confined region of the lateral rim of the cuneate nucleus. No retrograde staining of motoneurons was observed. The pattern of spinal cord labelling with its concentration in lamina II suggests that WGA/HRP may be selectively transported in small fibres (a possibility raised on the basis of similar evidence by Brushart and Mesulem, Neuroscience Letters 17, 1980).

No evidence was found in these experiments for the retrograde transport of WGA/HRP and no retrograde labelling of motoneurons was seen. In contrast, a sample of WGA/HRP obtained from Sigma and used in identical experimental conditions, showed no evidence of transganglionic transport to sites in the spinal dorsal horn, but was readily transported in a retrograde direction and intense staining appeared in motoneurons after exposure of neck muscle nerves.

Our conclusions are that the small fibres, which are unusually abundant in neck muscle nerves (Richmond et al., Can. J. Physiol., 1976) may have terminals confined to a restricted lateral region of the substantia gelatinosa (suggesting that the substantia gelatinosa in this area may be somatotopically organized). In addition, it should be noted that the axonal transport properties of particular WGA/HRP conjugates may be dependent on the source of supply.

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199.3 PROPERTIES OF NEURONS IN LAMINA X AND THE MIDLINE DORSAL HORN OF THE SACROCOCCYGEAL SPINAL CORD OF THE CAT. C.N. Honda and E.R. Perl. Neurobiology Program and Dept. of Physiology, University of North Carolina, Chapel Hill, N.C. 27514.

Light and Perl(JCN 186:133,1979) showed that in addition to the marginal zone, SG_O, and Rexed's lamina V, myelinated mechanical nociceptors often had terminal arborizations within the gray matter surrounding and dorsal to the central canal. Our preliminary results describe some details of the function and morphology of neurons located in the substantia grisea centralis (Rexed's lamina X) and the dorsally adjacent gray matter, part of the central canal region receiving this projection.

In anemically-decerebrate, unanesthetized cats, glass microelectrodes filled with horseradish peroxidase (HRP) were used to record unitary activity in the lower sacral and coccygeal spinal cords. Seventy-five units were functionally characterized and attempts were made to iontophorese HRP into them. In 29 cases where HRP marks were histologically recovered, we found 13 cells whose morphology was extensively delineated with HRP.

Three main categories of neurons were identified within lamina X and the region immediately dorsal to it. One group (27%) was excited only by low threshold mechanoreceptors associated with skin and hair; this group included units responsive only to very slowly moving stimuli. The second and largest group (46%) was excited by both innocuous and noxious stimuli; effective innocuous stimuli were gentle manipulations of skin and hair, or more rarely, subcutaneous tissue. Nociceptive properties of the second group were expressed as either 1) an increased sustained activity in response to noxious mechanical or heat stimulation of the low threshold receptive field or 2) noxious intensities of stimulation for a threshold response in adjacent areas. The third group (27%) was excited only by intense mechanical and/or noxious thermal stimulation of the skin. Units in all three groups usually had receptive fields crossing the midline. Background activity appeared in 20% of the sample, and included units responsive to slowly moving stimuli and those excited by high threshold mechanoreceptors from the skin and deep tissue.

The intracellularly marked neurons formed a heterogeneous population of cells with respect to perikaryal size (8 to 35 μm diam.), dendritic orientation, and localization. Their geometries gave no indication of how morphological features might correlate with functional properties. It appears that neurons of the midline dorsal horn region, described by Rexed as a medial fusion of the dorsal horns, are functionally similar to those of lamina X.

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199.4 LIGHT AND ELECTRON MICROSCOPIC IMMUNOCYTOCHEMICAL DEMONSTRATION OF ENKEPHALINERGIC INPUT ONTO DORSAL HORN THALAMIC PROJECTION NEURONS. M.A. Ruda. Neurobiology & Anesthesiology Branch, NIDR, NIH, Bethesda, MD 20205.

Thalamic projection neurons (TPN) represent a distinct population of dorsal horn neurons, most of which respond to noxious stimulation. These neurons convey noxious input to higher centers of the neuraxis. Enkephalin (ENK), an opiate peptide, is thought to mediate inhibition of the response of these neurons to noxious stimulation. Identification of the site of interaction between these two systems is thus crucial to our understanding of the mechanisms of pain and analgesia. This study identifies one site of synaptic interaction by simultaneously labeling ENK axonal endings and thalamic projection neurons in the medullary and spinal dorsal horns. Several large injections of a 50% solution of HRP were made into the thalamus of 4 adult cats. Following a survival time of 68-72 hrs, the animals were perfused with 4% paraformaldehyde and 0.2% glutaraldehyde and the medulla and spinal cords sectioned on a vibratome. Tissue was processed first with cobalt chloride intensified diaminobenzidine to produce black HRP granules in retrogradely labeled dorsal horn neurons, and subsequently for leu-ENK using the peroxidase anti-peroxidase method of Sternberger to produce red-brown labeled varicosities. TPNs are concentrated in laminae I and V where they represent different morphological types of neurons. At the light microscopic level, numerous ENK varicosities outline the soma and proximal dendrites of large, multipolar TPNs in lamina V. However, in lamina I, only an occasional ENK varicosity is adjacent to the soma of a TPN. At the EM level, numerous ENK immunoreactive endings containing round, agranular synaptic vesicles, form asymmetrical synapses on the soma and proximal dendrites of lamina V TPNs. These neurons also receive a dense innervation from unlabeled endings, many of which contain flattened vesicles. In contrast, the soma of the lamina I TPNs receives few synapses, mainly from endings containing flattened vesicles, and are largely enveloped by glial processes. To date, no ENK endings have been observed synapsing on the soma of lamina I TPNs. These results indicate that lamina I and V TPNs differ with respect to the afferent input received on their soma. ENK synapses on lamina V TPNs demonstrate that one major site of opiate modulation of noxious input in the dorsal horn occurs directly on projection neurons. Moreover, this data indicates that opiates act, at least in part, on postsynaptic receptors located on TPNs.

199.5 ACTION POTENTIAL CHARACTERISTICS OF MYELINATED AXONS IN THE RAT SPINAL CORD: AN INTRA-AXONAL ANALYSIS. J. D. Kocsis, S. G. Waxman, and M. Bickford*. Dept. of Neurology, Stanford Sch. of Med., and V. A. Med. Ctr., Palo Alto, CA 94304.

Intra-axonal recordings were obtained in vivo from dorsal column (d.c.) axons of the rat spinal cord. The rats were anesthetized with urethane, artificially ventilated, and surgically prepared for acute electrophysiological experiments. Intra-axonal impalements were obtained with glass microelectrodes filled with KCl or K-citrate. The d.c.'s were stimulated locally with teflon-coated platinum-iridium wires. Axons having resting potentials of at least -60 mV and action potential amplitudes of 60 mV or greater were selected for analysis. Intra-axonal impalement was identified by the presence of resting potential and the ability of intra-axonal current passage to modulate action potential amplitude; hyperpolarizing current increasing and depolarizing current decreasing spike amplitude.

Impulse fractionation occurred spontaneously in depolarized axons and could be induced by depolarizing current injection. Fractionation also occurred at critical intervals during the relative refractory period (RRP). Threshold increased following depolarizing prepulses, and decreased following hyperpolarizing prepulses. These changes were both voltage- and time-dependent. Anode break excitation was often observed following hyperpolarizing prepulses. During passage of low level depolarizing currents, threshold reductions were observed in some axons.

Spike amplitude was reduced and spike width increased during the RRP. In about half the tested axons a supernormal period (SNP) was present as evidenced by a reduction in threshold following a previous action potential. The SNP duration was about 150 msec. The spikes were followed by either a depolarizing or hyperpolarizing after-potential. The SNP could be present during either type of after-potential. In some axons a hyperpolarizing potential of up to 20 mV amplitude with a time course of greater than a second, intermittently followed the action potential. Although the hyperpolarizing potential was often entrained to spike activity, it could occur independent of spike activity. The origin of this potential is not certain.

In mammalian dorsal column axons, threshold reductions have been observed 1) following a previous action potential, 2) during passage of low level depolarizing pulses, and 3) following hyperpolarizing prepulses. These results suggest that in addition to membrane potential levels, sodium inactivation may be an important determinant of membrane excitability for central myelinated axons. (Supported by the V.A. Medical Research Service and grants from the Multiple Sclerosis Society).

199.6 RESPONSES OF POST-SYNAPTIC DORSAL COLUMN NEURONS IN THE CAT. R.E.W. Fyffe*, P.B. Brown, Lillian M. Pubols and A.G. Brown*. Dept. of Vet. Physiology, Univ. of Edinburgh, Edinburgh EH9 1QH, U.K.

Single axons in the L5 dorsal columns were recorded with microelectrodes in chloralose-anesthetized, gallamine triethiodide-paralysed cats. All responded antidromically to stimulation of the dorsal columns at C3-C4 but failed to respond from C1-C2 above a section of the dorsal columns at C2-C3. Responses were recorded from 48 axons. Their conduction velocities ranged from 22 to 61ms^{-1} (38.3 ± 8.9 mean ±s.d.). Most (71%) had spontaneous discharges of short bursts of high frequency firing separated by a few hundred ms or even seconds of silence. Excitatory fields were of several types: 1. 21 units were excited by hair movement, displacement of glabrous skin (input from Pacinian corpuscles and/or Krause corpuscles and Type I slowly adapting receptors) and high threshold mechanoreceptors from within the area; 2. 11 units were similar but had no high threshold components; 3. 6 units had no glabrous skin component and responded to hair movement and light pressure and pinch of the skin and underlying tissues; 4. 2 units had responses indicating input from Pacinian corpuscles only; 5. 2 units responded only to hair movement. Many units (17%) (especially those with excitatory fields including foot and toe pads) had receptive fields made up of disparate parts. A feature of some (10%) units was the expansion in size of the excitatory field during recording, often after natural stimulation of the field and the area around it. Inhibitory fields were common (18 units) and were mainly of two kinds: 1. The inhibition, (usually elicited by noxious mechanical stimulation) was evoked from within the excitatory field with most of the inhibitory field proximal to it. Inhibition could be elicited by electrical stimulation of both ipsilateral and contralateral cutaneous nerves. Characteristically, it had a time course similar to that of primary afferent depolarization in the spinal cord, although in some units the inhibition was long-lasting, e.g. more than 300ms with a peak at about 120ms.

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199.7 THE MORPHOLOGY AND LOCATION OF THE CELLS OF ORIGIN OF THE DORSAL COLUMN POSTSYNAPTIC TRACT (DCPST). G.J. Bennett, Z. Seltzer*, M.J. Hoffert, G.W. Lu*, N. Nishikawa* and R. Dubner. Neurobiology and Anesthesiology Branch, NIDR, NIH, Bethesda, MD 20205.

We have labeled the cells of origin of the DCPST in the lumbosacral enlargement of cats using retrogradely transported and intracellularly injected horseradish peroxidase (HRP). In the retrograde transport studies, the dorsal columns of a caudal thoracic segment were partially transected. A small piece of HRP-containing polyacrylamide gel was placed into the transection. The cats were sacrificed and perfused 2-3 days later and their spinal cords were sectioned serially in the sagittal plane. HRP reaction product was developed with either cobalt chloride-intensified diaminobenzidine or with tetramethylbenzidine (TMB). Retrogradely labeled perikarya (12-50 μm) were most numerous within a band that extended dorsoventrally for 115 μm. The band was largely within layer IV (with a small excursion into layer III) throughout most of the dorsal horn. Near the horn's medial border, however, the band shifted ventrally into layer V. Labeled cells were also found in the gray matter around the central canal and, very rarely, in layer I. The ventral horn neurons that are labeled by HRP injections into the dorsal column nuclei (Rustioni and Kaufman, '77; Brain Res., 27) were not found. No cells were labeled when the dorsal columns were completely transected caudal to the HRP placement. In one experiment, all of the cells that had clearly visible nuclei were counted in TMB-reacted sections from segment L_7. We found an average of 41.5 cells (unilaterally) in each millimeter of the segment's rostrocaudal extent. This is about equal to the estimated density of spinocervical tract neurons (Brown, et al., '80; J. Physiol., 300) and about one-third the estimated density of spinothalamic tract neurons (Carstens and Trevino, '78; J. Comp. Neurol., 182). In the intracellular experiments, neurons were antidromically driven from cervical dorsal columns that were dissected free, severed rostrally, and insulated from the rest of the spinal cord. To date, all of the cells that we have stained have been in layer IV. They were all wide dynamic range neurons, i.e., they responded to noxious as well as to innocuous stimulation of their cutaneous receptive fields. Their dendritic arbors were elongated rostrocaudally (up to 1,450 μm) but relatively compressed mediolaterally (generally about 450 μm). Some cells had apical dendrites extending into the substantia gelatinosa. We conclude that the DCPST is an ascending pathway important in the transmission of information about noxious stimuli.

199.8 TASK-RELATED RESPONSES OF MONKEY MEDULLARY DORSAL HORN NEURONS. M.C. Bushnell, R. Dubner, D.S. Hoffman*, R.L. Hayes* and M.B. Taylor*. Neurobiology & Anesthesiology Branch, NIDR, NIH, Bethesda, MD 20205.

In previous studies using behaving monkeys we described thermosensitive and mechanosensitive medullary dorsal horn neurons with additional responses independent of stimulus parameters or stimulus modality. The present study investigated these task-related responses in more detail and identified one output pathway in which they are involved. Rhesus monkeys were trained to release a panel when they detected one of the following events: (1) the termination of a warming stimulus (37°-43°C) on the face; (2) the onset of a noxious thermal stimulus (45°-49°C) on the face; (3) the onset of a light on the response panel. Single unit activity was recorded in the medullary dorsal horn (trigeminal nucleus caudalis) during the performance of the behavioral tasks, and projection neurons were identified by antidromic responses to stimulation of the ventral posterior medial thalamic nucleus. EMG activity was recorded from the lip musculature.

Medullary dorsal horn neurons responding to mechanical or mechanical and thermal stimuli also exhibited responses associated with the initiation of the trial, or the sensory cue for panel release, or both. These responses occurred only during performance of the task and were related to sensory events that led to the successful completion of the task, independent of modality, intensity, or location of the relevant stimulus. Such responses were not correlated with facial or arm and hand movements.

Several types of task-related responses were identified, and neurons of each variety could be antidromically activated from the thalamus. In some cells the task-related response occurred only at trial initiation, while in others the response occurred only after the signal for panel release, irrespective of whether that signal was a thermal or visual stimulus. However, the most common pattern of task-related activity was a transient or sustained response at trial initiation with an additional neuronal burst discharge in response to the signal to release the panel. Task-related responses always were associated with stimuli that were components of the sequence of behavioral events leading to reception of a liquid reward. Since neurons with task-related responses project to the thalamus, they could provide a gain-control mechanism for somatosensory information that the animal must use for successful completion of the task. Alternatively, they could be involved in the transmission of behavioral information to motor cortex to facilitate appropriate goal-directed motor behaviors.

199.9 TASK-RELATED NEURONAL RESPONSES AND BEHAVIORAL PERFORMANCE COVARY WITH THE RELIABILITY OF RELEVANT SENSORY CUES IN PREDICTING REINFORCEMENT IN A REACTION-TIME TASK. R. Dubner, D.S. Hoffman*, R.L. Hayes* and M.C. Bushnell. Neurobiology and Anesthesiology Branch, National Institute of Dental Research, National Institutes of Health, Bethesda, MD 20205.

During the monkey's performance of thermal and visual reaction-time tasks, neurons in the medullary dorsal horn (trigeminal nucleus caudalis) exhibit responses associated with the detection of sensory cues relevant to the successful completion of the task. These task-related responses do not code stimulus features and are not related to parameters of movement necessary for acquisition of the liquid reward (Bushnell et al., Neuroscience Abstracts, this volume). This report examines the effect of manipulating variables that alter the reliability of relevant sensory cues in predicting reinforcement on task-related neuronal responses and behavioral performance.

The reliability of occurrence of relevant sensory cues increases as a function of foreperiod length, the time between panel press and the onset of the cue. Reaction time, the time to release of the response panel following onset of the relevant sensory cue, is a measure of the monkey's performance proficiency. Task performance improved significantly (shorter reaction times) on long foreperiod trials (6.5-8.0 sec) as compared to short foreperiod trials (2.0-3.5 sec). We found that the magnitude of task-related neuronal responses following the onset of relevant sensory cues (a temperature decrease or light stimulus) also increased on long foreperiod trials.

The monkey's ability to predict reinforcement was reduced on experimenter-initiated trials in which the monkey had no control over panel release and reinforcement availability. Task-related neuronal responses usually were of less magnitude on reinforced experimenter-initiated trials, and were reduced further, or absent, when reinforcement was withheld. Lip movements, the behavioral performance associated with reception of the liquid reinforcement, also were reduced or absent on experimenter-initiated trials.

We conclude that task-related neuronal responses in reaction-time tasks are evoked by sensory cues that reliably predict reinforcement. More reliable sensory cues produce both increases in the magnitude of task-related neuronal responses and improvement in task performance.

199.10 STRETCH TRAUMA OF THE SPINAL CORD--OXIDATIVE METABOLISM AND ULTRASTRUCTURAL CHANGES. S. Yamada, D. Knierim,*G. Maeda,* R. Schultz.* Section of Neurosurgery, Loma Linda University Med. Cntr., Loma Linda, CA 92350

Dual wavelength spectrophotometry (Jöbsis) allows continuous recording of reduction/oxidation ratio (redox) of cytochrome a,a_3 in cerebral or cord mitochondria. A trauma of the lumbosacral cord was produced in experimental cats by applying traction of various weights on the filum terminale. Double beams, (590 nm-as reference, 605 nm-as sample) were projected to the sacral cord under traction with 0, 1g, 2g, 3g or 5g weight. 1) Transient oxidation of cytochrome a,a_3 in sacral cord mitochondria was measured in response to stimulation of the posterior nerve roots corresponding to the cord segments under examination. 2) After 6-minute period of traction with respectively 2g, 3g and 5g, each cat was sacrificed by fixative perfusion through the descending aorta for ultrastructural study.

Addition of each weight starting from 1g, resulted in increasing reduction of cytochrome a,a_3. Transient oxidation of cytochrome a,a_3 was diminished to 2/3 of normal amplitude under 1g, 2g, and 3g traction. After traction of 1g, 2g and 3g was released, normal transient oxidation returned. However, during 5g traction no oxidation was noted. For 5 minutes after 5g traction was released, transient oxidation of low amplitude occurred, but thereafter, no oxidation was elicited. The ultrastructural changes were prominent only after 5g traction; numerous membrane breaks were noted in neuronal and glial cells.

Low grade stretching (1, 2, and 3g traction) results in metabolic changes i.e. reduction of cytochrome a,a_3. High grade stretching produces structural changes, mainly membrane breaks, which contribute to ion leakage and energy loss. This may lead to irreversible neuronal damage.

199.11 EFFECTS OF SYMPATHECTOMY ON BLOOD FLOW, EVOKED POTENTIALS, EXTRACELLULAR POTASSIUM AND CALCIUM IONS IN EXPERIMENTAL SPINAL INJURY. W. Young*, I. Koreh*, E. S. Flamm. Dept. Neurosurgery, NYU Med. Center, New York, N.Y. 10016.

Contusion of cat spinal cord produces a characteristic pattern of neurophysiological loss [Young, W., et al, J. Neurosurg. 52:64, 1980]. Action potential conduction across the impact site is immediately lost, recovers transiently 1-2 hours later, but only to disappear again 2-3 hours after injury. We report experimental evidence for the role of extracellular potassium (K+) and post-traumatic ischemia in the initial and delayed loss of evoked potentials.

Two groups of 5 cats, anesthetized with 25 mg/kg IV pentobarbital, were studied. The T1-12 sympathetic ganglia were removed in one group 2 hours prior to injury and not in the other (control). Ion-selective microelectrodes were used to measure extracellular K+ and Ca++ ionic activities in the lateral columns of the thoracic cord. Blood flow was measured close to the site of ionic recordings with the hydrogen clearance method [Young, W., Stroke 11:552, 1980]. White matter conduction was assessed with somatosensory evoked potentials (SEP). The spinal cords were injured by a 20 gm weight dropped 20 cm onto the T7 cord. Systemic pressure and respiratory parameters were maintained within normal range.

In control cats, extracellular K+ rose precipitously from a pre-injury level of 3.2 mM to a mean of 52 mM, clearing out with a half-time of 45 minutes. SEP were lost virtually immediately after contusion but recovered transiently 1-2 hours after injury, as extracellular K+ returned towards baseline. Blood flow, however, began to decrease as K+ fell below 12 mM. SEP disappeared again when blood flow reached 50% of preinjury levels by the 3rd hour after injury. Extracellular K+ did not increase above 12 mM again despite the ischemia. In sympathectomized cats, extracellular K+ behaved in a similar manner except for a shorter clearance half-time of 30 minutes. The pattern of SEP change was identical to the controls except that there was no secondary loss at 2-3 hours. Ischemia did not occur as K+ normalized. Extracellular Ca++ ions in both groups fell from a pre-injury mean of 1.2 to 0.3 mM, recovering to baseline with a half-time of >1.0 hour.

These results suggest that contusion of spinal tissues causes massive exchange of ions across cellular membranes. The extracellular levels of both K+ and Ca++ ions return slowly towards pre-injury levels. Transient recovery of evoked potentials occurs as K+ falls, only to be lost again when ischemia sets in. Sympathectomy ameliorates the delayed post-traumatic ischemia and prevents the secondary loss of SEP.

199.12 PSYCHOPHYSICAL EVALUATION OF SOMATOSENSORY IMPAIRMENT IMPLICATING DORSAL (COLUMN) FUNICULUS INVOLVEMENT. Richard J. Schneider and Ronald F. Burke.* Maryland Institute for Emergency Medical Services Systems, Baltimore, Maryland 21201.

We trained monkeys and humans to discriminate between hair follicle displacements or electrocutaneous stimuli. In the former task, the amplitude or frequency of displacement of patches of hair on the anterior calf were varied. In the latter, current pulse trains of different frequency were applied to the skin over the peroneal or sural nerve. The ambiguity of these discriminations was manipulated. The subject was required to depress a manipulandum in response to one amplitude (frequency) and to refrain in response to the other. The stimuli were presented successively (10 sec. ISI) and pseudorandomly. Thus, four responses were possible - a correct depression (hit), an incorrect one (false alarm), a correct refrain (correct rejection), and an incorrect refrain (miss). These were used to calculate d-prime (sensitivity) and beta (response bias) scores according to signal detection theory (TSD). These measures were established in normal subjects and compared to either the same animals with peripheral nerve block, dorsal funiculus (DF) lesions or to human patients with multiple sclerosis (MS).

Discriminative capacity (d') was reliable and independent of response bias (β). Normal subjects established a given sensitivity level comparable for humans and monkeys, and not affected by hair density, sex or age. When (local anesthetic) peripheral nerve block was applied to monkeys, responding to both stimuli ceased below the affected area, but not above it or on the contralateral leg. Following DF tractotomy, however, d' was markedly diminished only to hair displacement below the level of the lesion on the ipsilateral side. Diminution in electrocutaneous d' was small in comparison. MS patients have a lower hair displacement d' than do normals. As long as the stimulator does not touch the skin, this holds true. Differences between normal and MS groups diminish when skin is also stimulated.

These conclusions are suggested: (1) Hair follicle displacement has particular advantages in evaluating DF damage in monkeys. (2) Electrocutaneous stimulus discrimination is not as useful in making such an assessment. (3) MS patients have less sensitivity to hair follicle stimuli than to electrocutaneous or touch stimuli.

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200.1 REGIONAL VARIATION IN BZ_1 and BZ_2 BENZODIAZEPINE RECEPTOR SUBCLASSES IN 40 BRAIN AREAS OF THE MONKEY (CERCOPITHECUS AETHIOPS). M. Nielsen*, H. Simonsen*, I. Divac and C. Braestrup*. Psychopharmacological Research Lab., St. Hans Mental Hospital, DK-4000 Roskilde; Neurophys. Lab., Panum Inst., Copenhagen and A/S Ferrosan, Soeborg, Denmark.

Derivatives of β-carboline-3-carboxylic acid, including the methyl, ethyl and propyl esters have been shown to be potent inhibitors of brain benzodiazepine (BZ) receptor binding. ^{3}H-propyl β-carboline-3-carboxylate (^{3}H-PrCC) binds specifically to the benzodiazepine recognition site of BZ receptors in cerebellum (the BZ_1-receptor subclass). In other brain regions ^{3}H-PrCC labels only a subfraction of the total BZ receptor populations as labelled by ^{3}H-flunitrazepam (^{3}H-FNM). In the rat brain, BZ_1 receptors (identified by ^{3}H-PrCC binding) constitute ca. 50% of hippocampal and ca. 70% of cortical BZ receptor population. Remaining receptors are classified as BZ_2 receptors. By simultaneous estimations of ^{3}H-PrCC (0.3 nM final concentration) and ^{3}H-FNM (1 nM final concentration) binding, the fraction of BZ_2 receptors to total BZ receptors can be calculated (Braestrup and Nielsen, J. Neurochem. in press) provided that no variations in K_D values occur. The fraction of BZ_2 receptors has been estimated in 40 areas of the brain of Cercopithecus aethiops. We found high levels of BZ_2 receptors (30-50% of total BZ receptors) in some cortical areas, e.g. the prefrontal cortex and the hippocampus, in the amygdala and in the anterior portion of the neostriatum. The majority of cortical areas contain intermediate levels of BZ_2 receptors. Interestingly, low levels of BZ_2 receptors (less than 10% of the total BZ receptor population) was found in the posterior part of the neostriatum and in the globus pallidus. It is striking that high relative concentrations of the BZ_2 receptor subclass occur in the limbic system.

200.2 PROPERTIES OF THE BENZODIAZEPINE (BZ) RECEPTOR FROM DIFFERENT BRAIN REGIONS. Mathew M.S. Lo and Solomon H. Snyder, Johns Hopkins University, School of Medicine, Dept. of Neuroscience, Pharmacology and Psychiatry, Baltimore, Maryland 21205.

The pharmacological and biochemical properties of the BZ and GABA receptors were studied in different regions of calf brain. Soluble receptors were quantitated with a new and novel assay method which utilizes concanavalin-A to immobilize receptor glycoproteins. Soluble BZ receptors from different brain regions (as measured by [^{3}H] flunitrazapam (FNZ) or [^{3}H] propyl-β-carboline-3-carboxylate (PCC) binding) appeared to have similar pharmacologic properties. On the other hand, a large population of BZ receptors (e.g. cerebellum, ~ 90%; hippocampus, ~ 80%; cortex, ~ 60%; striatum, ~ 80%) remained in the detergent insoluble fraction: these were not solubilized even after repeated extraction with high concentration of detergent.

The stoichiometry of high affinity GABA:BZ sites is 2 in the soluble detergent extract and 1-4 in the insoluble fragments depending on the brain region studied. In contrast, the number of [^{3}H]FNZ sites always equals that for [^{3}H]PCC in either the soluble or insoluble fractions. Enhancement of [^{3}H]FNZ binding by GABAergic agonists was observed in the soluble and insoluble BZ receptors. Dependency on Cl^- ions was also found in both cases.

Insoluble receptor in certain brain regions exhibited pharmacological properties distinct from soluble receptors from the whole brain. For example, displacement of [^{3}H]FNZ by CL 218872 in the soluble BZ receptors from different regions appears to be a single class of sites with a low affinity (IC_{50} = 20 μM) - this ligand has been shown to bind preferentially to BZ type 1 receptors. However, high and low affinity binding of CL 218872 was observed in the insoluble membrane fragment of cortex, striatum, hypothalamus, superior and inferior colliculus. These results suggest the existence of a heterogeneous population of BZ receptors with one form preferentially isolated after solubilization with detergents.

200.3 THE LIGHT MICROSCOPIC IN VITRO AUTORADIOGRAPHIC LOCALIZATION OF ADENOSINE (A1) RECEPTORS. R.R. Goodman and S.H. Snyder. Johns Hopkins University, Sch. of Med., Dept. of Neuroscience, Pharmacology and Psychiatry, Baltimore, MD 21205.

Adenosine displays a number of receptor-mediated physiological actions, including dilation of coronary vessels, inhibition of platelet aggregation and inhibition of lipolysis. Adenosine also behaves as a neuromodulator or neurotransmitter, inhibiting neuronal firing and synaptic transmission and altering cyclic AMP concentrations in brain tissue. Adenosine regulates adenylate cyclase activity via two apparently distinct receptors, the A_1 receptor activation lowers adenylate cyclase activity, while A_2 receptors mediate augmentation of adenylate cyclase. The two receptors have different substrate specificities, and recently it has become possible to label A_1 receptors in brain membranes with ^{3}H-N^6-cyclohexyladenosine (^{3}H-CHA) (Bruns, R.F., Daly, J.W. and Snyder, S.H., Proc. Natl. Acad. Sci. USA, 77:5547-5551, 1980). We have utilized this ligand with the recently developed technique for the light microscopic in vitro autoradiography of neurotransmitter receptors (Young, W.S.,III and Kuhar, M.J., Brain Res., 179: 255-270, 1979), to study the localization of adenosine receptors in rat brain. Adenosine receptors are widely distributed throughout the grey matter areas of the central nervous system, but in the brain levels studied thus far occur in high concentrations only in certain brain areas. The highest density of adenosine receptors is found in the molecular layer of the cerebellum, with relatively low levels in the granule and pyramidal cell layers. Areas with very high concentrations of receptors include the marginal nucleus of the medial geniculate body, the superficial layer of the superior colliculus, the molecular layer of the hippocampus, the piriform cortex and layers I and IV of the cerebral cortex. Moderately high concentrations of adenosine receptors are found in the dentate gyrus of the hippocampus, several thalamic nuclei and in layers V and VI of the dorsal cerebral cortex. A moderate concentration of adenosine receptors was found diffusely throughout the corpus striatum. Low levels were found in layers II and III of the cerebral cortex, the amygdala and the hypothalamus. Lowest levels were found in white matter areas, particularly in the corpus callosum. The localization of high concentrations of adenosine receptors in specific synaptic-rich brain regions, as well as the finding of quite low concentrations of receptors in white matter areas, supports the role of adenosine as a central nervous system neuromodulator or neurotransmitter.

200.4 DOPAMINE RECEPTORS IN RAT AND DOG STOMACH: IDENTIFICATION WITH [^{3}H]-SPIROPERIDOL IN VITRO. A. M. Marchisio*, Y. Taché and R. Collu. Neuroendocrine Research Laboratory, Pediatric Research Center, Hôpital Ste-Justine and Université de Montréal, Montréal, Québec, H3T 1C5.

The role of dopamine (DA) as a neurotransmitter in the central nervous system has been known for several years. More recently, it has become increasingly evident that DA acts as a neurotransmitter also in the peripheral nervous system. Significant amounts of DA have been found throughout the mammalian body and in particular in the stomach mucosa. The neurotransmitter has recently been found to be able of reducing both basal and stimulated gastric acid secretion in man (Caldara et al., Gut 19: 724, 1978). In rats, cysteamine-induced duodenal ulcers and gastric-acid output were antagonized by dopaminergic agents and enhanced by DA receptor antagonists (Szabo, The Lancet 2: 880, 1979). More recently, the elevation of gastric pH induced in rats by the intracisternal injection of bombesin was found to be reversed by DA receptor antagonists (Taché and Collu, Gastroenterology 80: 1298, 1981). These data suggest the existence in the mammalian stomach of receptors for DA, which might play a physiological role in the regulation of gastric secretion. In order to verify this hypothesis we have performed several experiments with both adult male Sprague-Dawley rats and adult male mongrel dogs. Binding studies were done with [^{3}H]-Spiroperidol ([^{3}H]-SPIR) as ligand and as previously reported. Specific binding was defined as the counts in the absence of 10^{-6} M d-butaclamol minus those in its presence. Preliminary studies were performed to determine the ideal incubation time and pH. No change in binding was observed between 15-60 min while a decrease occurred afterward, and the highest specific bindings were obtained at pH < 4.5. Scatchard analysis of binding data revealed the presence of a single class of high affinity [^{3}H]-SPIR binding sites in the mucosa of the antrum of rat (K_D = 2.97 nM; B_{max} = 41.15 fmol/mg protein) and dog (K_D = 2.35 nM; B_{max} = 41.33 fmol/mg protein). No specific binding was identified in the mucosa of the corpus. Displacement studies with various agonists and antagonists confirmed the dopaminergic nature of the binding sites. These results indicate that dopaminergic receptors are present in the mucosa of the antrum of both rat and dog stomach which might be involved in the regulation of gastric secretion.

200.5 DEVELOPMENTAL CHANGES IN THE REGIONAL DISTRIBUTION OF HERPESVIRUS RECEPTORS IN THE MOUSE BRAIN. D. J. McFarland*, J. Hotchin and F. Baker*. NY State Dept. of Health, Albany, NY 12201.

Viral infection is initiated with the binding of a virion to specific receptors on the host cell membranes. The purpose of this work was to determine the regional distribution of receptors for herpes simplex as this may be one determinant of the localization of infection within the CNS. The MacIntyre strain of herpes simplex Type I was grown in human embryonic tissue culture cells with ^{3}H deoxythymidine-5-triphosphate(methyl-^{3}H) added to the medium. Duplicate experiments with mock-infected cells, treated identically throughout with the exception of addition of virus, were also performed to provide material for blank counts. Infected and control cell homogenates were then subjected to differential centrifugation. Tissue homogenates were incubated with labelled virus or with the control extract for 30 min at 37°C, centrifuged, washed twice, and the radioactivity in the pellets was determined. The specific of the binding of these suspensions was confirmed by the observation that binding could be blocked by neutralization with antiherpes antibody.

Crude brain homogenates were prepared from mouse cerebral hemispheres, hippocampus, cerebellum, brainstem, and liver. Nylar mice 4, 6, 10, and 66 weeks of age were employed.

Results expressed as H3 counts/min. in the pellets are shown in the table below. Each value represents the mean of 4 independent observations. An analysis of variance indicated that there was a significant interaction between the age of the animal and the tissue source ($p.< .02$). This effect is due to an asymmetrical distribution of binding in the mouse brain and liver. The distribution also varied with the age of the animal. These results demonstrate that herpesvirus receptors are not uniformly distributed within the CNS.

Region	Age			
	4 wks	6 wks	10 wks	66 wks
Cerebral Hemispheres	2092.8[1]	2218.0[1]	2270.3[1]	2441.5[1]
Hippocampus	2180.5[1]	1829.0[2]	2111.3[1]	2246.0[1]
Cerebellum	1133.5[2]	1223.8[3]	1212.5[2,3]	1027.3[3]
Brainstem	1891.0[1]	1841.3[2]	1529.3[2]	1639.0[2]
Liver	1056.3[2]	655.3[4]	1028.8[3]	1265.3[3]

Note: Means with same superscript do not differ significantly. All $p<.01$ except 6 wk CX vs BS ($p<.05$)

200.6 RECEPTOR AUTORADIOGRAPHY WITH TRITIUM-SENSITIVE SHEET FILM: ANALYSIS BY COMPUTERIZED DENSITOMETRY. J.M. Palacios, J.R. Unnerstall, D.L. Niehoff, L. Buhle* and M.J. Kuhar. Dept. of Neurosci., Johns Hopkins Univ. Sch. of Med., Balto., MD 21205.

Receptor sites for neurotransmitters and drugs have been localized at the light microscopic level after in vitro labeling of slide-mounted tissue sections. Autoradiograms have been generated by apposing emulsion-coated coverslips (Young and Kuhar, Brain Res., 179:225, 1979). In this communication we describe the use of ^{3}H-sensitive sheet film (^{3}H-Ultrafilm, LKB) for the generation of autoradiograms, its advantages and disadvantages.

We initially characterized the behavior of the film under conditions normally used for the identification of receptors. Standards were prepared by mixing known amounts of non-volatile radioactivity with brain tissue ground to a paste. This tissue was handled in the same way as samples used for the localization of receptors. The film was exposed to these standards for varying lengths of time, and the autoradiograms thus generated were evaluated by microdensitometry. In some instances, optical density was also determined by computer-assisted densitometry (Goochee et al., Ann. Neurol., 7:359, 1980). Optical density increased proportionally with exposure, and the limits in which accurate determinations of receptor concentrations could be made were defined.

As a model, this method was applied to the study of multiple receptor systems. For example, multiple benzodiazepine (BZ) receptors were studied by generating displacement curves using propyl-beta-carboline carboxylate (beta-CC) and triazolopyridazines (TPZ), agents which have a high affinity for the cerebellar, or Type-1 BZ receptor. It was shown that both beta-CC and TPZ displaced in a monophasic manner ^{3}H-flunitrazepam in the molecular layer of the cerebellum, while a biphasic displacement was observed in other areas such as the dorsal CA1 region of the hippocampus. The proportion of the different receptor sites can be accurately determined by these methods. The densities of receptors and the relative proportions of the different subtypes agree well with results obtained in tissue homogenates. Similar analyses have been done for multiple serotonin, muscarinic cholinergic, beta-adrenergic and dopaminergic receptors.

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201.1 REQUIREMENT OF HYPOTHALAMIC SODIUM CURRENTS FOR THE LORDOSIS REFLEX. R.E. Harlan*, B.D. Shivers and D.W. Pfaff. The Rockefeller University, New York, N.Y. 10021.

Lordosis is an estrogen-dependent reflex necessary for reproduction in rats and many other species. Estrogen acts primarily on neurons in the hypothalamus, and in the ventromedial nucleus (VMN) in particular, to induce and maintain lordotic responsiveness. The mechanisms by which estrogen acts in the hypothalamus are inadequately defined. The present studies were designed to examine the role of sodium currents in the hypothalamus in the expression of lordosis in estrogen-treated rats. Drugs which interfere with sodium ion flux were infused (1 µl on each side) into the hypothalamus in awake animals, and the rats were tested periodically for lordosis and other postural responses. Infusion of vehicle (N=9) or the local anesthetics 0.5% bupivacaine (N=5) or 50% procaine (N=7) had no effect on lordosis or reflexes for righting, climbing or leg withdrawal. Even repeated infusions of 50% procaine (every 30 min for three infusions, N=3) had no effect.

Infusion of the neurotoxin tetrodotoxin (TTX) induced large deficits in lordotic, righting and climbing reflexes, though no decrement in leg withdrawal. Bilateral infusion of either 5 (N=7) or 10 (N=10) ng TTX decreased lordotic responsiveness significantly between 40 min and 8 h after infusion, with the nadir reached at 2 h (63% decrease for 5 ng) or 4 h (59% decrease for 10 ng). These doses also induced general sedation (though the rats were easily aroused by somatosensory stimuli) and loss of righting and climbing reflexes within 5-10 min following infusion. Recovery of these responses began 1-2 h after infusion, a time course clearly different from the effects on lordotic responsiveness. Infusion of 0.5 ng TTX (N=6) induced much smaller decrements in lordotic and other reflexes, while infusion of 25 ng TTX (N=2) resulted in death within 1 h. Control infusion of 10 ng TTX into the thalamus (N=6) resulted in a temporary loss in righting reflex, but no decrement in lordotic responsiveness.

These data suggest that local anesthetics either do not affect lordosis-relevant VMN neurons, or that the duration of disruption of sodium currents is too short to affect lordosis. The reversible decline in lordotic responsiveness following TTX infusion is probably due to sustained interruption of action potentials of lordosis-relevant neurons. Furthermore, these data are consistent with electrophysiological results (Bueno and Pfaff, 1976) indicating that estrogen increases the electrical activity of VMN neurons, and that these neurons must participate in lordosis control on a tonic rather than a reflex mount-by-mount basis.

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201.2 SEXUAL BEHAVIOR AND SCENT MARKING IN MALE GERBILS: INDEPENDENT CONTROL BY THE MEDIAL PREOPTIC AREA - ANTERIOR HYPOTHALAMUS. D. L. Commins* and P. Yahr (SPON: A. Starr). Dept. of Psychobiology, Univ. of California, Irvine, CA 92717

The anterior hypothalamus (AH) and medial preoptic area (MPOA) are implicated in the hormonal control of sexual behavior and ventral scent marking in male Mongolian gerbils (Meriones unguiculatus). However within the AH-MPOA, the loci involved in these two behaviors may not be the same. Testosterone (T) implants in the most anterior or most posterior portions of the MPOA of castrated males will reinstate scent marking. Similar implants in the middle portion of the MPOA do not.

In a related study, an attempt was made to selectively disrupt one or the other behavior by making radiofrequency lesions in a variety of sites within the AH-MPOA. Sexual behavior was most reliably disrupted by lesions in the middle part of the MPOA. Subcutaneous implantation of T at lesion or 6 wk later did not alleviate the decline. Some males that ceased mounting had been reliable markers preoperatively. Marking did not decline substantially after lesion surgery when these subjects were given supplemental T.

Three males showed impaired marking despite T treatment. Their sexual behavior also declined, although their lesions tended to be more anterior or posterior than the majority of the lesions that affected only sexual behavior.

A subsequent study attempted to verify that the anterior part of the MPOA (near the diagonal band of Broca) and the AH are particularly important in the control of marking. Gonadally intact males that marked reliably received lesions in one or the other location. In general, marking was not impaired. The same subjects were then relesioned to increase the size of the lesion. This resulted in a persistent decrease in marking in both anterior and posterior lesioned animals. These lesions typically included most of the MPOA, and in some cases may have disrupted gonadotropin release.

In a third study lesions were made simultaneously at these same anterior and posterior locations. Lesioned animals marked significantly less than sham-operated controls. After three weeks of testing, all subjects were castrated and implanted with 10-mm T capsules. Marking recovered at least partially in response to T therapy in some subjects, but not in others. Those that did not recover usually had extensive damage extending from the diagonal band of Broca to the AH.

The effects of lesions and of T implants suggest that the far anterior and far posterior MPOA are important in the neural control of scent marking. The areas involved are relatively widespread and overlap only partially the region of the MPOA that influences sexual behavior.

201.3 FEMALE SEX BEHAVIORS IN THE SOUTH AFRICAN CLAWED FROG, XENOPUS LAEVIS: GONADOTROPIN-RELEASING, GONADOTROPIC AND STEROID HORMONES. D. B. Kelley. Dept. of Psych., Princeton Univ., Princeton, NJ 08544

The goal of this study was to characterize certain female sex behaviors in the South African clawed frog, Xenopus laevis, and to explore the behavioral effects of endocrine manipulation. The responses of females to clasp assaults by sexually active males were observed. Two patterns of female responses predominated: in one, females exhibited extreme leg extensions and ticking vocalizations when clasped (unreceptive behaviors). In the other, the female responded to a clasp by adducting her thighs and increasing flexion at the knee; she did not vocalize (receptive behaviors). Clasp durations of pairs in which the female was unreceptive were short, generally less than 1 minute. With a receptive female, on the other hand, amplexus could last up to two days.

In intact females, injection of human chorionic gonadotropin (HCG) or of luteinizing hormone releasing hormone (LHRH) resulted in significant increases in receptivity. These hormones did not promote receptivity in ovariectomized females. Treatment with a combination of estradiol (E) and progesterone (P) restored receptivity to females. Either hormone when administered alone was ineffective in decreasing leg extension scores. The releasing hormone, LHRH, when given to ovariectomized, E + P treated females, further decreased their leg extension scores and led to the prolonged amplexus otherwise found only with HCG-injected intact females. The effects of LHRH on E + P treated, ovariectomized females may be independent of action on the pituitary since they are not mimicked by gonadotropin.

Hormones administered exogenously in this study are also present endogenously and have been shown to fluctuate with reproductive condition. Such hormonal fluctuations may serve to synchronize behavioral receptivity with ovulation and oviposition. One potential mechanism for the behavioral effects of such hormones is interaction with endocrine target cells in the central nervous system. Previous studies have outlined a system of estradiol-concentrating cells in X. laevis brain (Morrell, Kelley & Pfaff, 1975, J. Comp. Neurol.). The existence of neurons in X. laevis which are sensitive to progesterone and to LHRH remains to be investigated.

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201.4 OVARIAN HORMONES AND EATING BEHAVIOR IN RHESUS MONKEYS. Joseph W. Kemnitz, Wis. Reg. Primate Research Ctr., Univ. of Wis., Madison, WI 53706

Food intake of 13 adult female Macaca mulatta was measured during a total of 51 menstrual cycles (1-7 cycles/animal). Four phases of the cycle were defined on the basis of coloration of sex skin and menses: early follicular (EF), 5 days beginning with the first day of menstruation; preovulatory (PO), 2-4 days prior to color breakdown; midluteal (ML), 5 days halfway between the day of presumed ovulation and the first day of subsequent menstruation; and late luteal (LL), the last 4 days before menstruation. Intake was lowest during the PO phase in 58% of the cycles, whereas lowest intake occurred during the EF, ML, or LL phases in only 28%, 2% and 12% of the cycles, respectively. Mean intakes (gm/day, ± SEM) were 137 ± 8 for the PO phase, 167 ± 11 for the EF phase, 184 ± 7 for the ML phase, and 186 ± 7 for the LL phase. Five of these animals were weighed daily during a total of 26 cycles. Body weight decreased during the follicular phase reaching a nadir one day before color breakdown (i.e., one day after presumed ovulation), and then increased during the luteal phase.

Although intake was, on average, lowest during the PO phase for 10 of the 13 animals, prominent individual differences in intake were observed. For example, average intakes during the ML phase for individual animals ranged from 134 gm/day to 312 gm/day. These values were not discernably related to age (which ranged from 5 to 17 years), body weight (which ranged from 4 to 10 kg), or parity (which ranged from 0 to 5 offspring). Likewise, magnitude of suppression of intake (PO intake/ML intake) during the PO phase was not attributable to these variables or to overall level of intake during the menstrual cycle. However, animals with longer inter-menstrual intervals tended to have higher average daily food intake.

Ovariectomized monkeys treated with estradiol and progesterone have been studied in order to investigate more directly the mechanisms of hormone-induced changes in food intake as well as the basis for individual differences in responsiveness to hormonal changes. Silastic capsules were used to vary serum concentrations of estradiol and progesterone. When estradiol was maintained at levels characteristic of the midfollicular phase or progesterone at levels characteristic of the early luteal phase, few statistically reliable effects of these treatments on food intake, body weight, fasting plasma glucose, glucose tolerance or glycosylated hemoglobin were observed. However, treatment with higher doses of estradiol did suppress food intake and body weight.

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201.5 STUDIES ON THE EFFECTS OF PREGNANCY AND OF ESTROGENS ON ETHANOL INTAKE IN THE RAT. D. Sandberg* and J. Stewart (SPON: A. Giachetti). Dept. of Psychology, Concordia University, Montreal, Canada, H3G 1M8

Changes in consumption of a 10% ethanol solution and of tap water by pregnant rats was investigated over the course of gestation. Ethanol intake during the first two trimesters of pregnancy matched that of non-mated control animals. During the third trimester, however, ethanol consumption by the pregnant animals decreased. Water intake by the pregnant animals was elevated above that of control animals for most of the gestational period, and these changes did not appear to be related to decreases in ethanol consumption. In a second study decreases in consumption of an isocaloric sucrose solution were observed to occur during the same period of pregnancy, whereas consumption of a saccharin solution remained stable. These findings suggest that common factors control ethanol and carbohydrate intake in the pregnant rat and have led us to do a series of experiments on the effects of estrogens on ethanol consumption in the rat.

The effects of acute (one day) and chronic (18 day) subcutaneous administration of 5 µg estradiol benzoate (EB) on ethanol consumption were studied in ovariectomized rats in a free-choice situation. EB injections decreased ethanol consumption. A single injection led to a significant but transient inhibition of intake. Chronic injections caused an even more pronounced suppression. With continued EB administration, however, ethanol consumption returned to levels indistinguishable from those of control animals. This apparent tolerance to the suppressive effects of EB parallels that observed when food intake is measured in response to chronic estradiol treatment.

The final study tested the effects on ethanol consumption of MER-25, an anti-estrogen, which mimics estradiols effect on food intake and body weight. MER-25 administered daily subcutaneously in a dose of 10 mg/animal produced effects that were similar to those produced by EB, thus supporting the idea that the mechanism that leads to estradiol's inhibitory action on food intake is the same that leads to decrements in ethanol consumption. Additional studies demonstrating that neither EB nor MER-25 interfere with ethanol metabolism, and that EB does not produce a conditioned taste aversion in combination with ethanol further supports this conclusion. (Supported by a grant from the Natural Sciences and Engineering Research Council of Canada, AO-156.)

201.6 RATS HAVE REDUCED PREFERENCE FOR ALCOHOL DURING PREGNANCY AND FOLLOWING LACTATION. L. W. Means and H. B. Goy*. Depts. of Psychol. and Physiol. East Carolina Univ.,Greenville, NC 27834.

Recently it has been reported that non-alcoholic women (Little, R.E. et al. J. Stud. Alcohol, 1976, 37, 375), macaques (Elton, R.H. & Wilson, M.E. J. Stud. Alcohol, 1977, 38, 2181) and mice (Randall, C.L. et al. Pharmacol. Biochem, & Behav., 1980, 13, 150) show reduced alcohol consumption during pregnancy. The present study was conducted to examine alcohol preference in rats during cohabitation, pregnancy, lactation, and post-lactation. Eighteen 75-95 day old female Long-Evans rats were individually housed, given continuous free access to lab chow, and 10 hr. (8:30 AM-6:30 PM) daily access to a .01 M saccharine solution and a .01 M saccharine solution containing 5% ethanol (v/v) throughout the study. The solutions were presented in 100-ml calibrated drinking tubes whose positions were randomly changed. Following 3 days adaptation to the drinking schedule and 11 days of baseline preference readings, a comparably aged male was placed with each female during the 14 hours that the drinking solutions were not available. Upon determination of pregnancy of a female by presence of sperm plugs, the male was removed from her cage and another male was removed from the cage of a non-pregnant female. Thus, all males were removed by the end of the third night, when 9 females had been determined to be pregnant. Liquid consumption was monitored throughout pregnancy, lactation, and for 10 days post-lactation for the pregnant animals and during this same time period for the control animals. All pups were removed at 20 days of age.

Alcohol preference ratios (alcohol solution/total fluid) for both groups during each phase of the experiment are shown in the table.

Mean Alcohol Preference Ratio (Alcohol/Total Fluid)
± Standard Errors

Group	Baseline	Cohabitation	Pregnancy	Lactation	Post-Lact.
Pregnant	.76± .04	.60± .09	.36± .08	.50± .06	.35± .08
Control	.84± .04	.70± .09	.73± .06**	.62± .10	.60± .09*

**p < .01 * p < .05

The pregnant animals had significantly lower preference ratios than the control animals during both pregnancy ($t=3.80$, $df=16$, $p < .01$) and the 10-day post-lactation period ($t=2.15$, $df=16$, $p < .05$).

The strong decrease in alcohol preference during both pregnancy and following lactation strongly suggests that alcohol preference is modulated by reproductive hormones.

202.1 REDUCTION BY ATROPINE OF PHENCYCLIDINE-INDUCED HYPERTENSION. G. K. W. Yim, A. Malave* and M. P. Holsapple*. Dept. of Pharmacology and Toxicology, School of Pharmacy and Pharmacal Sciences Purdue University, West Lafayette, IN 47907.

The pressor effect of phencyclidine (PCP) has been attributed mainly to direct and indirect actions on the peripheral adrenergic system. The purpose of this investigation was to determine whether the anticholinesterase action of PCP might result in activation of central cholinergic pressor systems, and thereby contribute to the PCP-induced hypertension. Sprague Dawley rats, lightly anesthetized with urethane (1.25 g/kg i.p.) exhibited a dose-related pressor response (16.5 ± 2/3. 21.3 ± 3.7, 31.0 ± 2.6 and 26.8 ± 3.5% increases over control systolic blood pressure) following 0.1, 0.3, 1.0 and 3.0 mg/kg i.v. doses of PCP. After atropine pretreatment (0.8 mg/kg i.v.), the PCP dose-response was shifted to the right, and the magnitude of the pressor responses was smaller by about 50%. In contrast, methylatropine pretreatment (1.0 mg/kg i.v.) did not reduce the PCP-induced pressor responses. The bradycardia, which accompanied the PCP pressor response, was not affected either the atropine or methylatropine pretreatment. Apneusis was observed in 85% of 19 control animals receiving 1.0 to 3.0 mg/kg i.v. doses of PCP. The incidence of PCP-induced apneusis was 0% in 18 atropine pretreated rats, and 100% in 16 methylatropine pretreated rats. These results suggest that the central cholinergic actions of PCP contribute significantly to the hypertension and respiratory toxicity induced by PCP. (Supported by USPHS Grant DA 01916, a University of Puerto Rico Scholarship, and a Purdue Research Foundation Fellowship).

202.2 PHYSICOCHEMICAL PROPERTIES OF PHENCYCLIDINE AND PHENCYCLIDINE DERIVATIVES. M.R. Rosenfeld*, B. Pazhenchevsky*, H. Weinstein* and S. Maayani. Department of Pharmacology, Mount Sinai School of Medicine of the City University of New York, N.Y., 10029.

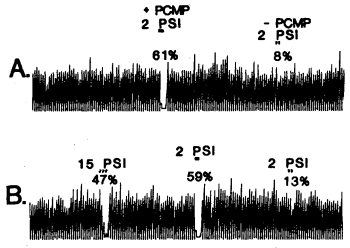
Some phencyclidine (PCP) derivatives were unexpectedly found to be devoid of PCP-like effects on behavior. Physicochemical measurements showed that their inactivity might have been due to low solubility, or decreased oil:water partitioning rather than to the pharmacodynamic properties of the drugs. In order to discriminate between these factors for PCP-like action we developed methods to determine the pK_a's and partition coefficients (P) of PCP analogs. Due to the relatively low aqueous solubility of both the protonated ($\leqslant 10^{-2}$M) and the non-protonated ($\leqslant 10^{-4}$M) species of PCP and its analogs, and because of the small U.V. extinction coefficient (ε= 420 at 263nm) neither a titrimetric method nor a spectrophotometric method could be used for the determination of these two parameters. Therefore, we devised a method that allows for the simultaneous determination of pK_a and P. It is based upon the distribution of radioactively labelled drug between an aqueous phase and an organic phase at varying pH. Four compounds were studied, ^{3}H-PCP, ^{3}H-m-amino PCP (NH_2PCP), ^{3}H-m-nitro PCP (NO_2PCP) and ^{3}H-PCP methiodide (CH_3IPCP). The choice of buffers--borate, HEPES or CAPS--was dependent upon the pH range to be tested. The organic phase was either iso-octane or 1-octanol. All compounds were routinely checked for purity by thin layer chromatography. An alternative method (method 2) was also used to estimate P. A summary of the results obtained at 25°C with iso-octane:borate or iso-octane: CAPS for NH_2PCP is given in the table below (mean ±S.D.).

	pK_a	P	P (method 2)
PCP	9.41 ± 0.04	6750 ± 200	6500 ± 120
NH_2PCP	9.57 ± 0.10	23 ± 6	33
NO_2PCP	8.23 ± 0.13	3337 ± 695	2362 ± 1309
CH_3IPCP	----	9.14 ± 0.62	1.50 ± 0.22

Both pK_a and P measurements for PCP were shown to be dependent upon temperature, i.e., increasing the temperature from 25°C to 37°C resulted in a 2.3% decrease in the pK_a. The method developed in these studies can be applied to other compounds which like PCP have an extremely low water solubility. This work was supported by NIDA grant DA02534.

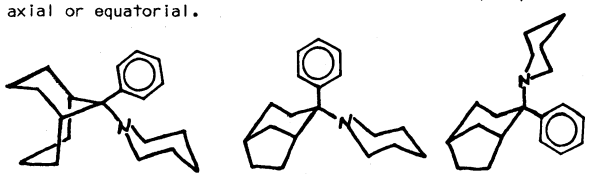
202.3 STEREOSPECIFICITY AND RECEPTOR SPECIFICITY OF PHENCYCLIDINE INTERACTIONS WITH RAT HIPPOCAMPAL PYRAMIDAL NEURONS. Paula C. Bickford, Michael R. Palmer, Kenner C. Rice*†, Barry J. Hoffer*, and Robert Freedman*. Dept. of Pharmacology, Univ. of Colorado Health Sciences Center, Denver, CO 80262, and †NIAMDD, Bethesda, MD 20205.

The effects of phencyclidine (PCP) on rat hippocampal pyramidal neurons were studied using electrophysiological techniques. The predominant response of most pyramidal cells to local micropressure application of PCP was a decrease in firing rate. Biphasic responses and pure excitations were occasionally observed. The stereospecificity of PCP responses was studied with (+) and (-) isomers of 3-methyl PCP (PCMP). The (+) isomer was 4-15 fold more potent than the (-) isomer.



Catecholamine depletion by reserpine pretreatment markedly increased the dose of PCP needed to elicit effects on pyramidal cell discharge. The α-adrenergic antagonist phentolamine reversibly antagonized PCP-induced inhibitions, while having no apparent effect on excitations. In contrast, timolol, a β-adrenergic antagonist, did not alter the depressions, but reversibly antagonized the excitations. Although the interaction of PCP with other neurotransmitter systems, in particular cholinergic systems, can not be ignored, these results suggest a major role of noradrenergic transmission in the observed effects of PCP on pyramidal cell activity. (Supported by USPHS Grant # DA-02429).

202.4 THE PHARMACOLOGY OF SOME NOVEL RIGID PCP ANALOGS. B. Pazhenchevsky,* S. Maayani, S.D. Glick and H. Weinstein* (SPON: J.P.Green). Dept. of Pharmacol., Mount Sinai Sch. Med., C.U.N.Y., New York, New York, New York, 10029. The PCP derivative in which the cyclohexyl portion was replaced by adamantyl (ADM-PCP), was found to be a more potent muscarinic antagonist than PCP. Since substitution of the phenyl group in PCP by nitro and amino groups produces marked changes in muscarinic potency, it was interesting to see whether comparable changes could be observed for the adamantyl PCP derivatives. We found that the 2-adamantyl derivative of PCP competes with ^{3}H-atropine binding in a non-competitive manner (Hill slope = 0.4), but that a meta-NH_2 substitution on the phenyl ring of ADM-PCP restores the competitive nature of the interaction. One of the possible explanations for the increased antimuscarinic activity of ADM-PCP, as compared to PCP, is in the position of the aryl moiety relative to the cyclohexyl ring. To investigate this hypothesis we prepared three new analogs of PCP. The [3.3.1]bicyclononane derivative contains the same structural characteristics as ADM-PCP. The [3.2.1]bicyclooctane derivatives contain a conformationally immobile cyclohexyl group and should be rigid models of the two conformers of PCP in which the phenyl is either axial or equatorial.



[3.3.1] [3.2.1] [3.2.1]

These compounds were also shown to have a much higher affinity than PCP for the muscarinic receptor. ADM-PCP was inactive in a behavioral test that characterizes PCP-like action (Glick et al., Eur. J. Pharmacol. 59:103, 1979). Because we have found that the ADM-PCP is only sparingly soluble in water it could not be expected to be potent in vivo. The solubility of the meta-NH_2 derivative of ADM-PCP was only slightly higher and did not yield an active compound. The physico-chemical properties of the other derivatives are being studied in conjunction with their central activities in order to discriminate between negative results related to uptake and distribution from measured inactivity resulting from the pharmaco-dynamic properties of the drugs. Supported by NIDA grant DA-02534.

202.5 BIOCHEMICAL AND BEHAVIORAL EVIDENCE FOR CHOLINERGIC AND ANTI-CHOLINERGIC PROPERTIES OF PHENCYCLIDINE IN THE RODENT. K. M. Johnson and S. C. Wilkenfeld*. Dept. of Pharmacol., Univ. Texas Med. Br., Galveston, TX 77550.

Anticholinergic properties of phencyclidine (PCP) have been identified in several isolated tissue preparations including rat brain membranes. Whether this in vitro effect is important in producing abnormal behavior in animals is uncertain. For example, the reports that muscarinic agonists inhibit and antagonists potentiate PCP-induced stereotypic behavior must be considered in the light that the same is true for apomorphine and amphetamine-induced stereotypy. Furthermore, mice made tolerant to PCP were found to be partially tolerant to some of the effects of physostigmine and oxotremorine (OXO), a muscarinic agonist.

In a preliminary evaluation of the effects of OXO on PCP-induced ataxia in the rat, we found that the combination of 1 mg/kg OXO & 10 mg/kg PCP produced profound exopthalmia, lacrimation, and salivation. Four of the six rats died within 45 min, apparently of asphyxiation. This effect was further characterized in experiments using ICR mice. OXO (1 mg/kg) in combination with 3, 10, and 30 mg/kg PCP caused death in 1/5, 5/5, and 10/10 mice, respectively. The lethality of this combination was completely blocked by 1 mg/kg methyl atropine (MA), an antagonist which does not easily enter the brain. This data suggests that in some peripheral tissues PCP is acting like a muscarinic agonist rather than an antagonist.

In order to test for a central muscarinic activity, we measured the effects of PCP and PCP in combination with low doses of OXO-MA (to block the peripheral effects of OXO) on forced, coordinated motor activity using an inverted screen test. PCP in doses of 3 mg/kg and above significantly impaired performance in this test and appears to be a good index of PCP-induced ataxia. Pretreatment with 0.3 mg/kg OXO-1.0 mg/kg MA significantly shifted the PCP dose-response curve to the left, suggesting a muscarinic agonist action of PCP in the CNS.

In direct distinction to these studies, we have confirmed that PCP has antagonistic properties in the rat striatum. In this experiment, we evaluated the effect of PCP on the ability of OXO (in combination with MA) to elevate striatal DOPAC levels. OXO-MA elevated striatal DOPAC levels 71% above saline controls. Pretreatment with 6 mg/kg PCP reduced this effect to a non-significant 13%. (PCP alone had no effect on striatal DOPAC compared to controls). By comparison, pretreatment with 6 mg/kg atropine sulfate reduced the OXO-MA elevation of DOPAC to 11% (N.S.). Thus, in the striatum, PCP does appear to have antimuscarinic effects. (Supported by DA-02073.)

203.1 GLUTAMATE AND POTASSIUM IONS CAUSE SWELLING OF MÜLLER FIBERS IN THE CHICK RETINA. A. Van Harreveld. Division of Biology, California Institute of Technology, Pasadena, CA 91125.

Spreading depression (SD) in the isolated chick retina is characterized by an increase in transparency of the tissue. An increase in transparency was also recorded after applying glutamate or potassium ions in the bathing solution. These compounds may be involved in glutamate- and K^+-based SDs (Van Harreveld, J. Neurobiol., 9:419, 1978). It was postulated that the increase in transparency is due to swelling of the Müller fibers, which have a radial position in the retina and which, acting as light guides, might in the swollen state enhance the amount of light transmitted through the retina.

Chick retinas were prepared by cutting the eye in the equatorial plane and removing the vitreous body. The eye was stored at 32°C in a physiological solution containing 10 mM $MgCl_2$ which suppresses spontaneous SDs. A block of the retina was then isolated and subjected to freeze substitution which preserves the water distribution in the tissue more faithfully than chemical fixation. Retinas treated for 2 min with 30 mM KCl or 1 mM L-glutamate were compared with controls frozen without such treatments. Similarly treated material was chemically fixed. The retinas were examined with the light- and electron microscope. The Müller fibers (measured halfway between the vitreal surface and the ganglion cell layer) in freeze-substituted preparations of glutamate- and K^+-treated retinas were much thicker than in the controls. The mean diameter of the Müller fibers of 4 preparations was 1.54 µM in K^+-treated retinas, 0.98 µM in glutamate-treated ones against 0.44 µM in the controls. This swelling may account for the increased transparency of the retinas observed both in glutamate- and K^+-based SDs.

The chemically fixed preparations of the same material showed only a slight difference in diameter of the K^+- and glutamate-treated preparations as compared with the controls. It seems that the swelling of Müller fibers which existed at the end of the 2 min treatment with K^+ and glutamate was reversed during chemical fixation.

This investigation was supported in part by NSF grant BNS 784142.

203.2 NEGATIVE FEED-BACK FROM HORIZONTAL CELLS TO CONES IN THE CARP RETINA. M. Murakami* (SPON: E. Cosmos). Dept. Physiol., Keio Univ. Sch. Med., Tokyo 160, Japan.

In the isolated, perfused retina of the carp, Cyprinus carpio, properties of a feed-back pathway from horizontal cells to cones were investigated by means of electrophysiological and neuropharmacological methods. When horizontal cells were hyperpolarized by peripheral annulus illumination, a depolarizing synaptic potential was produced in cones at the center, suggesting that horizontal cells receive inputs from cones and exert a negative feed-back to cones. On the other hand, when a transient depolarization (EPSP) was induced in horizontal cells by application of a transretinal current pulse flowing from the receptor side to the vitreal side, an IPSP was evoked in cones. The IPSP was abolished when the retina was perfused with a GABA-containing Ringer solution, probably because of desensitization of the feed-back synapse. GABA also hyperpolarized the cone cell membrane, indicating the presence of GABA-sensitive site in the cone. These results suggest that the GABA-mediated negative feed-back operates from horizontal cells to cones in the dark, and ceases to function in the light.

Effects of GABA and its antagonists, picrotoxin and bicuculline, were investigated on spectral responses of horizontal cells. When the GABA-mediated feed-back was desensitized or blocked by these chemicals, remarkable changes were observed in spectral responses, due to selective suppression of responses to long wave-lengths. In particular, the depolarizing responses in the biphasic C-type (H2) cell were abolished, while the hyperpolarizing responses to short wave-lengths were retained, resulting in a monophasic spectral response curve. From these results, it was concluded that the GABA-mediated negative feed-back plays an essential role in neural mechanisms which convert the trichromatic process at the level of cones into the opponent color process in horizontal cells.

(Supported by grants from the Ministry of Education of Japan, Nos. 421821, 448104 & 520920)

203.3 INTRACELLULAR RECORDING AND HORSERADISH PEROXIDASE MORPHOLOGY OF TREE SQUIRREL HORIZONTAL CELLS. J. Sherwood Charlton*, Harold F. Leeper* and Kenneth T. Brown. Univ. of California, San Francisco, CA 94143.

A new preparation has been developed for the study of mammalian retinal neurons using intracellular recording and horseradish peroxidase (HRP) injection. Several cell types have been recorded in the arterially-perfused eyecup preparation of the eastern grey (tree) squirrel, Sciurus carolinensis. This is a report of a study of horizontal cells (HC's). HC's respond to flashes of light with graded hyperpolarizations over at least a 4 log unit stimulus intensity range. Maximum responses of up to 35 mV amplitude were measured. In response to light stimuli of longer duration, hyperpolarizations decline to a plateau level. At the cessation of the stimulus, responses of most cells exhibit a rapid return to baseline (dark level). Others return slowly to baseline, possibly indicating rod input.

One morphological class of horizontal cell has been identified after staining with HRP. These cells exhibit the following characteristics: (1) The V-log I curves are single-limbed and the response returns rapidly to baseline at the cessation of illumination, suggesting that these cells are cone driven. (2) The dendritic fields of these cells are about 50 µm in diameter. (3) A single axon courses across the retina for more than 300 µm from the cell soma. (4) These cells have small receptive fields. Using a bar of light passed through the receptive field, length constants of 31 to 158 µm were measured. The mean was 95 µm, corresponding to a receptive field radius of about 400 µm, much smaller than found for HC's in mammalian and nonmammalian species.

The morphological features of these cells correspond to those of type 1 HC's identified in Golgi preparation by R. West (Vision Res., 18:129, 1978). West determined that this cell type contacts only cones. We measure a maximum spectral sensitivity in the green, with a λmax of 500 to 520 nm in these cone driven cells. This λmax is shifted from the 543 nm λmax of the only known cone pigment in this retina (Loew, E.R., J. Physiol., 251:48P, 1975). Our data suggest that the interactions responsible for an apparent blue sensitivity observed in our ganglion cell recordings and found in behavioral studies by P. Silver (Vision Res., 16:1235, 1976) may have begun by the horizontal cell level.

203.4 Development of Morphological and Biochemical Properties of the Dopaminergic System in Rabbit Retina. D.A. Redburn, C.K. Mitchell* and C.K. Hampton*. Dept. Neurobiol. & Anat. U. Tex. Med. Sch., Houston, TX 77025

Studies from our laboratory and others have demonstrated that in rabbit retina, the dopamine system is limited to a subset of amacrine cells with terminals located in a trilaminar arrangement in the inner plexiform layer. We have also shown that many of the retinal dopamine receptors are coupled to adenylate cyclase, thus suggesting that one consequence of dopamine transmission is an increase in intracellular cAMP formation. We have now analyzed the development of both pre- and post-synaptic markers of this dopamine system in rabbit retinas from one day to 30 days postnatal. Receptor activity was determined first in an in vitro binding assay using ^3H spiperone as a ligand and ADTN as a displacer. Secondly, dopamine stimulation of cAMP formation was assayed. Localization of endogenous dopamine was demonstrated by histofluorescence using the glyoxylic acid method. Specific increases in fluorescence after incubation in micromolar concentrations of dopamine were used as an indication of the high affinity uptake system for dopamine. Our results showed that at birth, endogenous and exogenously accumulated levels of dopamine were not detectable by histofluorescence, no appreciable amount of specific ^3H spiperone binding was observed, and dopamine had no effect on cAMP production. However, all pre- and post-synaptic dopamine markers showed dramatic increases in activity between post-natal days 5 and 9. Adult patterns of endogenous and exogenously-accumulated dopamine fluorescence were observed by day 12. In contrast, we observed a significant overshoot in receptor binding and dopamine-stimulated adenylate cyclase activity at days 7-9, followed by a decrease to adult levels by day 11. The apparent down regulation of dopamine receptors coincides with, and may be functionally related to, two other developmental processes which occur at day 11. First is the appearance of maximal, adult levels of GABA receptor activity. The second is the appearance of receptive field responses in ganglion cells which are directly and indirectly postsynaptic to both GABAergic and dopaminergic amacrine cells.

203.5 ULTRASTRUCTURAL LOCALIZATION OF SOMATOSTATIN-LIKE IMMUNOREACTIVITY IN GOLDFISH RETINAL AMACRINE CELLS. D. Marshak*, D. Lightfoot*, T. Yamada*, and W. Stell (Spon. B. Bastian) Jules Stein Eye Inst., UCLA School of Medicine, Los Angeles, CA 90024.

The retina is a well-characterized neural network in which we have identified amacrine cells containing somatostatin-like immunoreactivity (SLI) (Yamada et al. (1980) Proc. Nat. Acad. Sci., 77: 1691-95). We have studied their ultrastructure and synaptic connections by electron microscopic analysis of serial sections.

Retinas of dark-adapted goldfish were fixed by immersion for 2 hrs at 4°C in .08M phosphate buffer pH7.8 with 4% paraformaldehyde and .05% glutaraldehyde. Tissue-chopped sections (100 um) were stained by the peroxidase-antiperoxidase method using a rabbit antiserum directed against the midportion of somatostatin 14; therefore, the serum recognized both large and small forms of SLI. After labeling, osmication, and embedding, serial sections were cut both parallel and perpendicular to the distal sublamina of the inner plexiform layer, in which the labeled processes ramified. Some sections were stained with lead citrate and uranyl acetate.

Labeled, varicose dendrites with ultrastructure characteristic of amacrine cells were found within a few micrometers of the edge of the thick section. Electron-dense labeling was concentrated on the outer aspects of clear synaptic vesicle membranes, on the outer membranes of mitochondria, and on synaptic densities. Large, dense-cored vesicles, which were not associated with synaptic specializations, were seen in a few labeled processes; smaller, electron-lucent vesicles were more abundant in labeled processes and concentrated opposite synaptic densities. Bipolar cells, identified by their synaptic ribbons, received synapses from SLI-containing cells at points distal to the ribbons. Labeled cells were also presynaptic to processes which, unlike those of amacrine cells, were relatively electron-lucent, had few organelles, and were not presynaptic. These appeared to be dendrites of retinal ganglion cells, but their identities could not be confirmed. Labeled cells were both presynaptic and postsynaptic to processes from other, unlabeled amacrine cells, which were identified by their presynaptic specializations. Control sections incubated with antiserum preadsorbed with synthetic somatostatin were not labeled.

These results suggest that neurons containing somatostatin-like immunoreactivity act through at least three different pathways in the inner plexiform layer of the retina.

Supported by USPHS EY1190 to W. Stell

203.6 DISPLACED AMACRINE CELLS OF THE RETINA. E. V. Famiglietti, Jr. Dept. Anatomy, Wayne State Univ. Sch. of Med., Detroit, MI 48201.

It is universally accepted that there exist in the retina neurons generally similar in morphology to retinal ganglion cells, but lacking axons. Many have found it less easy, however, to accept the evidence that these "amacrine cells" may have their cell bodies "displaced" from the inner nuclear layer (INL) into the inner plexiform layer (IPL) or the ganglion cell layer (GCL). Most of the evidence to date on the existence of displaced amacrine cells is of a negative kind: lack of axonal staining in Golgi preparations, and failure of axonal transport to small cell bodies of tracers from the optic nerve. Recently, much stronger evidence has developed for the existence of one class of displaced amacrine cells which synthesize acetylcholine (Masland & Mills, '79; Hayden et al., '80). We have provided evidence from Golgi preparations that these are type b "starburst" amacrines, which always have cell bodies displaced to the GCL and branching in sublamina b of the IPL (Famiglietti & Siegfried, '80; Famiglietti, '81). Moreover, in an extensive survey of rabbit and cat retinas, we find a number of different morphological classes of displaced amacrine cells, both of type a and of type b: 3 classes in rabbit retina and 8 classes in cat retina. In addition, cell bodies of other amacrines are occasionally displaced from their conventional position in the INL.

Rabbit and cat retinas were prepared as whole mounts and processed according to a modified Golgi-Kopsch-Colonnier technique. Golgi-impregnated amacrine cells were studied in flat view. Their morphology and dendritic stratification was analyzed, in some cases with the aid of an image processing computer.

Rabbit retina contains at least two kinds of displaced amacrine cells, in addition to the type b starburst amacrines. One general class of amacrines, made up largely of displaced cells, is the "pseudopolyaxonal" amacrine (Famiglietti & Siegfried, '80). One subvariety has its cell body in the GCL and all of its branches in stratum (S) 5 of the IPL, while the other has its cell body most often in the middle of the IPL with its processes in S3. A third general class of displaced amacrine cell is morphologically less distinctive: wide-field and sparsely branched in S3.

In cat retina, medium-wide field, sparsely branched amacrine cells with cell bodies in the ganglion cell layer are of at least 2 varieties, and their dendrites are narrowly stratified in S1, in S3, or in S5. Type b starburst amacrines have also been found in cat, but thus far no "pseudopolyaxonal" amacrines. 5 other classes of displaced amacrines are medium- or narrow-field, have a variety of unusual morphologies, and branch either narrowly or broadly at various levels, collectively involving all 5 strata of the IPL. Because few examples of these 5 have been found, the occasional presence of their cell bodies in the INL cannot be excluded.

203.7 THE SYNAPTIC BASIS OF SURROUND ANTAGONISM IN OFF-CENTER GANGLION CELLS OF THE MUDPUPPY RETINA. Jack H. Belgum*, John S. McReynolds and David R. Dvorak* (SPON: L.T. Rutledge). Dept. of Physiol., University of Michigan, Ann Arbor, MI 48109.

Intracellular recordings were used to measure current-voltage relations for off-center ganglion cells under the following conditions: maintained darkness, central illumination, and surround illumination combined with existing center illumination. We have previously reported that center illumination causes a hyperpolarization associated with a large tonic conductance increase, and that this response has a reversal potential more negative than the dark membrane potential. While this response is predominantly due to activation of a tonic inhibitory input, a reduction in excitatory input from hyperpolarizing bipolars is also probably involved.

When surround illumination is added to existing center illumination, membrane potential is depolarized toward the normal dark potential, clearly antagonizing the center response. Current-voltage relations under these conditions show that there is also a decrease in conductance, and that the reversal potential for this depolarizing response is essentially the same as for the hyperpolarizing center response. This suggests that the surround stimulus acts through the same synaptic mechanism as the center response, shifting both inhibition and excitation back toward dark levels. In fact, at least some of the conductance increase must occur by reducing the light-activated inhibition, since the conductance decrease produced by surround stimuli can exceed the entire conductance of the cell in the dark.

Since the surround must, in part, reduce the light-activated inhibition, and since the center and surround effects reverse at the same hyperpolarized membrane potential, the most obvious conclusion is that surround illumination acts through the excitatory and inhibitory inputs that produce the response to center stimuli. Moreover, the changes in these two inputs in response to surround illumination must be in essentially the same proportion as the changes resulting from center stimulation. Since inhibition dominates the center response, it is apparent that disinhibition dominates the surround response. Because hyperpolarizing bipolars probably mediate the excitatory input, it is not surprising that this input possesses an antagonistic center-surround organization. The cells providing tonic inhibition have not been determined, but our results suggest that their receptive fields also have an antagonistic surround.

This work was supported by NIH grants EY 01653 and EY 07022.

203.8 CAT RETINAL GANGLION CELL RESPONSES TO SPATIOTEMPORAL WHITE NOISE. M. C. Citron, J. P. Kroeker* and W. R. Levick*. Bioinformation Systems, California Institute of Technology, Pasadena, CA 91125.

Until recently modelling of ganglion cell responses has been based mainly on linear analysis. This is satisfactory for those cells in which the processes of spatial summation of excitation are approximately linear (X-cells) but it fails for Y-cells where spatial summation is very nonlinear. Weiner kernel analysis is a general mathematical method of dealing with nonlinear systems by which the output as a function of time is represented as a power series. The coefficients are kernels of corresponding order. Our experiments demonstrate the praticality and effectiveness of applying Weiner kernel analysis to cat retinal ganglion cells.

To measure the kernels, a spatiotemporal white noise (STWN) stimulus was applied to the receptive field. The stimulus consisted of a 16 by 16 array of square picture elements (pixels) the intensity of each independently modulated by a 16 level uniform, random signal. The train of impulses constituting the ganglion cell's response was binned synchronously at the picture change rate (31.5 Hz).

The first order kernel for each pixel was estimated from the crosscorrelation function of the response with the stimulus intensity sequence at the pixel. The second order kernel for each pair of pixels was estimated from the crosscorrelation of the response with the product of the intensities at the two pixels. Higher order kernels were not calculated.

From the arrays of calculated kernels we predicted the cell's response to several traditional stimuli, such as: (1) a small spot flashed on for 0.5 sec against a uniform background at each of 121 position over the receptive field (2) alternating gratings of several spatial frequencies, Actual responses to these stimuli were also recorded for comparison. The predicted receptive field map, based on 24 min of recorded response to the STWN stimulus closely matched the recorded receptive field map based on 16 min of sequential testing. In addition, the distictive bimodal character of the recorded response to the alternating grating was clearly present in our predicted response (based on the same 24 min response as above) and could be attributed to the second order kernel component. Thus, within the limitations of the Weiner kernel analysis, a range of ganglion cell behavior can be predicted. The use of the resulting first and second order kernels may be sufficient to classify many different types of ganglion cells.

Supported in part by NSF grant BNS 7924730 and NIH grant EY 03462 to M.C.C.

203.9 THE NEUROPHYSIOLOGICAL BASIS OF PRESSURE PHOSPHENES; RESPONSES OF CAT RETINAL GANGLION CELLS TO EYEBALL DEFORMATION.
O.-J. Grüsser, U. Grüsser-Cornehls and U. Schreiter.
Lab. of Neurophysiology, Department Physiology, Freie Universität, Berlin, Germany (West) (SPON: M.M. LaVail)

Action potentials of single on-center and off-center neurons (X, Y) were recorded from the optic tract of anesthetized cats. The eyeball was mechanically deformed in total darkness at the temporal sclera for periods lasting 1 to 30 sec. Similar stimuli elicit in man pressure phosphenes first described by Alcmaion of Croton (5th century B.C.)

Two classes of neuronal responses were observed: An early activation appearing with a short latency of about 15-300msec after the beginning of the eyeball indentation or after the release of deformation. About 15 percent of the on-center and off-center neurons responded with such an early activation. On-center neurons were more frequently activated at the beginning of deformation than after the cessation of deformation. The opposite was found for the off-center neurons.

All retinal ganglion cells showed a "late" response to maintained deformation of the eyeball appearing with a latency of 0.8-2.8 sec. All on-center neurons (X, Y) exhibited (in total darkness) an activation period lasting 10-25 sec. All off-center neurons decrease their activity to 0 impulses/sec within 1-3 sec after the beginning of the deformation.

When the eyeball deformation was released during the late activation period of on-center neurons, the latter ceased abruptly and after a discharge pause of several seconds the neuronal activity of the on-center neurons slowly returned to the pre-deformation level. Some on-center neurons showed a transient overshoot of neuronal activity during this recovery period. Off-center neurons responded to the end of eyeball deformation with an activation period during which the neuronal activity in most neurons was higher than the pre-deformation level.

The late activation periods of on-center neurons and the corresponding inhibition of off-center neuron spontaneous dark activity are correlated well with the phosphenes filling the visual field after some seconds of sclera indentation. We assume that this activation of on-center neurons and inhibition of off-center neurons are caused by stretching of the horizontal cell membrane, which would lead to a depolarization of horizontal cells. The latter causes a depolarization of on-center bipolarcells and a hyperpolarization of off-center bipolar cells, which triggers the corresponding responses of retinal ganglion cells.

203.10 MONOCLONAL ANTIBODIES WHICH REACT SELECTIVELY WITH LARGE GANGLION CELLS.
S.E. Kornguth, R. Auerbach*, J. Grieves* and L. Kahan*.
University of Wisconsin, Clinical Science Center, Madison, Wisconsin 53706.

This report describes the preparation of highly specific reagents (monoclonal antibodies) which react selectively with large ganglion cells in retina. Large ganglion cells of mammalian retina are of particular interest because they are presumed to be the major cell population which relays information about moving targets from the retina to the brain. Hybridomas were generated by fusion of NS-1 myeloma cells (P3-X63-Ag8; Salk Institute) with spleen cells from BALB/CAu mice immunized against a cell preparation consisting primarily of large ganglion cell (>28 μm soma diameter) isolated from ox retinas. Of the 3840 culture wells prepared from four separate fusions, about 1500 showed positive growth. Preliminary screening of supernatants using a microElisa assay led to the selection of 30 antibody-producing populations. When these were tested by an immunoperoxidase histochemical method, immunoglobulins in two supernatants were found to react selectively with large ganglion cells. Stable clones from these two wells were subsequently isolated and expanded. The two monoclonal antibodies, one selective for the cytoplasm of large ganglion cells and the other directed at a cell surface component, appear to be both cell-type and species-restricted. On the one hand neither antibody was reactive on liver sections; however both were localized to ganglion cells on tissue sections, and could be absorbed by isolated ganglion cells. At the same time, the reaction of both monoclonal antibodies was markedly attenuated when tested on ganglion cells obtained from either dog or cat retinas, in contrast to earlier results obtained with conventional antibodies prepared in rabbits. By employing a highly enriched fraction of large ganglion cells both for the initial immunization schedule and for the microElisa screening assay we may have been able to prejudice both the generation and selection of hybridomas toward a particular neuronal type.

Supported by funds from the University of Wisconsin Medical and Graduate Schools and by a gift from Dr. and Mrs. Leonard Weiss.

203.11 LUMINANCE DEPENDENCE OF CAT SPATIAL VISION AFTER LESIONS OF AREA CENTRALIS.
T. Pasternak and D. Zehl. Center for Visual Science, University of Rochester, Rochester, NY 14627

The spatial contrast sensitivity and visual acuity of cats were measured behaviorally before and after argon laser lesions of the central retina. The lesions were bilateral, centered on the area centralis and 10° in diameter. The exact location of the area centralis and the dimensions of the lesions were determined from fundus photographs.

Spatial vision was tested over a 6 log unit range of luminances. Cats were trained under a two-alternative spatial forced-choice paradigm to discriminate vertical sinusoidal gratings from uniform fields of the same mean luminance. At the highest luminance tested (16 cd/m^2), the lesions resulted in reduced contrast sensitivity at higher spatial frequencies. Visual acuity dropped approximately ½ octave. As the luminance was reduced to scotopic levels spatial vision showed a similar impairment. Only at the lowest luminance tested (16 x 10^{-6} cd/m^2) were no deficits observed.

This result suggests that even at scotopic luminances the area centralis contributes to spatial resolution and contrast sensitivity of the cat. This may be accomplished by a relatively large proportion of rods present in the area centralis of the cat retina.

Supported by NEI grants EY01875 & P30 EY01319.

203.12 VISUAL ACUITY AND FOVEAL CONE DENSITY IN THE RETINA OF THE AGED RHESUS MONKEY.
J.M. Ordy, and K.R. Brizzee. Delta Regional Primate Res, Ctr., Covington, La. 70433 and Tulane University, New Orleans, LA 70118

Visual acuity plays a fundamental role in visually guided behavior of man and all diurnal primates. Acuity depends on optical and neural mechanisms. Optical factors include mechanisms for focusing an image on the retina. Neural factors include foveal cone and ganglion cell density, their ratio and eccentricity from the fovea, and a magnification factor in the geniculostriate system. Acuity has received considerable attention in studies of development. Only a small number of studies have dealt with changes in acuity in relation to changes in foveal cone density of the retina during aging. Specific aims of this study were to examine age differences in visual acuity among young adult (5 yrs.), middle age (12 yrs.) and aged (22 yrs.) rhesus monkeys in relation to foveal cone density in the retina. Acuity has been defined as the reciprocal of the least resolvable spatial detail measured in minutes of visual angle. The minimum separable binocular acuity mean of the young group was 0.83 ± 0.11, the middle aged acuity mean was 0.86 ± 0.12, and the acuity mean of the aged monkeys was 2.0 ± 0.70 minutes of visual angle. According to analysis of variance and multiple range tests, the 0.83' acuity mean of the young, and the 0.86' acuity mean of the middle age group did not differ significantly, whereas the 2.0' acuity mean of the old group differed significantly from the 2 younger age groups. Foveal cone density was determined norphometrically by assessment of cone inner segment (CIS) width, and absolute CIS number per 100μM along the horizontal meridian of the pure cone, rod-free 1° x 1° foveola. Foveal cone density decreased significantly from 44.16 per 100μM in the middle age group to 39.00 per 100μM in the old macaque group. Since the diurnal macaque is of the same taxonomic order as man, and the Visual systems of the two species are directly comparable in terms of acuity and the central receptive field organization of the retino-geniculostriate system, it may be concluded that the macaque may represent an attractive and valid model for studies of aging in photopic and scotopic vision of diurnal primates.
(Supported by NIH Grants RR00164-19 & HD09942).

203.13 MAMMALIAN EYE TRANSPLANTATION. M.J. Perlow. Dept. Neurology, Mt. Sinai School of Medicine, New York, N.Y. 10029.

Previous studies have demonstrated that mammalian fetal nervous tissues, when transplanted into the substance of an adult brain can survive for prolonged periods of time without rejection, grow in size and mature into adult-appearing tissues. Transplanted neurons can synapse with host tissues and, in some cases, alter the behavior of the recipient animal in a manner consistent with the normal function of the grafted tissue (Perlow, Science 204:643, 1979; Gash, Science 210:1367, 1980).

As a first step in determining if eye transplantation can restore vision to blind mammals, whole fetal eyes were transplanted into the brain of enucleated adult rats. Whole eyes with some ocular adnexal tissues were obtained from 14-15 day gest. Sprague Dawley (SD) rats and sterotaxically injected into the brains of adult SD rats. The injections were made such that the tip of the injection needle terminated close to the lat. geniculate nucl. or sup. colliculus, in the substance of the cerebral cortex or in the lat. cerebral ventricle. The rats were enucleated several days after transplantation. Several months after transplantation the animals were killed. Frontal sections of the formaldehyde-perfused, paraffin-embedded brains were stained with cresyl violet. While there is considerable conn. tissue proliferation and cyst formation, examination of some of these sections, and sections from an earlier study (Freed and Perlow unpublished data), show that fetal rat eyes when homologously transplanted into an adult rat brain can (1) survive for prolonged periods of time without rejection and (2) grow in size and develop a structural organization similar to an adult eye. The individual tissues mature to such a degree that adult-appearing ocular and adnexal tissues are readily recognizable (cornea, lens, vitreous humor, hair follicles, sebacous glands). Retinal elements are frequently observed in the form of rosettes. A retina-like layered structure was only occasionally observed.

Coupled with previous studies on embryonic tissue transplantation and the recent demonstration that transplanted fetal retina can develop and make appropriate topographical projections into the brain stem of enucleated rats (McLoon and Lund, Exptl. Brain Res. 40:273, 1980), this study suggests that fetal eyes might eventually be transplanted to an enucleated adult animal in such a manner that they might be able to send efferent axons to the host brain. The ability of transplanted retina or whole eyes to transmit visual information to the host brain remains to be determined.

204.1 OPTOKINETIC AND VESTIBULAR RESPONSES IN MEDIAL PONTINE NUCLEUS NEURONS IN ALERT MONKEY. E. L. Keller and W. F. Crandall*. Smith-Kettlewell Institute of Visual Sciences, San Francisco, CA 94115.

The brainstem pathways responsible for optokinetic eye movements and visual sensitivity to the motion of large patterned fields in vestibular nucleus neurons have been recently the subject of intense investigation. A pathway from the pretectum to nucleus reticularis tegmenti pontis (NRTP) seems to be a major link in mediating this behavior in the rat, but studies in monkey have failed to find single neurons in NRTP whose behavior would support the existence of such a pathway in the primate. We now report that a group of neurons located ventral to NRTP in the medial pontine nuclei of monkey have a strong response to optokinetic stimuli. Unique features of the response of this group of cells include the following. The neurons responded with directional selectivity to the motion of large visual fields with the eyes fixed on a tiny stationary target. Both vertical and horizontal directional preferences have been noted in the recorded population of units. These cells do not respond to either moving or stationary discrete visual stimuli. They were modulated when the eyes pursued a discrete visual stimulus moving in the same direction as the preferred direction of OK motion. This indicates an eye movement sensitivity. During OK tests with abrupt onset of constant-velocity OK drum rotation and the eyes fixed, the unit response began about 120 ms following drum rotation and reached maximum discharge almost instantly. Sinusoidal tests with OK stimuli showed that the responses only slightly lag peak stimulus acceleration in the preferred direction even at frequencies up to 0.5 Hz. Most of these units were also tested for vestibular response both with the eyes fixed on a small stationary target rotating with the animal and during normal vestibulo-ocular response in the dark. All tested cells showed a vestibular response during suppression and during normal VOR. The directional preference of the vestibular response was always synergistic with the OK visual response. In contrast to the visual response, the vestibular response was close to being in phase with peak head velocity. In conclusion we suggest that a neural pathway through the pontine nuclei may be important in mediating OK behavior.

204.2 CAT NEURONS RELATED TO HORIZONTAL EYE POSITION LOCATED VENTRAL TO THE 6TH NUCLEUS. R. S. Remmel and R. D. Skinner. Depts. of Physiology and of Anatomy, Univ. of Ark. for Med. Sci., Little Rock, Ark. 72205.

Cats were prepared for alert recording by surgically implanting head-mounting bolts and a chamber over the cerebellum for micropipette insertion. A coil of wire was placed around the right eye for eye-position recording by the magnetic search coil technique. A bipolar electrode was permanently inserted into the right 6th-nerve root in the basis pontis.

In recording sessions the cat's head was bolted into position and an extracellular micropipette was lowered according to stereotaxic coordinates. The predominantly negative extracellular pulses and eye position signals were recorded on magnetic tape and photographed from an oscilloscope. The right 6th nucleus was located by observation of the field potential and of motoneurons antidromically excited by nerve stimulation. The depth of neurons could thus be accurately determined relative to this field potential. This report concerns neurons located within 1.5 mm ventral of the 6th nucleus. None of the described neurons could be antidromically excited from the 6th nerve.

Tonic neurons had firing rates which increased with rightward (ipsilateral) eye angle, with rates seldom exceeding 100 spikes/s. Tonic neurons usually had a threshold angle, to the right of which they began firing. Changes in firing rate usually preceded the start of saccades. Some tonic neurons showed a small increase or decrease in firing rate during saccades (a burst or pause), which was less pronounced than for most motoneurons. The correlation between eye position and firing rate was better for some neurons than for others.

Burst neurons were located near tonic neurons. The bursts for rightward (ipsilateral) movements typically had 18 spikes (26 maximum) and average frequencies during the burst of 170 spikes/s (230 maximum). Bursts began in general before and continued during saccades. Longer bursts with more spikes occurred for larger rightward saccades, while few or no spikes occurred for leftward or vertical saccades. Burst neurons were usually not tonically active. When the cat made a staircase of closely spaced saccades, the neuron produced corresponding bursts.

These neurons seem qualitatively similar to those in the paramedian pontine reticular formation of monkeys.

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204.3 OCULOMOTOR NEURAL RESPONSES TO SINUSOIDAL HORIZONTAL LINEAR ACCELERATION IN MONKEYS.

R.Eckmiller.Division of Biocybernetics,University of Düsseldorf,D-4000 Düsseldorf,West Germany.

Sinusoidal time courses of linear acceleration perpendicular to the gravity vector were applied to Java monkeys sitting upright in a primate chair, by means of a slide track with feedback control. Movement frequencies ranged from 0.1 to 1.0 Hz. Maximum acceleration reached values of 300 cm/sec^2. The angle δ between the monkey's X-axis(ant. - post.) and the slide track axis could be varied ($\delta = 90^o$ for pure right-left movement). Single unit activity in the abducens nuclei and their vicinity was recorded along with horizontal EOG's from each eye independently. The monkeys were kept under light barbiturate anesthesia in order to reduce spontaneous eye movements to occasional slow drift movements. In a few cases small electrolytic lesions were made, and were later used for histological verification of the recording site in Celloidin sections.

Many brain stem neurons with a regular tonic impulse rate(IR) showed a strong dynamic response to linear acceleration, although their IR was not modulated by eye movements or be rotatory accelerations. These neurons indicate the existence of pure otolith afferents close to the abducens nuclei. Quantitative analysis of presumed abducens motoneurons revealed: 1. IR of all these motoneurons showed a slight increase in response to a chair acceleration to the contralateral side(at $\delta = 90^o$) and a decrease in the opposite movement phase, comparable to a α - otolith response. Rotatory acceleration stimuli around the vertical axis always led to much stronger IR-modulation. 2. IR-modulation gradually increased with increasing movement frequency. The gain relative to acceleration, however, decreased with increasing frequency. 3. The phase lag of IR relative to acceleration increased from almost 0 deg at 0.1 Hz to over 90 deg at 1 Hz. The phase lag at a given frequency varied considerably for different neurons. 4. Sinusoidal linear acceleration at different angles δ between 0^o and 180^o revealed that maximal IR-modulation was reached at about $\delta = 90^o$.

The results suggest a significant utricular input to primate abducens motoneurons.

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204.4 MORPHOLOGY AND SYNAPTIC CONNECTIONS OF CAT OCULOMOTOR MOTONEURONE AXON COLLATERALS. R.F. Spencer, C. Evinger, and R. Baker, Dept. Anat., Med. Coll. of Virginia, Richmond, VA 23298, and Dept. Physiol. & Biophys., New York Univ. Med. Ctr., New York, NY 10016

Recent morphophysiological studies have demonstrated that all medial rectus and inferior rectus and some superior rectus motoneurones (MNs) in the cat oculomotor (Oc) nucleus have axon collaterals that arborize within the soma-dendritic domain of the MN of origin. Electrical stimulation of the IIIrd nerve, however, produces antidromic activation of Oc MNs without direct recurrent synaptic effects upon the MNs. The present study examined the morphology and synaptic connections of Oc MN axon collaterals stained by intracellular injection of horseradish peroxidase (HRP) and reconstructed from serial sections by light and electron microscopy.

In all cases, collaterals arose from the parent axon at a node of Ranvier and were unmyelinated throughout the extent of their course. The initial portion of the collateral was contacted by synaptic endings that contained either spheroidal or flattened synaptic vesicles. "Swellings" along the course of the collateral contained spheroidal synaptic contacts and, in some cases, presumed en passant synaptic contacts with postsynaptic membrane specializations were established predominantly with dendritic profiles. Collaterals were either unbranched or divided into 2 to 4 branches. Terminal branches established synaptic contacts with somata and dendrites of neurones both within the Oc nucleus and ventrolateral to the MLF.

The results indicate that a single MN axon collateral establishes synaptic connections with several Oc neurones. In contrast to spinal MNs, collateral synaptic endings of Oc MNs never were observed in relation to the soma-dendritic profiles of the MNs stained by intracellular HRP or other MNs labelled by retrograde HRP. In some cases, the postsynaptic neurone was identified as an Oc internuclear neurone (IN) labelled by retrograde HRP from posterior brain stem nuclei (i.e., abducens, prepositus hypoglossi). Given additional efferent projections of Oc INs to other brain stem nuclei (e.g., reticularis tegmenti pontis, facial, accessory abducens) and the cerebellum, synaptic connections mediated by Oc MN axon collaterals may be related to both oculomotor and facial motor systems.

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204.5 CONTRACTILE PROPERTIES OF SINGLY INNERVATED MUSCLE UNITS CONTROLLED BY OCULOMOTOR NUCLEUS MOTONEURONS IN THE CAT. M.A. Meredith and S.J. Goldberg. Dept. Anatomy, Med.Col.Va.-VCU, Richmond,VA.

Conjugate eye movements are precise and synchronous in nature. Therefore, it has been presumed that the extraocular muscles involved in the generation of such movements present similar contractile properties. While the characteristics of lateral rectus motor units have been reported, the present experiments were conducted to examine the contractile properties of muscle units controlled by motoneurons which reside in the oculomotor (OC) nucleus.

Cats were anesthetized and the OC nerve was stimulated in the brainstem. In the orbit, the medial (MR), superior (SR) and inferior rectus (IR) and inferior oblique (IO) muscles were freed from their insertions and attached to individual strain gauges. The length of each muscle was adjusted to maximize its isometric twitch contraction tension. Cells in the OC nucleus were identified by their antidromic response to OC nerve stimulation. Single OC cells were penetrated with a micropipette and those which elicited contractile responses in a muscle, when intracellularly stimulated, were identified as motoneurons (MN) of that muscle. A variety of stimulus patterns were presented to the OCMNs and the subsequent muscle unit responses were recorded.

100 OC nucleus motoneurons were identified; 60 of which innervated MR muscle units, 20 controlled IR units, 15 were SR units and 5 were IO units. The contractile properties of singly innervated units from each of these muscles were similar: mean contraction time = 5.64 msec (range = 5.35-5.89 msec) and mean fusion frequency = 245 Hz (range = 235-260 Hz). These values for oculomotor motor units, however, are faster and higher, respectively, than reported for lateral rectus motor units. A recent report indirectly confirmed the authenticity of these contractile distinctions between OC and AB units: OC innervated retractor bulbi muscle fibers were faster and presented higher fusion frequencies than abducens nerve controlled fibers in the same muscle.

It has been demonstrated that extraocular muscle units innervated by OCMNs exhibit different contractile properties than reported for units controlled by MNs in the accessory and principal abducens nuclei.

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204.6 AN IN VITRO PREPARATION OF THE MAMMALIAN OCULOMOTOR NUCLEUS. C. Evinger and J. Hounsgaard*. Dept. Physiology and Biophysics, New York Univ. Med. Ctr., 550 First Avenue, New York, N.Y. 10016

In alert animals the discharge patterns of oculomotor neurons are well documented. Nevertheless, little is known as to whether this pattern emerges solely from synaptic inputs or whether the motoneuron itself introduces some discharge characteristics in converting the synaptic input into action potentials. Resolution of this problem requires a detailed knowledge of motoneuron membrane properties. This abstract reports an in vitro slice preparation which has several advantages for studying oculomotor neuron properties and synaptic inputs. The guinea pig midbrain was sliced into 400 micron sagittal sections and bathed in an oxygenated Ringer's solution maintained at 37°C. These slices contained the oculomotor and trochlear nuclei and the medial longitudinal fasciculus (MLF). Despite the potential trauma of slicing, the neurons exhibited properties similar to those found in in vivo preparations. The input resistances, resting potentials, and spike height were normal. Intracellular injection of depolarizing current produced repetitive firing which adapted to a steady firing frequency within 50 msec of current onset. Consistent with in vivo studies, the neurons did not exhibit a primary range of repetitive firing. The slicing did not interrupt synaptic function since stimulation of the MLF generated postsynaptic responses in neurons and spontaneous synaptic potentials were observed. An advantage of the in vitro preparation was the stability provided by the absence of cardiovascular and respiratory pulsations. This allowed extended intracellular recordings from all elements in the oculomotor complex (including astroglia). Another advantage was the ease of altering the extracellular environment to study neuron properties. For example, application of tetrodotoxin (TTX) revealed the presence of a slow, TTX insensitive spike in oculomotor neurons. Thus, the normal behavior of neurons, the stability of recording, and the ease of modifying the extracellular environment suggest that the slice may be a useful tool in studying the oculomotor system.

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205.1 POSTEMBRYONIC MODIFICATIONS IN THE TELEOST RETINA. Pamela Raymond Johns. Dept. Neurobiol., Harvard Med. Sch., Boston, MA 02115.

Last year I reported that new rods are generated and inserted within differentiated regions of adult goldfish retina (Neurosci. Abst.6:639). Thymidine radioautography has revealed that dividing rod progenitor cells are scattered among mature rod nuclei in the outer nuclear layer (onl) in goldfish and also in Haplochromis burtoni, an African cichlid. I have now traced the ontogenesis of rods in the larval teleost retina and find that there, too, the formation of rods is unique.

Goldfish, newly-hatched to one-year-old, were injected with ^{3}H-thymidine and killed a few hours to several months later. Identification of photoreceptors in methacrylate sections was aided by bleaching the melanin in the pigmented epithelium with $KMnO_4$/oxalic acid, by staining with basic fuchsin and by assessing the level of incorporation of ^{3}H-fucose into outer segments.

At hatching, the photoreceptor layer in the goldfish retina is poorly differentiated. Outer segments are absent and the onl is a single row of columnar nuclei. These cells, which are post-mitotic, differentiate into cones. Rods appear at 3 days post-hatch and then gradually accumulate. The ratio of rods to cones is zero at hatching, 1:1 at 40-50 days, 6:1 at one yr. and 15:1 at 4 yrs. Rod nuclei throughout the retina were labeled with ^{3}H-thymidine injected at any age. Repeated divisions of labeled rod progenitors resulted after several months in about a 10-fold increase in the number of labeled rods over the number at 24 h following a pulse label. Dividing rod progenitors found in the onl of the larval retina were similar to those described previously in the adult. These cells derive from what appear to be clusters of undifferentiated neuroepithelial germinal cells whose nuclei are sequestered in the inner nuclear layer (inl). These neurogenic clusters are readily seen in larval retinas; they are easily overlooked in retinas from older fish where the "cluster" has often been depleted to a single remaining cell whose shape is sometimes like a Muller cell. Beginning at 3 days post-hatch, and continuing into adult life, proliferating cells appear to cross the outer plexiform layer from inl to onl. In the onl they become rod progenitors. This migration of cells is reflected in a progressive increase in the proportion of labeled nuclei in the onl compared with the inl, both with longer survival times and with advancing age at injection.

Preliminary observations of retinal histogenesis in another fish, the Japanese Medaka (Oryzias latipes), indicate that the retina is more precocious in this species: at hatching rods have already begun to accumulate, though they have not yet attained their final density. Delayed formation and continued addition of rods is probably a universal feature of retinal histogenesis in teleost fish. (Supported by EY00301).

205.2 TRANSPLANTATION OF NEURAL TISSUES IN THE SPINAL CORD OF THE ADULT RATS. G. D. Das* (SPON: R. W. Wallace). Dept. of Biological Sciences, Purdue University, West Lafayette, Indiana 47907.

During past several years we have demonstrated that embryonic neural tissues can be successfully transplanted invarious regions of the brains of neonatal or adult host animals. The transplants in the cerebellum or the forebrain region of the host animal grow differentiate and become anatomically integrated with the host brain. They are intraparenchymal transplants. This report is addressed to the nature of growth and anatomical integration of the neural transplants in the spinal cord of the adult rats.

In this study Long-Evans hooded rats were used. From embryos of 16 or 18 days gestational stages neural tissues from neocortical region, tectum, cerebellum and spinal cord were removed for transplantation. They were transplanted in the cervical or thoracic regions of the spinal cord of 4-6 months old host animals. The techniques of preparation of embryonic neural tissues, host animals, and transplantation were similar to those described earlier (Das, 1974; Das et al., 1979). Most of the host animals were sacrificed 4-6 months after transplantation, and some were kept for longer survival intervals. From each condition of transplantation portions of spinal cord, where the transplants were deposited, were removed and prepared for cresyl-violet stain Bodian stain, or Golgi-Cox impregnation. In order to study afferents to the transplants, in some animals surgical lesions were made rostral or caudal to the site of transplantation, and the spinal cords containing the transplants were processed for Fink-Heimer method of staining degenerating fibers.

Neural tissues from tectum, cerebellum or spinal cord of embryos did not survive in the spinal cord of the host animals. They were isolated and necrotic. The neural tissue from embryonic neocortical region survived, grew and became anatomically integrated with the host spinal cord. It contained normal-looking well-differentiated pyramidal and stellate cells. It was anatomically continuous with the gray matter of the spinal cord, and a number of fibers could be seen to course through the interface. At the regions where the transplant was apposed to the white matter of the host spinal cord a band of neuroglial cells was seen. Fibers were not seen to penetrate through this glial mass. Comparison between neural transplants in the spinal and those in the cerebellum or forebrain regions of the host animals indicated that the bands of glial cells observed in these cases were due to the modification in the technique which involved making lesions in the spinal cords just prior to the insertion of the transplants.

These findings showed that it is possible to transplant neural tissues in the spinal cord successfully, and that they receive afferents from the immediately available fiber tracts of the spinal cord.

205.3 TRANSPLANTATION OF ADRENAL CHROMAFFIN CELLS TO THE ADULT RAT BRAIN, W.J. Freed, J.M. Morihisa,* H.E. Cannon-Spoor,* L. Olson, A. Seiger, B.J. Hoffer,*, and R.J. Wyatt. (SPON: J.R. Stevens) Adult Psychiatry Branch, National Institute of Mental Health, Washington, D.C. 20032.

Adrenal Chromaffin Cells develop elongated processes and become morphologically similar to neurons when grafted to the anterior eye chamber or when grown in culture in the absence of corticosteroids (Olson, L., et al., Exp. Neurol. 70:414, 1980; Unsicker, K., et al., Proc. Natl. Acad. Sci. USA 75:3498, 1978). We have previously found that homografts of fetal rat substantia nigra (SN) in the lateral ventricle of rats with unilateral SN lesions can partially reinnervate the caudate nucleus and reduce apomorphine-induced rotational behavior. The purpose of the present study was to determine whether adrenal medulla grafts in the rat brain lateral ventricle can also reduce rotational behavior by reinnervating the caudate nucleus.

Unilateral SN lesions were produced in Sprague-Dawley rats by stereotaxic administration of 6-hydroxydopamine. Only rats with baseline rates of rotational behavior of at least 80 turns per 40 minutes after S.C. injections of 0.1 or 0.25 mg/kg of apomorphine HCl were used. Some of these animals (N=13) received intraventricular grafts of pieces of adrenal medulla and other (control) animals (N=15) received grafts of pieces of sciatic nerve. Two months after grafting, rotational behavior was again studied, and was found to be decreased in the animals that had received adrenal medulla grafts.

Falck-Hillarp fluorescence histochemistry showed surviving grafts in five of the six animals examined. Many of the cells had become elongated or had developed short, coarse processes, but few thin nerve fiber-like processes had been formed. Very few of these fibers had penetrated into the host caudate nucleus.

The findings indicate that grafts of adrenal medulla can reduce lesion-induced rotational behavior without reinnervating the caudate nucleus. This suggests that the behavioral effect was due to secretion by the graft of catecholamines or other substances which passively diffused to receptor sites in the caudate nucleus.

205.4 ALTERATION IN CORTICOSPINAL MICROCIRCUITRY FOLLOWING PYRAMIDOTOMY IN RAT. Deborah L. Claman* and Jerald J. Bernstein. Lab. CNS Inj. & Regen. V.A. Medical Center, Washington, D.C., Depts of Neurosurg. & Physiol., George Washington Univ. Sch. of Med.

Rat corticospinal tract (CS) somata lie in layer Vb somatomotor cortex. CS subpopulations may project to forebrain, midbrain, and pontine nuclei in addition to having multisegmental spinal cord projections. While most spinal cord injury affects both ascending and descending pathways, the CS tract can be transected without primary injury to the afferents on the receptive surface of the corticospinal neurons; somatic deafferentation is then due to axotomy. The present experiments continue a series of light and electron microscopic observations on the response of cortical layer Vb neurons to pyramidotomy at midmedulla. Since some CS neurons were partially deafferented by three days postoperatively (DPO) 4 additional groups of animals (N=5 per group) and 2 normals were prepared for electron microscopic analysis from 3 hours through 30 days after the pyramidal tract was unilaterally transected. The immediate effects of axotomy including the time course of somatic presynaptic bouton loss and glial mobilization are presently examined. Several features of the cellular reaction are apparent by 24 hours after surgery. Pathological nuclear crenations with abnormal condensations of nuclear chromatin on the inner nuclear membrane and associated vacuolated cytoplasm along the outer membrane are observed. There is also an abnormal clumping of nuclear chromatin in axotomized CS neurons. Presynaptic bouton ghosts with associated membrane profiles (somatic stripping) may be found by 1 DPO. Reactive and dividing neuroglia and increases in microglia are apparent. From 3 through 14 DPO an increasing number of neurons react to pyramidotomy. There are 3 correlations between bouton loss and neuroglial reaction: neuroglial cell processes may intervene between presynaptic bouton and bouton somata; neuroglial cell processes may be contiguous with postsynaptic specializaions without presynaptic boutons; neuroglial somata may overlie former postsynaptic specializations. The appearance of postsynaptic membrane specializations continues through 30 DPO. Between 14 and 30 DPO axonal growth cones are observed in depressions on the CS cell surface; they appear in areas of degeneration where there is vacated space. The persistence of postsynaptic membrane specializations and the observation of growth cones indicate that the CS neurons of origin may be receptive to reinnervation. Reinnervation might be a determinant for neuronal survival amongst those CS cells which do not necrose forming the basis of a reorganized spinal projecting neuronal pool for regeneration. (Supported by (NS-16979).

205.5 ABERRANT AXONS FROM THE DORSAL ROOTS AFTER CHRONIC SPINAL HEMISECTION IN THE CAT: THE DORSAL HORN, M.S. Beattie, J.C. Bresnahan and F.J. Liuzzi. Div. Neurosurg. and Dept. Anat., Ohio State Univ. Sch. of Med., Columbus, OH 43210

McCouch, et. al. (J. Neurophysiol, 21:205, 1958) were the first to describe apparent axonal sprouting of the dorsal root system in response to a rostral spinal hemisection. These observations were supplemented by those of Murray and Golberger (JCN, 158:19, 1974). The latter authors found evidence for terminal proliferation mainly in the base of the dorsal horn. In an attempt to further these observations of apparent axonal sprouting in the spinal cord following hemisection, we have employed the method of anterograde injury filling of the dorsal roots with horseradish peroxidase (HRP; Proshansky and Egger, Neurosci. Lett., 5: 103, 1977; Light and Perl, JCN, 186:117, 1979; Beattie, et. al., Brain Res., 153:127, 1978). This method provides a Golgi-like appearance of dorsal root axons suitable for observing axonal trajectories and morphological features. Additionally, the method allows subsequent electron microscopic analysis of the tissue.

HRP was applied bilaterally to cut dorsal rootlets rostral and caudal to a spinal hemisection (T13-L2) in 9 cats which had survived for 6 weeks to 3 months postoperatively. Most of the lesions included significant damage to the ipsilateral dorsal columns.

We observed two types of apparently aberrant axons at levels from one to seven segments caudal to the lesion. In several cases, large collaterals from labelled dorsal column axons were seen to diverge from their normal dorsal-ventral trajectories through the superficial dorsal horn to traverse laminae I and II in a lateral to medial orientation. These axons exhibited some large swellings along their course. Smaller straight fibers were seen to radiate from the apex of the dorsal horn in an atypical fashion, often ignoring their typical laminar distribution to the dorsal spinal gray. These axons also had occasional swellings along their course. Such axons were very rarely observed in normal material or contralateral to the lesion.

Since axons projecting rostral to the lesion were cut in these cases, it is not yet clear whether these abnormalities represent collateral axonal sprouting in response to denervation, collateral 'pruning', or both. Electron microscopic studies are underway to determine if these aberrant axons make synaptic contact within the superficial dorsal horn.

(Supported by Grants NS-14457 and 10165).

205.6 ACTIVITY OF MOTONEURONS AND INTERNUCLEAR NEURONS IN THE ABDUCENS NUCLEUS FOLLOWING PERIPHERAL AXOTOMY OF THE VIth NERVE. J.M. Delgado-Garcia*, R. Serra*, J. Ribas* and R. Baker (SPON: U. Kuhnt). Dept. Physiol. & Biophys., Seville Med. Sch., Seville, Spain and New York Univ. Med. Ctr., New York, NY 10016.

Our prior study demonstrated that the antidromic field potential recorded in the abducens nucleus following VIth nerve stimulation was reduced by more than 80% following peripheral axotomy. These changes began 2-4 days after axotomy, peaked at 10-15 days and returned to control values about 25-30 days later in parallel with recovery of muscle function. The present experiments were designed to record the activity of antidromically identified motoneurons and internuclear neurons in the abducens nucleus from the onset of axotomy to reinnervation. In seven cats stimulating electrodes were implanted intracranially upon the VIth nerve and in the medial rectus subdivision of the oculomotor nucleus. Eye movements were recorded in both eyes with the magnetic search coil technique. During the critical axotomized period, it was only possible to antidromically activate abducens motoneurons when adequate visual (optokinetic) and/or vestibular (head rotation) stimulation produced eye movements towards and/or maintained eye positions in the on-direction (i.e. abduction). Even then motoneurons showed a remarkable variability in antidromic latency as well as response to double shock stimulation indicating the need for additional synaptic input to compensate for the decrease in initial-segment excitability. Even so, the activity of axotomized motoneurons was qualitatively normal during most eye movements such as saccades, vestibulo-ocular reflex and fixation; however they clearly differed in their quick fatigability during fixed eye position and notably higher recruitment thresholds. Few motoneurons exhibited tonic activity for eye positions in mid-oculomotor range. On occasion, axotomized abducens motoneurons discharged before saccades in the off-direction without exhibiting any tonic activity for the preceding eye position. Quite in contrast, the activity of internuclear neurons and all eye movements produced by both the ipsi- and contralateral medial rectus muscles remained normal. Given the intermingled distribution of motoneurons and internuclear neurons in the abducens nucleus, we conclude that in spite of the altered physiological responses of motoneurons, the effects of axotomy are restricted to those cells. In addition, since these cells share common afferent input we conclude that supranuclear cells (e.g. inhibitory reticular and vestibular neurons) maintain normal physiological activity in spite of morphological and functional detachment of their synapses from motoneurons during the critical period.

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205.7 DIVERGENT NEUROPHYSIOLOGICAL AND AUTORADIOGRAPHIC SIGNS OF LESION-INDUCED REORGANIZATION IN THE ADULT CAT LATERAL GENICULATE NUCLEUS. U.Th. Eysel, U. Mayer* and U. Wolfhard*. Institute of Physiology, University of Essen, D-4300 Essen 1, West Germany.

Neurophysiological mapping of the retino-geniculate topography in layers A and A_1 of the adult cat lateral geniculate nucleus (LGN) more than 30 days after partial deafferentation by nasal retinal lesions yielded lesion-induced spreading of excitation from the normally innervated region into the formerly deafferented part of layer A by 200 µm beyond normal (Eysel et al., Brain Research, 181:285, 1980).

An autoradiographic approach was chosen to investigate possible morphological correlates. A strip of retina with blood supply and intact innervation was limited in the upper part by chronic photocoagulator lesions (survival-times 60 and more days) continued by subacute lesions (5-7 days survival-time) in the lower part. The width of this strip was constant. The intersection of the two types of lesions was at the elevation of the zero horizontal meridian. The projection of this area onto the LGN was marked by electrolytic lesions prior to the injection of 1mCi 3H-Proline into the eye. Autoradiographs of paraffin embedded serial sections were analysed. The width of the band of label in layer A of the contralateral LGN anterior to the electrolytic lesion (chronic deafferentation) was compared to measurements in the posterior part (subacute deafferentation). No increase of the spread of label in the range of 200 µm could be observed at the border of chronic deafferentation.

In the LGN of the cat, relay cell dendritic fields and optic tract input arborizations are overlapping in the range of 200 µm. Subthreshold peripheral inputs at the dendrites of deafferented cells may become able to excite the cells as a result of only small presynaptic changes (axonal sprouting, translocation of synapses towards the cell soma) or even without changes demonstrable with autoradiography (denervation supersensitivity). A completely different, possible mechanism could be the "axonization" of relay cell dendrites (Hâmori and Silakov, Neurosci.,5:2073,1980).The result emphasizes that structures not showing extended axonal sprouting may utilize other mechanisms to reactivate deafferented cells at quite a distance from the remaining normal cells. (Supported by the DFG, Ey 8/9).

205.8 FORELIMB PLACING DEFICITS CAUSED BY DAMAGE TO THE INTERNAL CAPSULE ARE REVERSED BY CORTICAL SPREADING DEPRESSION. David L. Wolgin and Priscilla Kehoe,* Dept. of Psychology, Florida Atlantic University, Boca Raton, Fla. 33431

Following unilateral damage to the internal capsule, rats failed to use the contralateral forelimb in reflexive placing reactions. In the most severely impaired animals, the deficit included loss of proprioceptive, tactile, whisker, chin, and visual placing. Spreading depression induced by applying potassium chloride to the sensorimotor cortex contralateral to the lesion reinstated placing in the impaired limb and abolished placing in the normal limb. As the spreading depression dissipated, placing in the impaired limb gradually deteriorated, while placing in the normal limb returned. In contrast, potassium chloride applied to the ipsilateral cortex did not reinstate placing. These findings suggest that the loss of forelimb placing following capsular lesions is due to tonic inhibition from the cortex contralateral to the lesion.

205.9 SUSTAINED FACILITATION OF EVOKED POTENTIALS IN THE CEREBRAL CORTEX FOLLOWING SHORT TRAINS OF HIGH-FREQUENCY STIMULATION, K.S. Lee. Max Planck Institute for Psychiatry, Munich, West Germany

An in vitro slice preparation containing the hippocampus and portions of the cerebral cortex was developed to examine the effect of brief bursts of high-frequency stimulation on cortical evoked potentials. Extracellular recordings were taken from layer III or IV of six cortical areas and evoked potentials were monitored in response to stimulation of the subjacent white matter. Following the delivery of short trains of repetitive stimulation (100-200 per sec for 0.5 sec), the peak negativity of the cortical potentials (i.e. the first calcium-sensitive component of the waveform) was increased in amplitude and usually showed a decreased latency. The increase in amplitude was usually non-decremental after 15-20 minutes post-stimulation; and, its stability was closely correlated with the capacity to induce a stable potentiation of hippocampal evoked potentials in the same slice. While the majority of experiments examined area 18, five other cortical areas exhibited similar changes. In some cases, these changes persisted for up to 4 hours, which was the longest post-tetanic interval tested. The non-decremental nature of the facilitation during this interval, however, suggests that the enhancement of cortical potentials could persist for a much longer period.

205.10 CHRONIC LITHIUM TREATMENT ATTENUATES RECOVERY FOLLOWING DAMAGE TO THE MESOTELENCEPHALIC DOPAMINERGIC SYSTEM. M. R. Kozlowski, J. Grisham*, J. F. Marshall. Department of Psychobiology, University of California, Irvine, CA 92717

This study examines the effect of chronic lithium treatment on behavioral recovery following damage to the mesotelencephalic dopamine (DA)-containing system. Male Sprague-Dawley rats were given lithium carbonate (1.25 mg/ml) in their drinking water for 3 weeks. The animals were then given an injection of 6-hydroxydopamine (6-OH-DA; 4.5 μg/2.25 μl) into the left ventral tegmental area in order to damage the ascending dopaminergic fibers. One half of the animals were maintained on lithium-free water for 4 weeks postoperatively (control group) while the other animals continued to receive water containing lithium (lithium group). All animals were tested for their orientation to somatosensory stimulation by applying a 4 g von Frey hair to 11 regions on each body surface. Orientation contralateral to the 6-OH-DA injection was expressed as percentage of ipsilateral orientation.

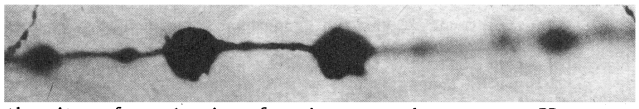
The intracerebral 6-OH-DA injection caused a large decrease in contralateral orientation to somatosensory stimuli in both groups at 3 days postoperatively (contralateral orientation 15% of ipsilateral). However, animals in the control group showed significantly greater contralateral orientation at 4 weeks postoperatively (90% of ipsilateral) than the lithium group (36%; t-test, $p < .01$).

The treatment of the two groups was reversed at four weeks postoperatively so that the lithium group now received unadulterated water while the control group was given water containing lithium. No change in the extent of recovery of contralateral orientation was seen in the control group during the subsequent 4 weeks. In contrast, the lithium group showed a 40% increase in orientation to contralateral stimulation during this time. This improvement was evident by 5 d after termination of lithium treatment and plateaued by 3-4 weeks. The extent of contralateral orientation in the lithium group at 8 weeks postoperatively (85% of ipsilateral orientation) was significantly greater than before termination of the lithium (t-test, $p < .001$) and did not differ significantly from that of the control group.

These results suggest that lithium interferes in a reversible fashion with the development of the neural changes underlying recovery after DA system damage. Chronic lithium administration is known to block striatal DA receptor proliferation following interruption of DAergic neurotransmission. Its effects on other forms of neuronal compensation for the injury are unknown. However, the present results are at least consistent with the hypothesis that DA receptor proliferation contributes to recovery following DA system damage.

206.1 EPILEPTIFORM HIPPOCAMPAL GRANULE CELL ACTIVITY IS NEUROTOXIC TO CELLS WHICH RECEIVE MOSSY FIBER INPUT. Robert S. Sloviter, Dept. of Pharmacology, Penn State Univ., Col of Med, Hershey PA 17033.

Sustained intermittent electrical stimulation of the perforant path (PP) of urethane-anesthetized rats for 24 hrs evokes epileptiform granule cell discharges and reproduces the selective pattern of hippocampal damage caused by kainic acid and other chemical convulsants (Sloviter and Damiano, Fed. Proc. 40:309, 1981; Neuropharm., in press, 1981). The present studies describe the nature of acute hippocampal pathology caused by continuous, relatively short duration PP stimulation at 20 Hz. Two hours of granule cell spiking results in abolished recurrent inhibition (RI) of granule cells as measured with the twin-pulse technique. Light microscopy of Nissl-stained sections also reveals severe damage to hilar interneurons. In the CA3 pyramidal cell region, a distinct band of pericellular spaces (presumably swollen glial elements) encompasses the cell body and proximal apical dendritic regions which receive mossy fiber input. Similar clear spaces often surround the somata in the CA1 region. In addition, relatively clear circular profiles are evident in the dentate molecular layer and in the distal apical- and distal basal dendritic regions of CA1. Light microscopy of hippocampi stained with the Rapid Golgi method shows these round, clear profiles to be cross-sections of spherical dendritic expansions (photo) which occur at



the sites of termination of excitatory pathways, e.g., PP, commissural and mossy fibers. If 2 hr-stimulated rats are allowed a 2 hr "rest" after stimulation and before perfusion, the dendritic expansions and pericellular swellings in CA3 and CA1 disappear, leaving hilar interneurons and CA3 pyramidal cells irreversibly damaged. *RI is still abolished at this time.* Rats were also stimulated at 20 Hz for 15 min to determine which of the two cell types are damaged first. This resulted in decreased RI and damage to hilar interneurons without involvement of CA3. Stimulation without granule cell spiking does not cause this pattern of damage. These results indicate that excessive activity in the hippocampal mossy fiber pathway is neurotoxic to the cells which receive this activity. In addition, dentate interneurons which may mediate recurrent inhibition of granule cells are the cells most sensitive to the neurotoxic effects of granule cell discharge. These results confirm the hypothesis of a causative relationship between epileptic neuronal activity and epileptic brain damage.

206.2 LOSS OF INHIBITORY SYNAPSES AT THE EDGES OF CHRONIC CORTICAL SLABS CAUSES THEIR EPILEPTIC SUSCEPTIBILITY. R. J. Reiffenstein and C. E. Ribak. Anaesthesia Research, McGill Univ., Montréal, PQ H3G 1Y6, and Dept of Anatomy, Univ. of California, Irvine, CA 92717.

Chronically denervated slabs of cat cerebral cortex that exhibit epileptiform activity have been studied by electron microscopy. Although previous ultrastructural, electrophysiological, and biochemical studies had indicated a persistence of GABAergic inhibitory synapses, the data from the present study indicates that the density of these synapses depends upon the location in the cortical slab. Somata of pyramidal neurons located adjacent to the edges of the slabs had few, if any, symmetric (GABAergic inhibitory) synapses. Instead, these neurons' somata were almost completely enveloped by layers of processes from reactive astrocytes. In contrast, pyramidal neurons in the center of the slabs appeared to have an almost normal number of terminals forming inhibitory, symmetric, axosomatic synapses, and no astrocytic layers surrounding them. Pyramidal somata located about 1 mm from the edges of the slabs had an intermediate number of such terminals. This loss of inhibitory synapses appears to have been selective, because the number of terminals forming asymmetric axodendritic (excitatory) synapses remains fairly constant all across the slabs, although fewer than in normal cortex. This correlates with the biochemical studies which have shown a loss of glutamate in chronic slabs. The selective loss of terminals forming symmetric synapses at the edges of the slabs indicates a localized loss of GABAergic inhibition. This is consistent with data showing a reduction in GABA content in the chronic slabs. Thus this loss of GABA probably occurs mainly at the edges of the slabs. Also the epileptic "focus" in the chronic slabs is likely to be pyramidal neurons that are (1) located at the edges of the slabs, (2) denuded of most or all of their axosomatic inhibitory terminals, and (3) projecting relatively active excitatory axons toward the centers of the slabs. It may be that the aspinous stellate cells which give rise to the GABAergic terminals are more sensitive than other neurons to the local anoxia which probably accompanies the isolation procedure.

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206.3 A SELECTIVE LOSS OF INHIBITORY, GABA TERMINALS IN MONKEY EPILEPTIC FOCI. C.E. Ribak, R.M. Bradburne*, and A.B. Harris, Dept. of Anatomy, Univ. of Calif., Irvine, CA 92717 and Dept. of Neurol. Surgery, Univ. of Wash., Seattle, WA 98195

Previous immunocytochemical results of five monkeys with cortical focal epilepsy produced by alumina gel showed a severe decrease at seizure foci of axon terminals that contained glutamic acid decarboxylase (GAD), the synthesizing enzyme for the inhibitory neurotransmitter, GABA. These data indicated a functional loss of GABAergic inhibitory synapses at seizure foci, but did not show 1) whether this loss was caused by GABAergic nerve terminal degeneration or by a lack of GAD synthesis in GABAergic neurons and 2) whether this loss was selective for only this terminal type. To answer these questions, epileptic and non-epileptic cortical tissue from 3 of the 5 monkeys used in the previous study were re-examined in the electron microscope. The terminals that formed axosomatic synapses with layer V pyramidal cells and those terminals and glia in the adjacent neuropil were outlined on electron micrographs and measured with a Zeiss Videoplan image analysis system. The following statistically significant changes were noted. First, axosomatic symmetric synapse decreased from an average of 1.32 per 10 μm of somal surface in nonepileptic cortex to 0.25 per 10 μm of somal surface in epileptic cortex. This reduction represents an average loss of 80% of these terminals. Second, in the neuropil adjacent to these pyramidal somata, the number of terminals forming asymmetric and symmetric synapses in epileptic cortex was reduced 20% and 50%, respectively, in comparison to nonepileptic cortex. Lastly, a 50% increase of glial profiles occurred in epileptic cortex both in the neuropil and at sites adjacent to pyramidal cell somata. These data indicate that the previously observed loss of GABAergic nerve terminals at sites of focal epilepsy is caused by terminal degeneration. Also, since GABAergic terminals are more severely reduced at epileptic foci than other terminals, it appears that this selective loss of GABAergic terminals could cause seizure activity due to a selective loss of inhibitory function at epileptic foci. Finally, the most severe loss of GABAergic terminals occurred adjacent to pyramidal cell somata. Since these terminals chiefly arise from basket cells which may have high physiological activities, their loss suggests that these cells are most sensitive to trauma and/or ischemia. This notion is consistent with recent data from hypoxic monkeys which had selective degeneration of GABAergic terminals.

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206.4 ANALYSIS OF NEURONAL INTERACTIONS IN NORMAL AND EPILEPTOGENIC BARREL CORTEX OF THE RAT. O.M. Sgro and R.N. Harner. Dept. of Neurology, Graduate Hospital, Univ. of Pennsylvania, Philadelphia, PA 19146.

Spontaneous and stimulus-related extracellular units were recorded at 420-1145 μ from the barrel cortex of 10 pentobarbital-anesthetized rats (6 mg/100 g) using a quadruple probe of high impedance (10 Megohms at 1 KHz) tungsten microelectrodes (spaced 50-200 μ apart) before and after the topical application of sodium penicillin (100,000 U/ml) to the cortex. Units were stimulated at rates of 0.5-1.0/sec by contralateral vibrissa displacement using a galvo-pen assembly. Unit activity was stored on FM tape for subsequent write-out and analysis using PDP-11/34 programs for the detection and classification of units and their interactions. Units were heterogeneous with respect to amplitude, duration, spontaneous bursting, relation to surface ECoG activity and stimulus sensitivity. Of the 34 pre-penicillin units investigated, 18 showed post-stimulus latencies of 9.9-27.8 msec with only 9 out of a possible 61 spontaneous or stimulus-induced interactions. After penicillin, 30 of 37 units were stimulus sensitive with post-stimulus latencies of 9.9-40.9 msec. Cross-interval histograms revealed 51 "tight" or "variable" one-way and two-way interactions with peak latencies of 0.5-12.0 msec.

Neuronal interactions recorded from nearby microelectrodes in normal somatosensory cortex were infrequent as was found in auditory cortex (Dickson, J.W. and G.L. Gerstein, J. Neurophysiol., 37:1239-1261, 1974). However, topical application of penicillin to the cortex strikingly increases interactions among nearby neurons. This method of analysis provides a reliable classification of units and their interactions which may prove useful in the further understanding of normal and epileptogenic mechanisms in cerebral cortex.

206.5 AN INVESTIGATION INTO THE MECHANISM OF ANTICONVULSANT INDUCED ELEVATIONS IN ENDOGENOUS γ-HYDROXYBUTYRATE CONCENTRATION IN BRAIN. O. C. Snead, III. Department of Pediatrics, Neuroscience Program, University of Alabama in Birmingham, Birmingham, Alabama.

Ethosuximide (ETX) and sodium valproate (SV) are anticonvulsants used specifically for petit mal epilepsy. Single dose administration of these drugs results in an elevation of brain concentration of γ-hydroxybutyric acid (GHB) a naturally occurring epileptogenic metabolite of γ-aminobutyric acid (GABA) (Snead, Neuropharmacology 19:47, 1980). The object of these experiments was to examine possible mechanisms of these anticonvulsant-induced changes in GHB concentration in rat brain.

SV and ETX were given alone and in combination with the GABA-T inhibitors aminoxyacetic acid (AOAA), γ-vinyl GABA (GVG) and γ-acetylenic GABA (GAB) and whole brain concentrations of GHB determined by electron capture gas liquid chromatography. In addition, the effect of SV, ETX and the enzyme inhibitors on the formation of ^{3} {H} GHB from ^{3} {H} GABA given intercerebro ventricularly (ICV)(Gold and Roth, J. Neurochem. 28:1069, 1977) was determined. Also the effect of ICV GABA, putrescine, and 1,4 Butanediol on endogenous brain concentrations of GHB was examined.

SV and ETX both produced a 60-70% increase in steady state brain levels of GHB. GAG and GVG administration also significantly elevated brain GHB concentration. The GABA-T inhibitors potentiated the anticonvulsant-induced increase in brain concentrations of GHB. The formation of ^{3} {H} GHB from ^{3} {H} GABA was augmented by SV, unchanged by ETX, and depressed by the GABA-T inhibitors. GABA and putrescine both produced significant elevations in brain GHB content but this increase was blocked by the GABA-T inhibitors. The 1,4 Butanediol also produced a significant elevation of GHB concentration. However, this was not blocked by GABA-T inhibitors.

These data suggest that ETX and SV may differ in the way in which they interact with GHB. The experimental results also raise the possibility that there may be a precursor for GHB in brain other than GABA.

206.6 EPILEPTIFORM BURST TERMINATION AND THE AHP IN HIPPOCAMPAL CA1 PYRAMIDAL CELLS. B. E. Alger and R. A. Nicoll, Depts. of Pharmacology and Physiology, UCSF, San Francisco, CA 94143.

Epileptiform bursting can be produced in the hippocampal slice preparation by perfusing slices with GABA antagonists. The burst descharge is followed by a potassium dependent after-hyperpolarization (AHP). We have recently reported that the AHP in hippocampal CA1 cells is substantially reduced by intracellular diffusion of the calcium chelator EGTA, the duration of the AHP being shortened by 60 - 80%, suggesting that the epileptiform burst AHP in CA1 cells is in large part a calcium dependent potassium potential. However two related issues remained unresolved: First, the nature of the early part of the AHP and second, the mechanism of burst termination. We wished to determine if the early part of the AHP is truly resistant to EGTA, i.e., due to another mechanism, or if it is generated at a site remote from the somatic EGTA injection. We have examined the early component of the AHP using bevelled electrodes containing 3 M KCl and 0.2M EGTA. We have confirmed our original report that the AHP is drastically shortened when EGTA diffuses into the cells over a period of 10 - 20 minutes. However in a number of cells the early phase is abolished, presumably due to the infusion of larger quantities of EGTA with these electrodes. This suggests that in these cells a remote calcium-dependent potassium potential contributes to the early phase of the AHP. Nevertheless, even in cells in which EGTA blocked the AHP, the duration of the depolarizing burst was not prolonged. Another mechanism might therefore be responsible for burst termination. Thus in 7 cells once the full effect of EGTA had been obtained we switched to a perfusion containing 2.5 - 5 mM tetraethylammonium (TEA). TEA reversibly prolonged the burst and no trace of the AHP remained. As reported by Schwartzkroin and Prince (Brain Res. 185:169, 1980), we find that TEA application to cells recorded without EGTA in the electrode increases the duration of the burst depolarization but does not affect the late portion of the postburst AHP.

We conclude that while the major part of the AHP is a calcium dependent potassium potential, the evidence suggests that potassium potentials play two roles following the epileptiform burst: 1) a TEA sensitive potential acts primarily to limit burst duration and 2) a late, calcium dependent potassium potential, while possibly contributing to burst termination acts primarily to limit the frequency of bursting.

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206.7 MECHANISMS OF DEPOLARIZATION SHIFT GENERATION IN THE PENICILLIN-TREATED NEOCORTICAL SLICE. M.J. Gutnick*, B.W. Connors and D.A. Prince. (SPON: R.W. Angel)Stanford Med. Sch., Stanford,CA 94305.

The cellular manifestations of interictal epileptogenesis are synchronous, large amplitude, prolonged depolarization shifts (DSs) in neurons of the epileptic aggregate. To investigate these potentials further, we obtained intracellular recordings from coronal slices (350-500 μm) of guinea pig sensory-motor cortex maintained in vitro (37°C). Penicillin (3.4 mM) increased the amplitude and duration of postsynaptic potentials (PSPs) evoked by focal stimulation of the cortical surface or underlying white matter. Spontaneous DSs were never seen; however, stimuli evoked DSs which were very similar to those recorded in vivo. Large amplitude (20-50 mV) long duration (50-300 msec) DSs usually triggered short trains of fast spikes and were followed by a long (up to 2 sec) afterdepolarization associated with a large conductance increase. DSs occurred in an all-or-none manner, and by adjusting stimulus parameters alternation of DS generation and failure could be obtained. In these instances each stimulus generated a PSP of invariant latency. The intervals between PSPs and subsequent DSs fluctuated widely and were as long as 100 msec. Simultaneous recordings from pairs of neurons up to 1000 μm apart (n = 15) showed a remarkable synchrony of DS generations, failures and latency shifts. Paired neuronal and glial recordings suggested that large (2-3 mM) slowly decaying (1-2 sec) increases in $[K^+]_o$ occurred simultaneous with DS generation. The data indicated that DSs occurred synchronously in a radially-oriented column of neurons. Intracellular current injections did not alter DS latency or probability of occurrence. When voltage dependent Na^+ conductances were blocked with intracellular QX-314, changes in DS amplitude during intracellular current injection indicated a very high peak conductance and an extrapolated reversal potential of about -10mV. Following Cs^+ injection neurons could be depolarized above 0 mV, and stimuli then evoked short latency inverted PSPs followed by paroxysmal hyperpolarizing potentials with a latency and frequency of occurrence identical to those of DSs evoked at rest. Our data indicate that synaptic currents play a major role in DS generation in neocortical neurons. These and other data from normal neocortex (Connors, Gutnick & Prince, this vol.) lead us to suggest that a small, interconnected subpopulation of neurons with intrinsic bursting capabilities, located in the middle cortical layers, serves as the pacemaker for DS generation. Convulsant-induced disinhibition may allow these endogenous bursters to activate synchronously and favor the spread of ramifying, recurrent excitatory drives to deeper and more superficial neurons.

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206.8 PHENYTOIN EFFECTS ON ACTION POTENTIALS OF FETAL MOUSE SPINAL CORD NEURONS IN CELL CULTURE. M.J. McLean* and R.L. Macdonald, Dept. of Neurology, University of Michigan, Ann Arbor, MI 48109

Effects of phenytoin (DILANTIN,DPH) on action potentials and strychnine-induced paroxysmal activity were investigated using mouse spinal cord (SC) and dorsal root ganglion (DRG) neurons in primary dissociated cell culture. Cell were mechanically dissociated from fetal (12-13.5 days gestation) tissue and cultured for 1-3 months prior to electrophysiological study. Experiments were performed in phosphate-buffered balanced salt solutions (PBS). Intracellular recordings were made using 4M KAC containing glass micropipettes.

Recording from SC neurons bathed in 1 mM $MgCl_2$-containing PBS revealed spontaneous synaptic activity and variable rates of action potential bursting. Large paroxysmal discharges (PDE) were recorded from most cells after exposure to strychnine (10^{-6}M). DPH abolished PDE and reduced spontaneous activity to quiescence in a dose and time dependent manner starting from 2.5 μg/ml (~10 μM) at 90 min. Higher doses produced maximal effect earlier and prior to reduction of maximal rates of rise of action potentials ($\dot{V}_{max}$). Action potentials could be elicited from quiescent cells by intracellular current pulses, but repetitive firing was limited. Preincubation with DPH prevented strychnine-induced PDE.

To investigate the mechanism of these effects, we tested DPH in clinically relevant anticonvulsant concentrations on action potentials recorded in 10 mM $MgCl_2$-containing PBS to reduce spontaneous activity. $\dot{V}_{max}$ of both SC and DRG neurons was ~215 V/sec. DPH reduced this value for both cell types to ~100 V/sec in a dose and time dependent manner with maximal reduction at about 90 min and concentrations ranging from 2.5-40 μg/ml (~10-160 μM). Hyperpolarization by intracellular current pulses (using a bridge circuit or two microelectrodes) increased $\dot{V}_{max}$ to about initial levels. Long depolarizing pulses in SC neurons produced trains of action potentials sustained for the duration of the pulse (up to 500 msec). DRG neurons were not observed to fire repetitively unless injured. DPH reduced repetitive firing to only a few action potentials which decreased in rate of rise and amplitude without changes in resting membrane potential. Limitation of repetitive firing occurred prior to the reduction of $\dot{V}_{max}$. These changes may be due to accumulation of voltage-sensitive sodium channels in the inactivated state.

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206.9 ELECTROPHYSIOLOGICAL AND BIOCHEMICAL CORRELATES OF FELINE GENERALIZED PENICILLIN EPILEPSY. Y.S. Siatitsas*, N.M. van Gelder and C. Menini* (SPON: Y. Lamarre). Montreal Neurological Institute and Université de Montréal, Quebec, Canada.

One or more of the following amino acid anomalies are associated with seizure prone nervous tissue: (1) a diminution of nervous tissue glutamic acid; (2) an exaggerated increase of glutamic acid in the biofluids or cortical superfusates; and (3) Some evidence exists that in association with the development of epilepsy, a disturbance occurs in the regulation of taurine and glutamic acid levels which normally are balanced relative to each other in nervous tissue. However no attempt has been made to test whether such metabolic changes may also accompany feline generalized penicillin epilepsy. If metabolic changes of this type are to be considered as close correlates of an epileptic condition, they must be demonstrated to precede the electrographic manifestation of the epilepsy. To test this hypothesis a sensitive method is needed to predict in terms of a physiologic parameter the onset of seizures and to correlate this with the onset of metabolic changes thought to lead eventually to seizures. Recent studies have demonstrated (35th Ann. Meet. Am. EEG Soc., Chicago, 1981) that an increase in the amplitude of the visual evoked potentials can predict with a 10 min accuracy the first electrographic appearance of epileptic discharges induced by systemic penicillin in the cat. This made it possible to biochemically sample cortical areas from the same animal before, at onset and after development of epilepsy. It was found that both glutamic acid and aspartic acid levels consistently diminished before the electrographic occurrence of epilepsy. After the development of epilepsy nervous tissue levels of these amino acids were further decreased, this now also being accompanied by a decrease in tissue taurine content and an increase in GABA. These findings, as well as the observation that a strong imbalance between tissue content of taurine and glutamate precedes the electrographic manifestations of epilepsy, suggest once more an involvement of glutamic acid in the epileptic process. Moreover, the temporal coincidence of changes in glutamic acid and the first increase of the visual evoked potentials seems to support a suggestion of a "cause and consequence" relationship.

206.10 TAURINE, GLUTAMATE AND EPILEPSY. Douglas W. Bonhaus*, Hugh E. Laird and Ryan J. Huxtable*. Dept. of Pharmacology, College of Medicine and Department of Pharmacology and Toxicology, College of Pharmacy, University of Arizona, Tucson, AZ 85721.

Taurine (2 amino ethane sulfonic acid) is a neuroinhibitory amino acid which may be a physiological modulator of neuronal excitability. Van Gelder has found correlations between the concentrations of taurine and glutamate in the brain and has proposed that taurine effects its neuroinhibitory action by altering the metabolism of glutamate. However, no biochemical relationship between taurine and glutamate has been established. We have found correlations in the concentrations of a variety of amino acid pairs in the brain (see Table), including those which are apparently not metabolically related. For example, the correlation coefficient between concentrations of taurine and glycine in rat brain cortex was 0.78, for the taurine-glutamate pair the correlation coefficient was 0.69. These correlations between concentrations of metabolically unrelated amino acids suggest that the relationship between taurine and glutamate may be due to the sharing of the same kinetic compartment. Thus, an increase in a kinetic compartment shared by glutamate and taurine would result in increased concentrations of both of these neuroactive amino acids. In the epileptic rat differences in amino acid correlations are found in brain regions having low electroshock threshold, such as the inferior colliculi, as compared to brain areas having higher electroshock threshold such as cortex. If these differences in correlations represent differences in compartmentalization of amino acids, then the proper compartmentalization of neuroactive amino acids such as taurine and glutamate may be important in the modulation of neuronal activity. The epileptic rat has a defect in taurine transport. In vitro synaptosomal uptake is decreased 50% and in vivo uptake of taurine into certain brain areas is decreased as much as 70%. Since some human epileptics have a defect in taurine transport and because the only biochemical difference that we have found between epileptic rats and normal rats is a difference in taurine uptake, we are currently investigating whether this defect is responsible for the seizure susceptibility of epileptic rats.
(Supported by USPHS grants NS 14405 and HL 13994)

AMINO ACID CORRELATIONS

Amino Acid Pairs	C.Coef	Amino Acid Pairs	C.Coef.
Taurine-Glutamate	0.69	Serine-Alanine	0.62
Alanine-GABA	0.85	Serine-GABA	0.60
PEA-Glutamate	0.66	Serine-Glycine	0.61
Threonine-glycine	0.69	Aspartate-Taurine	0.67

206.11 ISONIAZID INDUCED SEIZURES AND REGIONAL CEREBRAL ENERGY METABOLISM. D.W. McCandless*, M.S. Abel*, and F.C. Schwartzenburg, Jr.* (Spon: J. DeFrance). Dept. of Neurobiology and Anatomy, Univ. of Texas Medical School at Houston, Houston, Texas 77030.

Isoniazid is a useful chemical convulsant in that it produces a reproducible seizure pattern, and unlike maximal electroshock, the preseizure state can be examined. In the present study, we have induced seizures in mice using isoniazid and examined energy metabolism in highly discrete brain regions. 20-22 gram female Swiss-Albino mice were injected IP with 200mg/kg isoniazid, and sacrificed 25 minutes after injection (preseizure), or at the onset of seizures (40 minutes). The brains were removed at -20°, and cerebral cortex and cerebellum were sectioned into 20 micron thick sections, and freeze-dried overnight at -40°. Subsequently the sections were brought to room temperature under vacuum and freehand dissected into layer I and pyramidal layer from the cerebral cortex, and molecular and Purkinje cell rich layers from the cerebellum. Using enzymatic cycling, selected energy metabolites were measured directly in these 4 discrete brain regions in controls, and preseizure and seizing isoniazid injected mice. Results from this study showed a decrease in ATP in the pyramidal layer of preseizing animals. ATP in the remaining areas was not decreased as compared to controls. Phosphoncreatine in preseizing mice was decreased in both cortical layers, but not in the cerebellum. In seizing mice, both ATP and phosphocreatine were decreased in all layers examined, but the percent decrease in metabolites was greatest in the cortex. Within the cortex, the greatest decrease in ATP and phosphocreatine occured in the pyramidal cell region. These data indicate that the cortex and cerebellum respond differently to isoniazid induced seizures, and may reflect the role of each in seizure production.

206.12 COLCHICINE INDUCED SEIZURES: MICROTUBULES AND EPILEPSY. J.C. Oakley*, S.R. Hameroff, and A.F. Reynolds, Div. of Neurol. Surgery and Dept. of Anesthesiology, Univ. of Arizona, Tucson AZ, 85724

We have initially tested the hypothesis that effects upon intraneuronal microtubules (MT), calmodulin, or associated proteins may produce epileptiform activity in rat cerebral cortex. Concentrations of colchicine 10^{-3}M and greater placed in a solution of 1-1.5% agar were applied to the pial surface of sensorimotor cortex in awake, locally anesthetized, paralyzed 0.2-0.4 kg rats. Electrical activity was characterized by epidural recording from four stainless steel screw electrodes located around the site of colchicine application and two in the homotopic contralateral hemisphere. The focal discharges were additionally characterized by extracellular single unit recordings in the region of the focus, which revealed typical burst discharges. Concentrations of colchicine 10^{-2} in agar or less required an incubation time of at least 2 hours to produce epileptiform activity. As concentrations were increased, the time to produce electrographic spiking decreased where a 10^{-1}M solution in agar generated activity within 1 minute. Agar alone, or vinblastine in agar did not cause seizures. Colchicine induced seizures were also observed in rats treated with neostigmine, a cholinesterase inhibitor.

Convulsant preparations attempting to model focal human epilepsy include topical convulsant metals, drugs, antimetabolites, freeze lesions, and electrical stimulation. To date no single mechanism unifies all models, however data from topical penicillin and alumina cream preparations suggest rises in intra-neuronal Ca^{2+} may mediate epileptic effects (Wyler & Schwartzkroin, Annals of Neurology 7:95, 1980). Colchicine inhibits MT polymerization and functions including axoplasmic transport and causes disassembly of MT-calmodulin arrays (Means & Dedman, Nature 285:73, 1980). Calmodulin mediates Ca^{2+} effects on MT and other organelles and regulates intracellular Ca^{2+} by membrane, MT, and Ca^{2+} binding effects. Some seizure causing metals (Al^{3+}, Co^{2+}, Fe^{3+}, Fe^{2+}, Hg^{2+}, tungstate) bind to MT, displace Ca^{2+}, and may cause MT dysfunction and depolymerization (Purpura et al, Exp. Models of Epilepsy, Raven Press, 1972; Reid & Sypert, Brain Res 188:531, 1980; Bonhaus et al, Toxical Letts 6:141, 1980). Anticonvulsant diphenylhydantoins and some barbiturates are known to bind to MT associated proteins and prevent colchicine induced disassembly (MacKinney, et al, J Pharm Exp Ther 204:189, 1978; Edstrom et al, Cell Tiss Res 162:35, 1975). Colchicine induced seizures could be mediated by alterations in MT trophic regulation of membrane proteins, or increased intra-neuronal calcium ion concentration caused by membrane protein effects or disassembly and release of MT-calmodulin bound Ca^{2+}.

207.1 THE A5 CATECHOLAMINE CELL GROUP: AN ELECTRICAL AND CHEMICAL STIMULATION STUDY. J.J. Neil and A.D. Loewy, Dept. Anat. & Neurobiol., Washington Univ. School of Medicine, St. Louis, MO 63110

The A5 catecholamine cell group projects to the intermediolateral cell column and to medullary sites (Brain Research, 174: 309 [1979]). Electrical stimulation of the A5 region elicits a rapid rise in blood pressure with no change in heart rate. However, electrical stimulation excites axons of passage as well as cell bodies. To activate the A5 cells more selectively, we have stimulated with monosodium-L-glutamate (L-glu).

The L-glu was mixed in artificial CSF (containing procion yellow to mark the injection sites) and pressure injected from a double-barrelled pipet with a tip 60-90 μ m across. One barrel, filled with agar/KCl, was used for electrical stimulation. In midbrain-hemisected rats anesthetized with Nembutal, electrical stimulation of the A5 region (10 μ A, 60 Hz, 0.5 ms pulses, 5s trains) consistently elicited a pressor response with no change in heart rate, as before. However, application of L-glu in 30 nl volumes led to a dose-related decrease in blood pressure and heart rate. The threshold for the depressor response was about 10 mM L-glu, and a maximal depressor response of 30 mm Hg was elicited at 500 mM. Similar L-glu concentrations were necessary to activate the motoneurons of the rat facial nucleus. The fibers of the seventh nerve, passing quite close to the area of injection, were not activated by the L-glu. Injections of 1.0 M NaCl in procion yellow or procion yellow alone had no effect on heart rate or blood pressure.

To determine if either the pressor or depressor response involved catecholamines, these experiments were repeated in midbrain-hemisected animals whose catecholaminergic neurons were destroyed with intraventricular 6-hydroxydopamine. While the pressor response to electrical stimulation was still present in these animals, the bradycardia and depressor response to L-glu were abolished.

These results suggest that activation of cells of the A5 region leads to a decrease in blood pressure and heart rate. (Supported by USPHS grant HL-25449 and GM 07200 and a grant-in-aid # 80-723 from the American Heart Association)

207.2 HYPOTENSION AND BRADYCARDIA AFTER STIMULATION OF THE RABBIT BRAINSTEM REGION CONTAINING THE A1 CATECHOLAMINE NEURONS: A NEW CNS VASODEPRESSOR AREA. W.W. Blessing and D.J. Reis. Lab. of Neurobiology, Cornell Univ. Med. Coll., New York, NY 10021

Lesions of the ventrolateral medulla at the level of the area postrema cause hypertension and pulmonary edema in rabbits (Blessing et al., Circ. Res. in press 1981). Since the region contains A1 catecholamine cells it is possible that these neurons may inhibit sympathetic vasomotor activity. In the present study we tested this hypothesis by determining whether stimulation of the A1 region would cause a fall in mean arterial pressure (MAP).

In rabbits anesthetized with urethane, paralyzed with curare and artificially ventilated, the ventrolateral medulla at the level of the area postrema was stimulated with 10 sec trains of cathodal pulses (0.1 ms, 75 μA, 10 and 50 Hz). MAP and heart rate (HR) were recorded from a femoral catheter. Stimulation restricted to a region 3 mm lateral to the midline and 3 mm ventral to the dorsal surface of the medulla caused falls in MAP and HR The fall in MAP was maximal at 10 - 25 Hz; a pressor response was obtained at higher frequencies.

Threshold was 10 uA and MAP fall (25Hz,75uA) was 22 ± 4 mm Hg, with a fall in HR of 23 ± 6 bpm. Bradycardia but not hypotension was abolished by methylscopolamine (50 ug/kg i.v.) and propranolol (0.5 mg/kg i.v.). Catecholamine histofluorescence procedures demonstrated that the area from which the hypotension could be elicited coincided with the region containing the A1 catecholamine neurons.

Microinjection of L-glutamate (0.25 uL over 3 sec) bilaterally into the A1 region elicited a dose dependent fall in MAP and bradycardia. Injection of saline vehicle did not cause the same response. Threshold dose was 0.01 nmol; with 10 nmol MAP fell 35 ± 2 mmHg and HR fell 34 ± 12 bpm. L-glu injected into regions dorsal to the A1 area failed to elicit the response.

Since L-glutamate excites cell bodies rather than fibers of passage it is likely that electrical stimulation produced similar cardiovascular responses by a similar mechanism. We conclude that the ventrolateral medulla at the level of the area postrema contains neurons whose excitation results in a fall in blood pressure. These findings are consistent with the hypothesis that A1 catecholamine neurons function as a vasodepressor system.

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207.3 CARDIOVASCULAR RESPONSES FOLLOWING 6-HYDROXYDOPAMINE LESIONS OF THE NORADRENERGIC A2 CELL GROUP. Healy, D.P.*, J.Y. Jew and A.C. Black, Jr. Dept. of Anatomy, University of Iowa, Iowa City, Iowa 52242

The noradrenergic A2 cell group, located wtihin the nucleus tractus solitarius (NTS) commissuralis, has been implicated in central cardiovascular (CV) control. We sought to investigate whether destruction of the A2 neurons using direct intraparenchymal injections of the neurotoxin 6-hydroxydopamine (6-OHDA) altered cardiovascular responses in normotensive animals. Although the adrenergic C2 cell group is located rostral to the A2 cell group and has also been implicated in CV control, C2 neurons should be unaffected since epinephrine-containing neurons are supposedly resistant to 6-OHDA. 4 μg/μl of 6-OHDA was injected bilaterally into the A2 area of the rat using a glass micropipette. Control animals received the same volume of the vehicle. Following these injections, measurements of heart rate, mean arterial pressure (MAP), MAP lability and baroreceptor reflexes were carried out in unanesthetized unrestrained animals for one week. The extent of the lesions was evaluated using fluorescence histochemistry and biochemical determinations of catecholamine content of tissue punched from NTS slices. It was noted that: 1. 6-OHDA lesioned animals displayed a significant bradycardia which lasted throughout the 7 day period. The bradycardia was reversed by i.v. atropine. 2. There were no differences in MAP or MAP lability between control and 6-OHDA treated animals. 3. The gain of the baroreflex was decreased after 6-OHDA (control 1.36±.11, 6-OHDA 0.77±.06 mmHg/msec.). 4. Catecholamine histofluorescent preparations from 6-OHDA animals revealed a marked reduction in fluorescent varicosities throughout the NTS. 5. Norepinephrine levels were decreased throughout the NTS of 6-OHDA animals. 6. After 6-OHDA treatment, there was a persistence of fluorescent varicosities which were derived presumably from the epinephrine-containing C2 cell group. NTS epinephrine levels were similar to those in controls. Knife cuts made rostral to the C2 cell group eliminated fluorescent terminals rostral to the knife cut, providing evidence that these terminals arise from the C2 cell group. Therefore, the adrenergic C2 cell group appears to be resistant to 6-OHDA, but the role of this cell group in NTS function still requires clarification. The results of our experiments indicate that the noradrenergic A2 cell group is involved in modulation of central vagal activity. (This work was supported by N.I.H. grants HL21914 and NS11650.)

207.4 ALTERED CARDIOVASCULAR CONTROL FOLLOWING SPECIFIC BRAINSTEM LESIONS IN THE RABBIT

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There are two groups of norepinephrine neurons in the medulla - the A1 group in the ventrolateral medulla and the A2 group in the nucleus tractus solitarius. Lesions of the A2 neurons produce transient hypertension, bradycardia and loss of baroreflexes, but the cardiovascular functions of the A1 neurons have not been previously studied. We have investigated haemodynamic parameters in the conscious rabbit following bilateral electrolytic lesions 1 mm in diameter at 3 contiguous rostrocaudal levels in the ventrolateral medulla coinciding with the A1 group of norepinephrine neurons. Arterial blood pressure, heart rate and distal aortic blood flow were measured for 2 weeks following the lesions. Baroreflex function was determined from changes in heart rate and distal aortic blood flow induced by graded alterations in central arterial pressure produced by inflation of vascular occluders around the aorta and inferior vena cava. Nasopharyngeal reflex function was determined from changes in heart rate and distal aortic blood flow following activation of this reflex with saturated formaldehyde vapour.

After lesioning there was an initial rise in mean arterial pressure of 40 mm Hg in the conscious animal, associated with an increased distal aortic vascular resistance and profound bradycardia. Distal aortic vascular resistance remained elevated throughout the 2 week observation period but the blood pressure and heart rate returned to normal levels. The gain of the baroreceptor-heart rate reflex was initially diminished by 40% but returned to normal at 2 weeks. The gain of the mean arterial pressure - distal aortic flow relationship, was reduced by 50% throughout the 2 week observation period, indicating severe attenuation of baroreceptor ability to induce vasoconstriction in the distal aortic vascular bed. Bradycardia and vasoconstriction normally observed with activation of the nasopharyngeal reflex were less marked after lesioning but the changes were transient.

The results suggest that A1 norepinephrine neurons modify heart rate effects elicited through baroreceptor and nasopharyngeal reflexes, but that while they play a major role in baroreceptor mediated vasoconstriction by inhibition of sympathetic vasoconstrictor tone, they have little influence on constriction mediated through the nasopharyngeal reflex.

207.5 HYPERTENSIVE EFFECTS OF CEREBELLAR NUCLEUS FASTIGIUS LESIONS AND SINO-AORTIC DENERVATION IN THE RAT. C. K. Haun*, M. DeCuir* and N. Alexander. Depts. of Anatomy and Medicine, Univ. of So. Calif. Sch. of Med., Los Angeles, CA 90033.

Hypertension produced in rats by sino-aortic denervation (SAD) is characterized by frequent pressure "dips" which are usually associated with head or body movements (Eur. J. Pharmacol., 63, 117, 1980). As source of these dips our attention was attracted to the Nucleus Fastigius (NF) through recognition of this site's modulatory involvement in 1) equilibrium-related adjustments of posture and movement, and 2) vasomotor reflexes (Brain Res. 13, 595, 1969).

In adult male rats under methohexital, bilateral stereotaxic lesions of the NF made with radio frequency current, were followed a week later by bilateral SAD and aortic canulation, under Innovar anesthesia. Controls received sham lesions (no current), or SAD first and then were lesioned and canulated a week later. Arterial pressure was recorded via strain gauge and inkwriter throughout the day of canulation, and periodically, on the following 2 to 3 days. The rats were then autopsied. Their brains were fixed, serially sectioned and examined for lesion localization.

Animals with verified NF lesions were found to have elevated arterial pressures exceeding those of shams and animals in which SAD preceded lesioning; and they also showed typical signs of congestive heart failure (which was a frequent cause of death) -- dilated heart and great veins, edematous and/or hemorrhagic lungs, and engorged liver. The characteristic pressure dips of SAD rats were dramatically reduced or absent. Controls' pressure recordings were typical of SAD rats; their viscera showed no signs of heart failure. That portion of the NF responsible for the hypertensive changes when followed by SAD seems to be at or near the rostral pole of the NF, a region in which electric stimulation causes acute elevations of arterial pressure (Brain Res., Ibid).

207.6 NEURAL AND HUMORAL COMPONENTS OF THE PRESSOR RESPONSE ELICITED BY ELECTRICAL STIMULATION OF FASTIGIAL NUCLEUS (FN) IN RATS BEFORE AND AFTER SYMPATHECTOMY. A. Del Bo*, C. Ross, J. Pardal*, J. Saavedra, and D.J. Reis (SPON: M. Kumada). Lab of Neurobiology, Cornell Univ. Med. Coll. New York, NY 10021 and NIMH, Bethesda, MD 20014.

We sought to determine whether; (a) electrical stimulation of the FN in rat will elevate circulating catecholamines (CA); (b) the increase is selective for norepinephrine (NE), epinephrine (E), or dopamine (DA); (c) CAs released from adrenal medulla contribute to the pressor response elicited by FN stimulation after chemical sympathectomy. Rats were anesthetized (chloralose 60 mg/kg i.v.), paralyzed and ventilated. The cerebellum was explored with 10 sec pulse trains. Stimulation of rostral FN increased mean arterial pressure (MAP) and heart rate (HR), the fastigial pressor response (FPR). The threshold current was 18 $\pm$ 2uA and the response graded. At optimal frequencies (50-100 Hz) at 5 x threshold FN stimulation increased MAP 62 $\pm$ 3 mmHg and HR 79 $\pm$ 11 bpm (n=20). The latency was < 2 sec. The FPR was stimulus-locked. A prolonged FN stimulation (30 sec) increased serum NE from 199 $\pm$ 12 to 309 $\pm$ 49 pg/ml (n=9, $P<0.05$), and E from 108 $\pm$ 20 to 230 $\pm$ 77 pg/ml (n=9, $p<0.05$), but not DA (97 $\pm$ 16 to 102 $\pm$ 16 pg/ml, n=9, n.s.). The magnitude and the characteristics of the FPR were not modified by adrenalectomy (n=9), nor by midcollicular decerebration (n=6). Administration of 6-hydroxydopamine (6 OHDA, 100 mg/kg i.v.) 24 h prior to testing effectively destroyed sypathetic terminals abolishing the pressor responses to tyramine (up to 400 ug/kg) and shifted the pressor/dose response curves of NE and E to the left. After chemosympathectomy: the FPR was enhanced (70 + 2 mmHg, n=26), had a longer latency (6 $\pm$ 1sec), persisted longer (up to 4 min), and its tachycardia was replaced by vagal bradycardia (-122 $\pm$ 11bpm). The stimulus-intensity/frequency characteristics of the response were unchanged. Adrenalectomy only partially reduced the FPR after chemosympathectomy (41 $\pm$ 3 vs 70 $\pm$ 3 mmHg, n=11, $p<0.001$). The residual pressor response was not affected by phentolamine (3 mg i.v., n=10), but completely abolished by midbrain transection (n=5). We conclude: (a) In rats with intact sympathetic nerves the FPR is mediated via pathways descending below the midbrain and is associated with increase in serum NE and E; adrenal CA's do not contribute to the magnitude of the FPR. (b) In the absence of sympathetic nerves, CAs of adrenal medullary origin released by FN stimulation only partially contribute to the hypertension. (c) A circulating pressor factor released by FN stimulation from areas above midbrain may significantly elevate MAP in the absence of sympathetic nerves and adrenal medulla. (NIH grant HL 18974)

207.7 CEREBROVASODILATATION ELICITED BY ELECTRICAL STIMULATION OF DORSAL MEDULLARY PRESSOR AREA IN RAT. C. Iadecola*, M. Nakai*, E. Arbit*, and D.J. Reis. Lab of Neurobiology, Cornell Univ. Med. Coll. New York, NY 10021.

The aim of the present study is to establish whether in rat electrical stimulation of vasopressor areas in the dosal medullary reticular formation (DMRF) will modify regional cerebral blood flow (CBF). Adult male Sprague-Dawley rats were anesthetized with chloralose, paralyzed and artificially ventilated. Arterial pressure, heart rate and body temperature were continuously monitored; pCO_2, pO_2 and pH were maintained within physiological range. The left DMRF was stimulated electrically with a 10 min (1 sec on/1 sec off) train at 50 Hz at approximately five times threshold for the pressor response (30-70uA). The elevation of AP produced by DMRF stimulation was maintained in the autoregulatory range by controlled hemorrhage. Regional CBF was measured using C-14-iodoantipyrine as indicator, with regional dissection of the brain.

Electrical stimulation of DMRF increased CBF significantly bilaterally in 12 of the 13 regions studied. The average increase was 183 $\pm$ 9% (n-6; $p<0.005$) of controls, the greatest value being in parietal cortex (240%) and the least in the pons (147%). Besides the cerebral cortex, increases> 180% were found in the thalamus (205%), hippocampus (180%), hypothalamus (180%), and subcortical white matter (205%). In the cerebellum flow did not change. After unilateral cervical sympathectomy, the increase CBF in cerebral cortex, caudate nucleus, and hypothalamus, was significantly greater in the denervated than intact side ($p<.01$- .05, paired t-test).

We conclude that in the rat: (a) electrical stimulation of the DMRF increases CBF globally; (b) after acute sympathectomy, the increase in CBF is greater in those regions that receive a strong vascular sympathetic innervation; (c) neurons within or projecting through DMRF mediate a potent cerebral vasodilation, probably via intrinsic pathways in brain, and which is opposed partially by sympathetic neurons contained in superior cervical ganglion.

(Supported by NIH grant HL 18974 and NS 03346.)

207.8 NEUROGENIC HYPERTENSION PRODUCED BY LESIONS OF THE NUCLEUS TRACTUS SOLITARII ALONE OR WITH SINOAORTIC DENERVATION IN THE CONSCIOUS DOG. Karen L. Barnes and Carlos M. Ferrario. Research Division, Cleveland Clinic Foundation, Cleveland, OH.

Although destruction of the nucleus tractus solitarii (NTS) in the rat produces fulminant hypertension, in other species central baroreceptor deafferentation has produced equivocal results. Increased lability of pressure without hypertension has been observed in the cat, while parallel studies of lability and hypertension have not yet been performed in the dog. A number of factors might explain these conflicting findings. One possibility is the recent evidence which suggests that the distribution within the brainstem of baroreceptor fibers includes the area postrema, nucleus ambiguus, and ventral reticular formation as well as the NTS, making selective and complete denervation difficult. With this in mind, we produced restrictive vs. extensive bilateral lesions of the NTS in dogs. During the 5 weeks following NTS lesions the degree of hypertension and the change in the variability of both mean arterial pressure (MAP) and heart rate (HR) were related to both position and size of the lesions within the NTS and surrounding brainstem. Lesions restricted to the medial NTS and solitary tracts between the obex and 1 mm anterior caused transient hypertension and tachycardia for 1 to 3 days; thereafter both MAP and HR returned to normal. In contrast, dogs with more extensive lesions of the NTS reaching at least 3 mm anterior to obex had mild hypertension for at least 15 days, increased lability of MAP, moderate tachycardia, and decreased variability of HR. Subsequently both MAP and its variability reverted toward normal, but tachycardia and decreased variability of HR persisted. Peripheral sinocervical aortic denervation in dogs with mild hypertension 28 to 42 days after NTS lesion yielded surprising results; severe hypertension and marked tachycardia in these dogs were followed by death from heart failure within 6 hours.

The mild hypertension, not necessarily sustained, following bilateral NTS lesions suggests that this procedure only partially interrupts central baroreceptor pathways. On the other hand, the development of fulminant hypertension following combined central and peripheral baroreceptor deafferentation reveals the importance of baroreceptor input for maintenance of normal blood pressure.

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207.9 THE ROLE OF THE ANTEROMEDIAL HYPOTHALAMUS IN DAHL HYPERTENSION. P. Ernsberger*, S. Azar*, and D.C. U'Prichard (SPON: W.D. Mink). Neuroscience Program and Dept. of Pharmacology Northwestern Univ. Chicago, IL 60611 and Dept. of Medicine, Univ. of Minnesota, Minneapolis, MN 55455

Anatomical and biochemical studies suggest that the paraventricular (PVN), periventricular (PVE), and suprachiasmatic (SCN) nuclei may participate in blood pressure (BP) regulation. Various radiofrequency lesions were placed in these structures in Dahl rats. The salt-sensitive (S) rat develops hypertension when given an 8% NaCl diet (high salt, HS), while the salt-resistant (R) rat remains normotensive. R and S rats were maintained from weaning on 0.3% NaCl chow, and put on HS 3 weeks postoperatively. BP was measured weekly through 14 weeks of HS and around the clock at 3 points. S rats with PVN-SCN lesions showed no change in BP with HS, with mean BP ranging from a maximum of 142±16 (week 5 of HS) to a minimum of 120±6 (week 13 of HS), values similar to those of R rats. In contrast, the BP of sham-operated S rats rose from 124±4 initially to 199±5 by 6 weeks of HS. In R rats with identical lesions, a transient increase in BP with HS was observed (lesion: 147±4, sham: 118±6, at 5 weeks of HS), which disappeared by 6 weeks of HS. The common extent of the lesions preventing hypertension in the S rats included the PVN, the SCN, and the PVE lying between them. The combined extent of the ineffective lesions included areas between the anterior optic chiasm and the ventral surface of the anterior commissure rostrally, and the SCN and adjacent PVE caudally. In no case was the PVN or the PVE ventral to it damaged. These lesions had no effect on BP in either R or S rats. Lesions of the PVN alone in S rats delayed the rise in BP with HS by several weeks. Lesions of the SCN alone had little effect on BP in either R or S rats. Plasma Na at time of sacrifice was unchanged after PVN-SCN lesions in S (lesion: 143±1, sham: 144±1 mEq/l) and R rats (lesion: 142±1, sham: 142±1). Similar results were observed with tail blood samples taken at two other times. At 11 and 12 weeks of HS, all rats were given injections of angiotensin (0.37 mg/kg, s.c.), and water intake was determined for 1 hour. PVN-SCN lesions had no effect on angiotensin-induced drinking in S (lesion: 8±2, sham: 12±2) and R rats (lesion: 9±2, sham: 13±2). PVN-SCN lesions prevented the renal hypertrophy which occurrs in S rats on HS (lesion: 6.8±.2, sham: 10.0±.4, renal mass/body weight, g/kg), while not affecting R rats (lesion: 8.7±.6, sham: 8.4±.3). The BP effects of these lesions are not due to alterations in circadian rhythms, since their hypotensive effect persists when BP is measured around the clock. The PVN and adjacent portions of the PVE may play a role in the initiation and development of Dahl hypertension.

207.10 ELEVATED SYMPATHETIC TONE IN NEONATAL SHR. D.C. Tucker and A.K. Johnson, Dept. of Psychology and Cardiovascular Center, University of Iowa, Iowa City, Iowa 52242.

Juvenile spontaneously hypertensive rats (SHR) have higher basal heart rates (HR) than Wistar Kyotos (WKY). In weanling SHR, both elevated sympathetic and depressed parasympathetic tone are observed (Hallback, in Onesti, Regulation of Blood Pressure by the CNS, 1976). The purpose of our present studies was to examine developmental changes in HR and to define the factors controlling this parameter in neonates of the SHR and WKY strains.

Rat pups were implanted with subcutaneous silver wire electrodes one day prior to testing. Recordings of EKG were made at 4, 8, 12, and 16 days of age from unanesthetized, unrestrained pups in their home cages. Pups from 8 WKY and 8 SHR litters were tested at each age. Injections of atenolol (1.0 mg/kg) and methylatropine (0.01 mg/kg) were made to determine the contribution of autonomic controls of HR. Both drug and saline control injections were standardized to a volume of 1 ml/kg.

While sympathetic influences were essentially equivalent in SHR and WKY at 4 and 8 days of age, by 12 days evidence of significantly elevated sympathetic tone in SHR was observed. Specifically, within 10 minutes after atenolol injection, the HR of 12-day old SHR decreased by 133±17 bpm (to 289 bpm); in 12-day old WKY, β-blockade resulted in a HR decrease of 70±12 bpm (to 292 bpm). Tonic parasympathetic control over heart rate was not observed prior to 16 days of age. In 16-day old pups, a methylatropine injection was followed by a HR increase of 55±8 bpm (to 460 bpm) in SHR and an increase of 70±11 bpm (to 451 bpm) in WKY. A combined muscarinic and β-adrenergic blockade was used to determine intrinsic HR. Similar developmental increases in intrinsic HR were seen in SHR and WKY pups.

The elevated heart rate seen in SHR during the second postnatal week seems thus to reflect high sympathetic tone rather than differences in parasympathetic influence or intrinsic heart rate. Since during the first postnatal week cardiac functioning in SHR seems relatively normal, this may be a period during which interventions altering maturation or adult functioning of the sympathetic nervous system might affect the developmental course of spontaneous hypertension.

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207.11 CARDIOVASCULAR, CATECHOLAMINE AND RENIN RESPONSES TO HEMORRHAGE IN BRATTLEBORO RATS. R.L. ZERBE*, G. FEUERSTEIN, D.K. MEYER*, J. KOPIN. (SPON:D.C. JIMERSON) Laboratory of Clinical Science, Section on Medicine, National Institute of Mental Health, Bethesda, MD 20205

Abnormal baroregulation in Brattleboro rats (Di/Di) (J. Physiol. 296:267) suggests that vasopressin (VP) has a function in blood pressure (BP) control. Whether VP acts by direct vasoconstriction or by potentiating other pressor mechanisms is unclear. The former seems incompatible with the relatively weak pressor effects of VP in intact animals, whereas the latter is given credence by the demonstration of VP in CNS areas important in baroregulation. To investigate the latter possibility, we measured blood pressure (BP), heart rate (HR), plasma epinephrine (E), norepinephrine (NE) and renin activity (PRA) before and after acute hemorrhage (5cc/300gm over 5 min) in 9 Di/Di, 9 weight matched (WMC) and 8 age matched (AMC) Long-Evans controls under halothane-anesthesia (0.8% in oxygen). Hemorrhage and cardiovascular recordings were done via a femoral catheter. For each group, the mean values (±SEM) before (PRE), immediately after (0') and 30 min. after (30') hemorrhage are shown below. Significant differences ($P<0.5$) from Di/Di are indicated by *, significant differences from baseline values within each group are underlined.

	Di/Di			WMC			AMC		
	PRE	0'	30'	PRE	0'	30'	PRE	0'	30'
BP mmHg	83 ±3	22 ±1	41 ±2	88 ±2	26* ±8	71* ±6	88 ±4	27* ±1	57* ±6
HR bpm	285 ±8	220 ±12	261 ±12	319* ±6	206 ±11	266 ±9	293 ±13	198 ±7	248 ±9
E pg/ml	60 ±15	550 ±78	374 ±68	13* ±3	122* ±24	121* ±20	24 ±8	236* ±92	143* ±27
NE pg/ml	154 ±23	170 ±15	162 ±16	115 ±15	96 ±12	114 ±13	94* ±10	100 ±17	119 ±19
PRA ngAI/min/ml	86 ±6	102 ±7	148 ±6	42* ±4	54* ±5	69* ±6	43* ±4	72* ±8	103* ±9

These data show that Di/Di: 1) have higher basal PRA and E, 2) recover more slowly from hemorrhage and, 3) have a normal or enhanced increase in E, NE, and PRA. We conclude that VP deficiency does not diminish the response of other pressor hormones, and that the blunted blood pressure recovery after hemorrhage in Di/Di is probably due to the loss of peripheral effects of VP or other consequences of chronic VP deficiency.

207.12 THE INITIATION, PROPAGATION, AND TERMINATION OF "PLATEAU" WAVES. M.J. Rosner* and D.P. Becker, Division of Neurological Surgery, Medical College of Virginia, Richmond, Virginia 23298.

In cats undergoing fluid percussion brain injury, plateau waves begin to occur 18-24 hrs after injury. Onset and behavior of these waves have proven to be stereotyped allowing development of a precise and testable model which we present herein.

Cats weighing 2.5 to 3.5 kg undergo continuous monitoring of systemic arterial (SABP), intracranial (ICP) and central venous pressures as well as heart rate, end-tidal CO_2, temperature (controlled at 39° C) and EEG. General anesthesia is maintained using N_2O/O_2 (70:30); muscle paralysis is obtained with pancuronium bromide. The animal is maintained on a ventilator for up to 72 hours after injury.

The substrate for plateau wave occurrence is elevation of the ICP, decrease in the intracranial compliance, and generally intact autoregulation. For several minutes just prior to the development of the plateau wave there occurs a gradual rise in the ICP from 20 to about 35 mmHg. This is always accompanied by a modest and gradual decline in the blood pressure from 120 to 90 mmHg. This SABP drop seems insignificant until one examines the cerebral perfusion pressure (CPP); the CPP drops to 60 mmHg, a value near the lower limits of autoregulation. We believe this gradual rise in ICP to reflect autoregulatory vasodilation which is actually responsible for the ICP increase.

At a CPP of 60 mmHg the plateau wave begins. The initiation of the plateau wave may correspond to an additional vasodilatory stimulus: decreased O_2 delivery. Both factors together result in this rapid increase in ICP. As the wave develops, perfusion pressure drops below 50 mmHg, and brainstem ischemia then initiates a "Cushing" response. Adrenergic discharge and hypertension (150 mm Hg) restore the CPP to 70-80 mmHg, and autoregulatory vasoconstriction ensues. Because autoregulatory vasoconstriction lags behind increasing blood pressure, the ICP transiently rises even farther (but with no further decrease in the CPP). After this final ICP peak, the plateau wave aborts, and ICP quickly returns to baseline levels.

In summary, when ICP is moderately elevate, normally trivial decrements in blood pressure result in large drops in perfusion pressure. A cycle of autoregulatory vasodilation is initiated further increasing ICP until CPP falls to ischemic levels (<50mmHg). Brainstem ischemia stimulates a "Cushing" response with restoration of the CPP. The ICP transiently rises further due to the lag between the hypertension and autoregulatory vasoconstriction, but then dramatically returns to baseline. This model has been substantiated by using mild hypertension to correct low CPP in animals and humans with subsequent abolition of the plateau wave.

208.1 DOSE-RESPONSE CHARACTERISTICS OF BENZODIAZEPINE POTENTIATION OF GABA CHEMOSENSITIVITY ON SINGLE CHICK SPINAL CORD NEURONS IN CULTURE. C. Y. Chan* and D. H. Farb* (SPON:R. T. Wang). Dept. of Anatomy and cell Biol., SUNY Downstate Med. CTR., Brooklyn, NY 11203.

Benzodiazepines (BZD's) potentiate GABA chemosensitivity of embryonic chick spinal cord (SC) neurons in cell culture (Choi, Farb and Fischbach, Nature 269:732, 1977). The dose-response characteristics of BZD potentiation of GABA chemosensitivity on single neurons were determined. Neurons were impaled for intracellular recording and passing of current. GABA and BZD's were applied extracellularly at known concentrations by pressure ejection. The GABA response (a rise in conductance) was calculated from the voltage deflection elicited by constant d.c. pulses of 0.1 nA across the cell membrane before and during GABA application. BZD potentiation of GABA responses was measured as a percentage increase in the GABA-induced conductance rise. A complete dose-response curve was obtained for each neuron studied. Lineweaver-Burk analysis of the data yielded EC_{50}'s of 0.16μM for flunitrazepam and 1μM for flurazepam. These are respectively 32 and 36 times the K_I's from adult rat brain binding data. EC_{50} for chlordiazepoxide obtained by statistical approach was about 17μM, which was 34 times the binding K_I. The BZD RO5-4864 is known not to exhibit central behavioral effects and binds with very low affinity to central BZD receptors. As expected, no GABA potentiating activity was found using 0.1μM to 100μM RO5-4864 on single SC neurons; rather, it inhibited GABA responses.

Dose-response curves for GABA chemosensitivity were measured on 3 neurons. We found EC_{50} of 40μM, which is similar to statistically derived EC_{50} of 17μM (Choi and Fischbach, J. Neurophysiol., in press). We investigated possible involvement of $GABA_B$ receptors in BZD potentiation of GABA responses using baclofen, a selective $GABA_B$ receptor ligand (Hill and Bowery, Nature 290:149, 1981). No conductance or potential changes were observed when baclofen was applied to cultured SC neurons, whether or not they were prepulsed with flurazepam. It also had no effect on GABA responses or flurazepam potentiation of GABA responses when it was applied prior to GABA or flurazepam pulses. Thus, $GABA_B$ receptors do not seem to be involved in this system.

(Supported by NSF grant BNS 80-04811 to D. H. Farb and a Muscular Dystrophy Assoc. Fellowship to C.Y. Chan.)

208.2 HIGH AND LOW AFFINITY BENZODIAZEPINE BINDING TO EMBRYONIC CHICK CNS MEMBRANES, T. T. Gibbs*, L. A. Borden*, G. Shifrin*, and D. H. Farb* (SPON: D. A. Fischman). Dept. of Anatomy & Cell Biology, SUNY Downstate Med. Ctr., Brooklyn, NY 11203.

Benzodiazepines (BZD's) enhance GABA chemosensitivity and potentiate GABA-like synaptic transmission in cell cultures of embryonic chick spinal cord (Choi, Farb & Fischbach, Nature 269 (1977)342). This enhancement requires higher BZD concentrations than expected from studies of BZD binding to rat brain membranes (Squires & Braestrup, Nature 266(1977)732). To investigate the differences between the EC_{50}'s determined by electrophysiological methods (see Chan & Farb, accompanying abstract) and the high-affinity K_I's determined by radioligand binding, standard radioligand techniques were used to characterize the binding of BZD's to washed P_2 membrane fractions derived from both late (20 day) and early (7 day) embryonic chick brain (EBM) or spinal cord (ESM). The rate constants for association (k_f=5.2±1.6 x 10^7 M^{-1} min^{-1}, n=2) and dissociation (k_r=0.096 min^{-1}) of ^{3}H-flunitrazepam (FNZM) determined with late EBM were consistent with a K_D of about 2 nM. This is in general agreement with the value of 5.8±1 nM determined at equilibrium (n=2). Flurazepam (FZ) and chlordiazepoxide (CDPX) competed for this site with respective K_I's of 34±3 nM (n=4) and 450 nM (n=1). Similar results were obtained with late ESM. Binding of ^{3}H-FNZM to early and late EBM was enhanced 60-80% in 100-200 μM GABA. Enhancement of ^{3}H-FNZM binding by GABA was blocked by bicuculine. In addition, the BZD-binding complex of EBM was irreversibly labeled with ^{3}H-FNZM by exposure to UV light (Battersby et al., Eur.J.Pharmac.57(1979)277) and comigrated with putative rat BZD receptor in SDS-PAGE. These results on late EBM and ESM are in agreement with published K_I values of adult rat membranes. Thus the discrepancy between electrophysiological EC_{50}'s and binding K_I's is not due to species differences between chick and rat BZD receptors. Whereas BZD binding to late EBM & ESM was consistent with Michaelis-Menten kinetics (Hill coeff. ≃1 for FNZM, FZ and CDPX), this was not the case with early embryonic membranes. Binding of BZD's to early EBM or ESM exhibited non-linear Scatchard plots and Hill coeff.'s of ca. 0.4. This is inconsistent with Michaelis-Menten binding at a single site, and suggests either multiple BZD binding sites or negative cooperativity. Computer analysis of the data by non-linear regression indicates that these results are well described by a 2-site model with a high-affinity site (K_1) similar to that observed in late EBM & ESM and a second, lower-affinity site (K_2). The values obtained are: clonazepam, K_1≃1.8 nM, 81% of total, K_2≃3 μM, 19% (n=2); FZ, K_1≃40 nM, 70%, K_2≃13 μM, 30% (n=2); and CDPX, K_1≃500 nM, 80%, K_2≃40 μM, 20%. (Supported by NSF grant BNS 80-0487 to D. H. Farb and a MDA fellowship to T. T. Gibbs)

208.3 PURIFICATION AND CHARACTERIZATION OF BENZODIAZEPINE RECEPTOR. J.-Y. Wu, Y. Y. T. Su*, W. M. Huang*, J. Pachter* and P. Seu* Dep. Cell Biology and Cullen Eye Institute, Baylor College of Medicine, Houston, Texas 77030

In our attempt to purify benzodiazepine (BDZP) receptor(s) from rat brain, we chose to use the crude mitochondrial fraction (P_2) pellet after lysis as the source of BDZP receptor and ^{3}H-flunitrazepam (FNZP) as ligand. The specific binding of FNZP to BDZP receptor was determined as 1.1 X 10^{-12} moles per mg protein of P_2 pellet. Clonozepam (10^{-4}M) was included in the incubation mixture as a control for non-specific binding. The purification of native BDZP receptor(s) involved the covalent labeling of the receptor with ^{3}H-FNZP by photoaffinity labeling as described [Battersby, Richards and Möhler, Eur. J. Pharmacol. 57:277 (1979)], followed by solubilization with 2% Triton X-100 and finally by column separation on gel filtration and hydroxylapatite. The subunit structure of BDZP receptor(s) was studied with ^{3}H-FNZP-labeled receptor preparations using SDS-polyacrylamide gel electrophoresis (SDS-PAGE). Upon SDS-PAGE, two protein components, I and II, labelled with ^{3}H-FNZP were observed with a molecular weight of 45,000 ± 5,000 and 10,000 ± 2,000, respectively. The ratio of I:II was about 1:2. Similar results were obtained on SDS-gel filtration. The Triton solubilized BDZP receptor(s) could be separated into two components on Triton-gel filtration column and both components migrated as a single species on SDS-PAGE, corresponding to the fast-moving component, II, (MW 10,000). Furthermore the slow-moving component, I, (MW 45,000) from SDS-column could be converted into component II by treatment with Triton prior to SDS-PAGE. Hence, it is concluded that the basic subunit of BDZP receptor is component II, a 10,000 dalton polypeptide and component I, is a polymeric form of II. The different forms of native BDZP receptor(s) observed on Triton-gel column may represent different polymeric forms of component II. (Supported by NIH grant NS-13224).

208.4 PURIFICATION AND CHARACTERIZATION OF AN ENDOGENOUS BRAIN PEPTIDE THAT COMPETES WITH ^{3}H-DIAZEPAM BINDING. A. Guidotti, B. Ebstein* and E. Costa. Lab. Preclin. Pharmacol., NIMH, St. Elizabeths Hosp., Washington, D.C. 20032.

An endogenous peptide which competes with ^{3}H-diazepam binding (EP) was extracted from fresh or microwaved rat brain in 20 volumes of hot (80°) 1 N acetic acid. After the pH was adjusted to 5, the 48,000 xg supernatant was dialyzed for 24 hrs against 0.1 N acetic acid. The dialyzate was concentrated and applied to a Sephadex G-100 column equilibrated in 0.1 N acetic acid. The fractions were tested for EP activity. Activity was associated with a protein peak which emerged after ribonuclease A (a marker for 14,000 daltons) and before β-mercaptoethanol (a marker for 78 daltons). An alternative method of extracting the EP consisted of treating frozen-thawed and well-washed crude synaptic membranes with 0.05% Triton X-100. The suspension was incubated for 30 min at 0°, sonicated for 30 sec and then centrifuged at 100,000 g for 60 min. From this crude extract EP was isolated by heat treatment at 95°C for 15 min and by chromatographing the subsequent 100,000 g supernatant on a G-100 column. This extraction procedure gives a profile similar to that shown after hot acetic acid extraction. The inhibitory material obtained from G-100 was further purified on a Biogel P-2 column equilibrated in 0.1 N acetic acid. The EP activity was associated with the protein peak emerging in the void volume and was destroyed by pronase and trypsin but not by phospholipase A_2, C or D. The purified EP, when subjected to electrophoresis on an SDS-12% acrylamide gel, revealed 2 major bands with molecular weight smaller than 14K. The EP extract from the Bio-Gel P-2 column was further purified by reverse phase HPLC on a BioRad ODS column with acetonitrile gradient. The protein peak and EP activity emerged at 40% acetonitrile. The purified EP inhibited ^{3}H-diazepam binding competitively. It does not inhibit the binding of ^{3}H-GABA, indicating that EP can be physically separated from GABA modulin. EP has no effect on the binding of ^{3}H-cyclohexyladenosine, ^{3}H-QNB, ^{3}H-etorphine or ^{3}H-alprenolol while it does inhibit effectively ^{3}H-propyl-β-carboline-3-carboxylate binding. EP displaces ^{3}H-flunitrazepam more effectively from cerebellar membranes than from hippocampal membranes.

208.5 OPTICAL ISOMERS OF 5-(1,3-DIMETHYL-BUTYL)-5-ETHYL BARBITURIC ACID (DMBB) BIND TO THE PICROTOXININ BINDING RECEPTOR. Maharaj K. Ticku, William C. Davis* and Cynthia A. Morency* (SPON: K. Blum). Dept. Pharmacology, Univ. Tex. Hlth. Sci. Ctr., San Antonio, TX 78284 and New England Nuclear Corp., Boston, MA 02118.

Depressant and convulsant barbiturates inhibit the binding of [^{3}H]-α-dihydropicrotoxinin (DHP) to membranes (Ticku & Olsen, Life Sci. 22:1643, 1978) and crude Lubrol-solubilized fraction (Davis & Ticku, J. Neurochem. in press, 1981). Gel filtration of the Lubrol fraction revealed that [^{3}H]-diazepam and [^{3}H]-DHP bind to two distinct fractions with apparent molecular weights of 61,000 and 185,000 daltons, respectively (Davis & Ticku, Neurosci. Absts. in press, 1981). [^{3}H]-DHP binding to the 185,000 dalton fraction was displaced by depressant and convulsant barbiturates. To investigate the possibility that the picrotoxinin binding site may represent a receptor site for barbiturates (Ticku, Br. Res. Bull. 5(2):919, 1980), we synthesized and radiolabeled the optical isomers of DMBB. [^{3}H]-DHP binding to the 185,000 dalton fraction was inhibited by both (+)DMBB (convulsant) and (-)DMBB (depressant), with shallow displacement curves. Specific binding of both [^{3}H]-(+)- and [^{3}H]-(-)DMBB was detected in the 185,000 dalton fraction. Scatchard analysis of the binding isotherms of [^{3}H]-(+)DMBB revealed downward curvature, suggesting heterogeneity of binding sites. [^{3}H]-(+)DMBB binding to the 185,000 dalton fraction was displaced by (-)DMBB, picrotoxinin and depressant and convulsant barbiturates. Likewise, [^{3}H]-(-)DMBB binding to the same fraction exhibited heterogeneity and was displaced by (+)DMBB, picrotoxinin and other barbiturates. These results indicate that barbiturates bind to the picrotoxinin binding fraction and support our earlier notion that the picrotoxinin site at the benzodiazepine-GABA receptor-ionophore complex may represent a receptor site for barbiturates. This site may be involved in allosteric regulation of the benzodiazepine-GABA receptor-linked chloride ionophores.

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208.6 A POSSIBLE ENDOGENOUS LIGAND FOR THE SPECIFIC ^{3}H-PHENYTOIN SITE IN THE RAT BRAIN. L. Spero* and P. Kunashko* (SPON: W.E. Corrigall). Dept. of Pharmacology, University of Toronto, Toronto, Ontario.

We recently made the observation that two specific binding sites for ^{3}H-phenytoin exists in a synaptosomal fraction of rat brain. Other anticonvulsants used to treat grand mal compete for these sites but not those used to treat petit mal (Burnham et al., C.J.P.P. 1981, April). A large number of neurotransmitters were tested and found not to compete with phenytoin. We have now isolated a fraction following removal of proteins, lipids and salts then G25 and G15 sephadex gel chromatography, with a molecular weight of approximately 500-700 Daltons, which competes for ^{3}H-phenytoin, ^{3}H-muscimol and ^{3}H-diazepam binding. The phenytoin competing activity is lost after incubation with trypsin and chymotrypsin, using soybean trypsin inhibitor, or heat inactivation to prevent membrane degradation. This enzyme treatment does not influence diazepam or muscimol competing activity in the fraction. Cation exchange HPLC chromatography in potassium phosphate buffer pH 6.5 gives further separation into two fractions, the binding characteristics of these fractions are currently under study. Serial combination experiments with cold phenytoin and the G25 fraction support a common binding site for the two, when tested against ^{3}H-phenytoin. Similar results were obtained for the G25 fraction combined with cold diazepam or cold muscimol against ^{3}H-diazepam and ^{3}H-muscimol respectively. Phenytoin itself does not compete against ^{3}H-diazepam or ^{3}H-muscimol. The G25 fraction does not compete for binding against a large number of other radio-ligands tested. Diazepam (1-20 um) which enhances phenytoin binding also enhances the G25 fraction binding, this has not been observed for other benzodiazepine analogues. However, we have shown (Okazaki et al., 1981, in prep.) that (+)-bicuculline but not (-)-bicuculline also enhances ^{3}H-phenytoin binding, and G25 fraction competition.

The G25 fraction has also been tested in mice using the standard NIH, Maximal Electroshock Technique. When administered i/p the fraction protects mice against MES within five minutes, and this protection lasts for fifteen to twenty minutes, and is absent by thirty minutes. The ED_{50} for this protection is approx. 1.5 mg/kg of the crude G25 extract. No marked change in the behaviour of the mice was observed during this period. (Supported by Medical Research Council of Canada.)

209.1 DISTRIBUTION OF SOMATOSTATIN-LIKE IMMUNOREACTIVE MATERIAL IN THE CNS AND PERIPHERY OF A GASTROPOD. Y. Grimm-Jørgensen, with the technical assistance of M.J. Borkowski*and C. Sowers-Clift,*Dept. of Anatomy, The University of Connecticut Health Center, Farmington, CT 06032.

Published immunocytochemical studies indicate that somatostatin-like immunoreactive material(IRSRIF)is found in the nervous system and peripheral organs of many vertebrates and invertebrates. In the CNS of the gastropod Lymnaea stagnalis IRSRIF is associated with a cluster of neurosecretory cells thought to be involved in body growth (Roubos et.al.,VIII International Symposium on Neurosecretion,1981,in press).

In order to gain more information on the possible physiological roles of IRSRIF in gastropods,we have studied the distribution of IRSRIF in various organs of the pond snail Physa spp. IRSRIF was quantitated by radioimmunoassay after extraction with 2 N acetic acid and lyophilization.IRSRIF was found in the circumesophageal ganglia,the gastrointestinal (GI) tract and the hemolymph.

Within the GI tract,the highest concentration of IRSRIF was observed in the esophagus.Although the IRSRIF concentration of the large digestive gland was lower than that of the esophagus, the total amount of IRSRIF in the digestive gland contributed a larger proportion of the total IRSRIF to the GI tract than did other parts.

The IRSRIF content of the hemolymph changed with age.The IRSRIF concentration in the hemolymph of young animals (prior to egg laying) was 4-5 fold higher than that in the hemolymph of old animals.When old animals were forced to regenerate their shell by removal of the shell edge, the IRSRIF concentration of the hemolymph increased 100%.These findings suggest that in Physa IRSRIF may be involved in the regulation of body growth.

Attempts were made to gain information on the chemical identity of IRSRIF in ganglia and hemolymph.Acetic acid-soluble extracts were subjected to liquid chromatography on Biogel P-2. IRSRIF eluted with the void volume fraction, indicating that Physa IRSRIF is larger than 1500 daltons.The acid-soluble material was also subjected to thin layer chromatography on reverse phase plates and the IRSRIF content of 1 cm sections of the plate determined.Various peaks of IRSRIF were observed. Whereas a small but significant part of the total immunoreactivity from ganglia co-migrated with synthetic somatostatin, none of the IRSRIF from the hemolymph co-migrated with synthetic somatostatin.These results indicate that multiple forms of IRSRIF are present in the tissues of Physa and that the bulk of the immunoreactive material is not chemically identical with mammalian somatostatin.Supported by NIH Grant RO1 AM20929-04.

209.2 A NEUROPEPTIDE INDUCING ENHANCEMENT OF NEURONAL ACTIVITY IN CRAYFISH. H. Arêchiga and A. Huberman*. Dept. Physiol. and Biophys. Ctr. Adv. Studies, México 14, D.F. and Dept. Biochem. Inst. Natl. Nutr., México 20, D.F.

The electrical activity of crustacean nervous system has been shown to be modulated by a blood-borne peptide which is released from the sinus gland a neurohaemal organ in the eyestalk and induces a lowering of excitability, hence its denomination as neurodepressing hormone (NDH) (see Arêchiga, H. and Huberman, A. Peptide modulation of neuronal activity in crustaceans In The Role of Peptides in Neuronal Function. J.F. Baker y T.H. Smith, Eds. Marcel Dekker Inc. 1980). The presence is here reported of another peptide in the central nervous system of the crayfish which induces an enhancement of excitability.

The experiments were carried out in adult specimens of the crayfish Procambarus bouvieri (Ortmann), without distinction of sex. The central nervous system was divided in segments and extracts from each were prepared and succesively passed through Sephadex G-25 and G-15 columns. In a fraction previous to that containing NDH, carrying molecular specimens between 1500-2000 daltons a peptide was determined which increases the spontaneous activity of motoneurons at various levels of the central nervous system, and that of peripheral mechanoreceptors. This peptide is inactivated by trypsin and by heating. It is more abundant in the eyestalk than anywhere else, but is also present in the supraoesophageal ganglion and the thoracic ganglion chain. Within the eyestalk it is stored in the sinus gland. The electrical stimulation of this organ, or its incubation in high potassium solutions results in the release of this peptide. Once depleted from its peptide content, the eyestalk is capable of resynthesizing it after incubation in a proper aminoacid mixture. The site of synthesis is the medulla terminalis, in the region containing the perikarya of the neurosecretory cells whose endings are located in the sinus gland. This peptide therefore appears to act as a neurohormone, performing a physiological action opposite to that of NDH.

209.3 MORE THAN ONE PEPTIDE NEUROTRANSMITTER MAY MEDIATE BAG CELL ACTIONS ON CENTRAL NEURONS OF APLYSIA. B.S. Rothman#, L. Padgett#* and E. Mayeri†# #Dept. Physiology, Univ. of Calif., San Francisco, CA 94143 and †Dept. Basic Sciences, California College of Podiatric Medicine, San Francisco, CA 94115

A burst discharge of impulse activity in bag cell neurons produces four types of responses in various identified neurons of the abdominal ganglion (Mayeri et al., JNP 42:1165). The bag cell peptide, ELH, is the putative transmitter for two of these types of responses, prolonged excitation and burst augmentation. ELH has no effect on neurons in which bag cell activity produces the other two types of responses, prolonged inhibition and transient excitation (Branton et al., PNAS 75:5732; Rothman et al., Nsci Abstr 5:260). To identify transmitters for the other two responses we have purified various factors from extracts of the bag cells, using as an assay neurons with known responses to bag cell activity. 50 to 100 bag cell clusters were surgically removed from the rest of the abdominal ganglion and combined with 2-4 clusters which had been preincubated with ^{3}H-leu and/or -arg and -lys in order to radiolabel ELH and other bag cell peptides. The clusters were extracted in 0.5 M formic acid, centrifuged, and the supernatant applied to a gel filtration column (G50 Sephadex). To obtain ELH and the factors described below, eluents were further purified either by cation exchange chromatography or high performance liquid chromatography (HPLC).

In the 3-11K Dalton range, as indicated by the gel filtration purification step, there was an inhibitory factor that produced prolonged inhibition of cells L3 and L6, which are also inhibited by bag cell activity. The factor was associated with a radiolabelled peak that was distinct from ELH, suggesting it is a bag cell peptide, but not ELH. The effect was dose-dependent, had a threshold of 30 µg/ml, and at higher concentrations lasted more than 1 hr. In the 0-3K Dalton range there were several factors, some radiolabelled, which also inhibited L3 and L6, but with a faster onset and shorter duration than for the larger inhibitory factor. In addition there was a radiolabelled factor which produced transient excitation of L1 and R1. At a concentration of 200 µg/ml it duplicated the slow depolarization of these cells normally produced by bag cell activity.

The results suggest that in addition to ELH, which mediates two types of responses,there are several candidate transmitters for prolonged inhibition and one for transient excitation. Thus, the bag cells may synthesize more than one peptide transmitter, each one mediating a distinct action in the central nervous system.

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209.4 MULTIPLE ACTIONS OF FMRF-AMIDE ON IDENTIFIED NEURONS IN THE ABDOMINAL GANGLION OF APLYSIA. L.S. STONE#* and E. MAYERI#†. #Dept. of Physiology, Univ. of California, San Francisco, CA 94143 and †Dept. Basic Sciences, California College of Podiatric Medicine, San Francisco, CA 94115.

FMRF-amide (Phenylalanyl-methionyl-arginyl-phenylalanine amide), first isolated from a molluscan ganglion (Price and Greenberg, Science 197:670) and later found immunocytochemically in the CNS of both invertebrates and vertebrates (Boer et al., Cell Tissue Res., in press), has been shown to produce excitation and inhibition in a variety of molluscan bivalve hearts (Greenberg and Price, Peptides: Int. of Cell and Tissue Function) and inhibition of a molluscan neuron (Cottrell, JP 284:130P).

We arterially perfused the abdominal ganglion of Aplysia californica with FMRF-amide dissolved in buffered sea water plus protease inhibitors. This produces widespread effects on identified cells of the ganglion, most notably: inhibition of left upper quadrant (LUQ) cells (L2,L3,L4,L6), inhibition of L10, and a biphasic exitatory-inhibitory response in L1 and R1. Massive vasodilation of the perfused artery also occurs.

The effects in the LUQ's, L1 and R1 were investigated in greater detail. When the peptide is applied via pressure ejection from small diameter polyethylene tubing placed adjacent to the soma of any one of these cells, the targetted cell, but not neighboring responsive cells, is affected. This indicates that the responses are direct and there are receptors on the somata. *The prolonged hyperpolarization of the LUQ's is dose-dependent with threshold below 100nM, saturates at about 10µM, and shows* little or no desensitization even during 30 minute applications. In L1 and R1 a rapid depolarization, usually with superimposed spikes, is followed by a prolonged, smaller hyperpolarizing phase. This is also dose-dependent with a somewhat higher threshold and saturation point (less than 1 and 100µM respectively).

The widespread effects of FMRF-amide in the ganglion suggests that it may play an important role as a neurotransmitter and/or neurohormone. It also provides evidence that peptides are capable of eliciting multiple and conjoint actions on central neurons just as has been found for non-peptidic transmitters.

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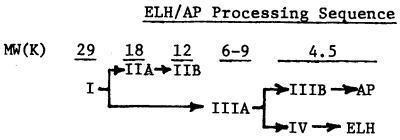
209.5 PROTEOLYTIC PROCESSING OF NEUROSECRETORY PEPTIDES BY THE BAG CELLS OF APLYSIA. R.W. Berry, J.T. Baylen*, and M.J. Trump*. Dept. of Cell Biology and Anatomy, Northwestern Univ. Sch. of Med., Chicago, IL 60611.

The neurosecretory bag cells of Aplysia produce and secrete two major peptide products: a highly basic 4,500 dalton (4.5K) egg-laying hormone (ELH), and a 4.5K acidic peptide (AP) of unknown function. Previous work has indicated that these peptides and an additional 12K product arise by cleavage of a 29K precursor, but the precise processing sequence and the structural relations among these proteins have yet to be established. Since detailed knowledge of the ELH/AP processing sequence might provide insight into multistep processing sequences in general, we have undertaken to identify the major members of this sequence by pulse-chase labelling, to characterize these proteins by sequential SDS-PAGE and isoelectric focusing, and to investigate their structural relationships by tryptic peptide mapping. The pulse-chase studies revealed eight major members of the processing sequence which have the precursor-product relations shown below:



All of these species except IIA and IIB are homogeneous by sequential SDS-PAGE and isoelectric focusing. The key features of the ELH/AP sequence are that the two secreted products arise from a single common precursor via three cleavage steps and a common intermediate form. The tryptic peptide mapping data are entirely consistent with this sequence and further indicate that the 12K material, which apparently represents a non-secreted endproduct, contains an additional copy of the AP peptide. Since the derivation of multiple secretory products from a common precursor, multistep processing, and unprocessed "extra" product copies are also found in the vertebrate processing sequence of proopiomelanocortin, these may be common features in the production of secretory peptides.

(Support: NIH NS 11519).

209.6 COEXISTANCE OF A NEUROPEPTIDE AND ACETYLCHOLINE IN AN IDENTIFIED MOLLUSKAN NEURON. P.E. Lloyd*, B. Masinovsky*, R.E. McCaman & A.O.D. Willows (SPON: R. Levine). Dept. Zoology, Univ. of Washington, Seattle, WA 98195, and City of Hope Medical Center, Duarte CA 91010.

Neuron B11 (formerly termed LDWC) is a large white appearing neuron located on the dorsal surface of each buccal ganglion in Tritonia. B11 is involved peripherally in the control of gut activity and centrally in the generation of cyclic bursting motor output from the buccal ganglia (Lloyd & Willows, Soc. Neurosci. Abstr. 6, 704; 1980).

B11 somata can be dissected free of the ganglia and their contents analyzed. They contain a cardioactive neural peptide that can be quantified by bioassay on an isolated snail heart. We have recently purified two cardioactive peptides from Aplysia nervous tissue that co-purify through gel filtration, aromatic affinity, and ion exchange chromatography. The two peptides can be resolved and purified to homogeneity as judged by quantitative amino acid analysis by reverse phase high performance liquid chromatography. The B11 peptide co-purifies through all steps with one of these peptides, termed small cardioactive peptide A (SCP_A), strongly suggesting that it is the same peptide. By comparing cardioactivity and amino acid analysis, we were able to determine the molar activity of SCP_A. The amount of SCP_A in a B11 soma is 1.9$\pm$ 0.3 pmoles (mean $\pm$S.D.: N>100).

A series of suggestive observations persuaded us to also assay for the presence of acetylcholine (ACh) in the somata of B11s. We used a radiochemical assay (McCaman & Stetzler, J. Neurochem. 28, 669; 1977) and found that a B11 soma contains 0.85$\pm$ 0.21 pmole ACh (mean $\pm$ S.D.: N=24).

The approximate volume of the B11 somata used for these analyses was 1 nl. Thus the concentrations of ACh and the peptide SCP_A in the cytoplasm of B11 is roughly similar at the 1-2 mM level. These are higher concentrations than those reported for transmitters (including ACh) in other Tritonia neurons.

B11 has a discrete peripheral target tissue, the foregut, which also contains ACh and SCP_A. Thus B11 and the foregut may provide an excellent system in which to physiologically study the actions of neurons that contain more than one bioactive substance.(NSF BNS 7906280 & NS 9339)

210.1 CONNECTIONS OF THE SUBFORNICAL ORGAN (SFO). R.W. Lind, G. W. Van Hoeson, & A.K. Johnson. Depts. of Psychology, Anatomy, & Neurology & The Cardiovascular Center, U. of Iowa, Iowa City,IA. 52242

The structures of the lamina terminalis (SFO, septum, median preoptic nucleus, and the organum vasculosum (ovlt)) have been independently studied over the years and each of them is thought to play a role in the control of bodily hydration. Anatomical studies of this area of the brain (Miselis, Neurosci. Abs. 1980; Hernesniemi, Acta Anat., 1972) have demonstrated that these structures are interconnected and Eng, et al, and Lind and Johnson (Neurosci. Abs., 1980) have reported that this circuitry is relevant to angiotensin-induced thirst. The present study investigated further the afferent and efferent connections of the SFO by using the horseradish peroxidase (HRP) technique.

Male rats received iontophoretic applications of HRP into the main body of the SFO. The marker was diluted to 20% in distilled water and pipettes with a tip of 20u were used. Injection parameters ranged from 0.5 uA x 30" to 2.0 uA x 150". Sections were reacted with tetramethyl benzidine and stained with neutral red.

A dense bundle of fibers was visualized issuing from the ventral stalk of the SFO containing both afferent and efferent connections. In agreement with Miselis' autoradiography data, we found that this bundle splits above the anterior commissure (AC), with some of the post-commissural fibers diverging caudally at the point where the columns of the fornix diverge laterally while others do not turn caudally until immediately above the AC. A substantial number of these post-commissural fibers appear to project to the paraventricular nucleus. (This observation was verified by the retrograde labeling of SFO neurons with HRP injections into PVN.) However, others by-pass this nucleus ventrally and were followed as far caudally as the median eminence.

The majority of fibers appear to pass anterior to the AC, tightly "hugging" it, with a few of them turning abruptly rostral at this point and continuing into the medial septum. As the main pre-commissural bundle continues ventrally through the median preoptic nucleus, it grows steadily more sparse, suggesting the presence of terminals throughout this nucleus. However, some fibers continue all the way to the ovlt where a few of them diverge laterally and project to the supraoptic nucleus.

Besides these efferent fibers, we also identified four sources of neural input to the SFO. The most prominent of these derived from cells in the triangular nucleus of the septum, with fibers entering the SFO by piercing the fornical commissure. Second, cells were observed in the medial septum. Third, neurons were labelled within the pre-commissural bundle anterior to the AC; fourth, cells were filled in the medial preoptic area.

210.2 LESIONS OF THE SUBFORNICAL ORGAN (SFO) BLOCK DRINKING TO PERIPHERAL BUT NOT CENTRAL ANGIOTENSIN. R.L Thunhorst*, R.W. Lind, & A.K. Johnson, (SPON: M.J. Brody) Dept. of Psychology and the Cardiovascular Center, University of Iowa, Iowa City, IA 52242

The SFO has been implicated in the control of fluid balance as a receptor for angiotensin (Simpson, et al., JCPP, 1978). While there is wide agreement that the SFO is sensitive to this hormone, its uniqueness as a central receptor is disputed (Buggy, et al., Science, 1975). The present study measures the ingestive behaviors of rats with lesions of the SFO, with particular attention paid to the thirst-inducing properties of angiotensin.

Thirty-three male rats that were screened for drinking to subcutaneous angiotensin (1.5 & 3.0 mg/kg) and hypertonic saline (4% & 12%, 10 ml/kg) received either an SFO (n=26) or a sham lesion (n=7). One week after surgery all animals were again tested with angiotensin and hypertonic saline at the same doses as pre-lesion. While control rats drank 2.4(±.7) mls and 3.7(±1.1) mls to the low and high doses of angiotensin, thirteen of the experimental rats were completely refractory to this stimulus. Furthermore, these thirteen rats drank significantly less than the controls to hypertonic saline at both the low dose (2.2 ± .8 mls vs 8.0 ± .9 mls) and high dose (9.0 ± 1.1 vs. 17.0 ± 1.5).

The rats that were refractory to peripheral angiotensin and the control animals were then implanted with intraventricular cannulae and tested for drinking responses to central angiotensin (50 & 500 ng). At the low dose the control animals drank 3.3 ± 1.0 mls vs. 2.4 ± .5 mls for the experimentals, and at the high dose the rats with sham lesions again drank more (4.7 ± 1.3 vs. 2.8 ± .4), though neither difference was significant. All rats that had received central angiotensin were then tested for feeding and drinking responses to food and water deprivation, respectively, and no group differences were observed (Food: $\bar{x}_e$=4.2 ± .6 g vs. $\bar{x}_c$=5.3 ±1.0 g; Water: $\bar{x}_e$=11.8 ± .9 mls vs. $\bar{x}_c$=13.4 ± 1.0 mls).

Brains were cut at 40μ with a freezing microtome and stained with cresyl violet. It was found that all experimental animals had some damage to the SFO but that the 13 animals that had failed to respond to peripheral angiotensin had lesions that included some part of the ventral stalk. Lesions in this location would sever neural connections between the SFO and periventricular structures of the anteroventral third ventricle (AV3V), which Miselis (Neurosci. Abs., 1980) and Lind, et al. (Neurosci. Abs., 1981) have described and implicated in angiotensin-induced thirst.

Our findings are consistent with the hypothesis that peripheral angiotensin induces thirst by acting in the SFO, and that angiotensin of central origin, which is also relevant for drinking behavior, acts in the region of the AV3V. A role for the SFO in osmotic thirst is indicated and requires further attention.

210.3 DISTURBANCES IN WATER BALANCE AFTER TRANSECTIONS OF SFO EFFERENT PROJECTIONS. Richard R. Miselis and Ricardo Eng. Anim. Biol., Inst. Neurol. Sci. Sch. Vet. Med., University of Penn., Phila., PA 19104.

The subfornical organ (SFO) of the rat has efferent neural projections to the nuclei of the anteroventral third ventricular area of the preoptic region and to some nuclei fo the hypothalamus, particularly the magnocellular nuclei (Miselis et al, Science, 1979). These terminal fields suggest a role in behavioral and physiological mechanisms of water balance. Transections of the SFO efferent projections in rats (SET rats) produce an obvious loss of drinking to i.v. angiotensin II (Eng, Miselis, and Salanga, Neurosci. Abst., 1980). Close examination of the water balance in this preparation reveals subtle problems in mechanisms of water conservation. First, SET rats (8 of 13) have a moderate polydipsia (18.9±1.4 vs. 12.1±1.1 ml of water/100g b.wt.) under ad libitum conditions. Their water-to-food ratios are elevated above controls (?.3±0.1 vs. 1.4±0.1). When food deprived all of the SET rats are polydipsic compared to control rats (7.4±1.2 vs. 3.9±0.6 ml/100g b.wt.). This polydipsia may be secondary to polyuria. In the immediate 24 hours post-operatively without access to water the SET rats are polyuric compared to controls (5.8±0.5 vs. 2.5±0.1 ml of urine/100g b.wt.). They remain polyuric although less so, and when tested at a later time under total deprivation still lose more water in the urine than control rats (3.3±0.4 vs. 2.3±0.2 ml urine/100ml b. wt.). When given access to water after a 24-hr total deprivation the SET rats drink the same amounts of water as the controls (2.6±0.4 vs. 2.5±0.4 ml water/100g b.wt.). Since they lost more water in the urine during the deprivation, their drinking response represents a deficit. The SET rats also do not conserve urine volume as well as control rats at 4 and 8 hours after a severe hypovolemic challenge caused by 5 ml of 30% PEG given s.c. They excrete 0.5±0.1 and 0.8±0.2 vs. 0.1±0.1 and 0.4±0.1 cumulative ml of urine/100g b.wt. at 4 and 8 hours respectively. These data reveal deficits in the control of renal mechanisms of water conservation when SFO efferent projections are disrupted. Since the SFO projects to magnocellular nuclei of the hypothalamus, the deficit in urine volume conservation may represent a loss of facilitation of antidiuretic hormone secretion.

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210.4 EFFECT OF KNIFE CUTS OF SUBFORNICAL ORGAN EFFERENT PROJECTIONS ON DRINKING BEHAVIORS. Ricardo Eng and Richard R. Miselis. Inst. Neur. Sci., Dept. Anim. Biol., Sch. Vet. Med., Univ. Penn., Phila delphia, PA. 19104

In the rat, the subfornical organ (SFO) has efferent projections to the preoptic area and areas of the hypothalamus that are involved in water balance. The efferent projections exit the SFO via its rostral component en route to the anteroventral third ventricular area (AV3V) and the ADH-producing paraventricular and supraoptic nuclei of the hypothalamus. We have previously shown that in the rat knife cuts of these efferents interfere with the drinking response to iv angiotensin II (AII) but not to high doses of hypertonic NaCl (Eng et al. 1980). We now report that the knife cuts also produce polydipsia, increased water/food ratios, polyuria, and disorders of water balance. The same knife cut rats that were unresponsive to iv AII responded to intracerebroventricular (icv) AII (10 ng); 10.5+1.4 vs. 10.6+1.5 ml for the controls with similar latencies. The latter results support the findings of Findlay, Elfont and Epstein (1980) in the opossum. The route-dependent differential drinking response to AII suggests that there are two sets of receptors for monitoring AII levels in the blood and the CSF. We also tested the knife-cut rats to stimuli that presumably activate the renin-angiotensin system, namely isoproterenol (ISO) and polyethylene glycol (PEG), to two doses of an osmotic stimulus (iv 2 M NaCl), and to 24 hr. total deprivation. The knife-cut rats do not drink to sc ISO, their response being comparable to drinking observed after control injections of isotonic saline vehicle. At 50 μg/kg ISO the knife-cut rats drank 0.7+0.2 vs. 1.2+0.1 ml/100 g BW for the controls. At 100 μg/kg ISO the respective responses were 0.8+0.3 vs. 1.6+0.2 ml/100 g BW ($p<.025$, df=16). The knife-cut rats did not differ from controls in the hypotensive response following 100 μg/kg ISO. Thus, the drinking deficits were not due to a general behavioral impairment following excessive hypotension. In contrast, the drinking to a high dose of PEG (5 ml, 30%, sc) was not impaired by the knife cuts. The knife cut rats had subtle deficits to iv 2 M NaCl at a low dose (0.5 ml/30 min.). During the one hour observation period, the knife-cut rats drank 0.7+0.4 vs. 2.5+1.2 ml for the controls ($p<0.05$, df=8). The subtle deficits to NaCl suggest that the SFO may also mediate osmotic thirst. However, at a higher dose (1.0 ml/30 min.) the knife-cut rats responded normally. The knife-cut rats' water intake did not differ from controls in a 2 hr. access after 24 hrs. of total deprivation. In view of increased urinary excretion by the knife-cut rats, this response actually represents a deficit. These findings support the hypothesis that the SFO and its projection fields are part of a neural network for the regulation of water balance.

210.5 EFFECTS OF GONADAL STEROIDS ON WATER AND SALT INTAKE INDUCED BY ANGIOTENSIN IN THE FEMALE RAT. J. Kucharczyk, Department of Physiology, School of Medicine, University of Oetawa, Ottawa, Ontario K1N 9A9.

Water intake, food intake and voluntary exercise in the female rat are predictably influenced by changes in endogenous levels of the two principal ovarian steroids, estradiol and progesterone. During proestrus and estrus, food and water intakes decrease while activity increases and rats lose weight. At metestrus and diestrus, when plasma estradiol is relatively low, the pattern of behaviors is reversed. Food and water intakes decrease, voluntary activity drops sharply and females gain weight.

Recent work suggests that ovarian hormones influence water intake by interacting with extracellular thirst mechanisms mediated by the renin-angiotensin system:

1. Water intake elicited by microinjection of 1 p-mole of angiotensin-II (ANG-II) into the preoptic region (POA) of cyclic rats is significantly lower on days of vaginal estrus or proestrus than on days of diestrus and metestrus.

2. Drinking in response to S.C. isoproteronol (a β-adrenergic agonist which increases ANG-II biosynthesis) is also significantly less at estrus than at diestrus.

3. Prepubertal female rats given single daily injections of ANG-II directly into the POA do not show cyclic patterns of water intake. After the onset of puberty, the same intracranial dose of ANG-II produces a cyclic pattern of elicited drinking, which in turn is abolished by bilateral ovariectomy.

4. Fluctuations in ANG-II and isoprenaline induced thirst are not secondary to changes in overall fluid and electrolyte balance, since the volume of water ingested in response to cellular dehydration (central and peripheral administration of hypertonic NaCl and sucrose) does not vary with the stage of the estrous cycle and is unaffected by ovariectomy.

5. Interaction between mechanisms for extracellular thirst and those controlling the estrous cycle likely occurs within the POA. There is evidence that this area of the brain contains receptors for ANG-II (Brain Res. 122:299) and estrogen (Brain Res. 101:67). While injections of p-mole doses of ANG-II into the subfornical organ, organum vasculosum of the lamina terminalis and lateral ventricles of cyclic rats also consistently produce drinking, the volumes ingested to not vary with the stage of the estrous cycle. (Supported by MRCC).

210.6 INFLUENCE OF OVARIAN STEROIDS ON ANGIOTENSIN-INDUCED DIPSOGENESIS AND PROSTAGLANDIN E_1-INDUCED ANTIDIPSOGENESIS AND THERMOGENESIS. K. M. Skoog and Nancy J. Kenney, Dept. of Psychology, University of Washington, Seattle, WA 98195.

Estrogens reduce the drinking (Fregly &Thrasher, 1978) but not the pressor (Skoog & Kenney, 1980) response to angiotensinII (AII). Both estrogens and progesterone attenuate the pressor response following central injection of prostaglandin E_1 (PGE_1; Skoog & Kenney, 1980). We now examine the effect of estrogen or progesterone replacement following ovariectomy on drinking to intracranial AII, on the PGE_1 suppression of AII-elicited water intake and on PGE_1-induced thermogenesis.

Ovariectomized (OVX) rats were given daily subcutaneous injections of estradiol benzoate (EB; 1 µg/rat), progesterone (PROG; 5mg/rat) or their seseme-oil vehicle beginning 7-8 days prior to testing and continuing throughout the experiment. On the test days, 5 ng AII was injected intracerebroventricularly (IVT) through indwelling stainless-steel cannulae. On days 1 and 3, the AIIinjection was preceeded by a 1 ul IVT injection of the PG carrier solution. On day 2, 100 ng PGE_1 was injected prior to the AII. Water intake was monitored for 30 min following the IVT injections. Core temperature was measured prior to the IVT injections and at the end of the drinking test.

In the absence of PGE_1, EB-treated rats drank significantly less than did oil-treated rats to AII ($p<.01$). PROG- and oil-treated females did not differ in average water intake to IVTAII. IVT PGE_1 significantly attenuated drinking to AII in PROG-treated OVX rats ($p<.01$) but not in oil- or EB-treated rats.

Baseline core temperatures of steroid-treated and oil-control animals did not differ. Likewise, steroid replacement had no effect on the increase of core temperature which typically follows IVT PGE_1 treatment. All rats showed marked core temperature increases following PGE_1 injection ($p<.01$).

Thus, a physiological dose of EB (Henderson et al., 1977) attenuates drinking to AII. PROG does not alter responsiveness to this dipsogen, however. The failure of PGE_1 to reliably suppress drinking of oil- and EB-treated OVX rats suggests that a lack of progesterone may result in the failure of female rats to exhibit PGE_1 antidipsogenesis. Finally, ovarian steroid replacement (either EB or PROG) in OVX rats does not affect PGE_1-induced thermogenesis.

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211.1 PRINCIPAL OLIVE (PO) COOLING IN BEHAVING MONKEYS PRODUCES MOVEMENT CHANGES RESEMBLING THOSE OF DENTATE DYSFUNCTION. Kennedy, P.R., Ross, H-G and Brooks, V.B. Dept. Physiology, University of Western Ontario, London, Canada N6A 5C1.

Selective cooling of PO (that projects largely to neocerebellum), caused replacement of brief, 'continuous' movements that oscillated at 6 Hz or above, by slower, 'discontinuous' ones that oscillated near 3 to 5 Hz. These changes of motor performance resemble those during cooling of cerebellar nuclei (J. Neurophysiol. 1973, 36:974).

A new gas cryoprobe with tip cooling was implanted in the flexible brainstem with x-ray assistance in 4 monkeys (J. Neurosci. Methods, 1979, 2:1 and 411). Monkeys were trained to make self-paced, intended, step-tracking movements consisting of elbow flexions (F) and extensions (X) between two targets that alternated at random intervals. In one monkey the cooling probe tip lay adjacent to the promontory of PO allowing selective cooling.

The most detailed evidence was obtained from 7 digitised cooling runs in which the temperature was adjusted to selectively cool 65% of arm area of PO and none of dorsal accessory olive (DAO) or reticular formation (RF). During this selective cooling, 13/20 F and 12/21 X movements oscillated at 3 to 5 Hz, compared with only 2/66 F and 3/66 X movements before and after cooling ($p<0.01$). Movetimes increased during selective cooling from 0.8 s for F and X, to 1.2 s for F and 1.1 for X ($p<0.01$). To outrule the effect of gas vibrating in the cooling probe, 2 controls were run with warm gas passing at the same rate as during cooling: Warm gas neither produced the 3 to 5 Hz oscillations nor the increased movetimes.

None of these effects were seen during cooling of DAO and/or RF in behaving monkeys. Instead, there was (1) a decrease in movement amplitude (or displacement amplitude in a different task), (2) drift of the arm towards flexion or extension depending on the tissue cooled, and (3) an increase in biceps EMG activity. These 3 changes were not seen during selective PO cooling.

The experiments demonstrate the importance of climbing fiber projections for correct cerebellar control of posture and movements, and suggest a role for PO different from that of DAO.

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211.2 COMPLEX RESPONSE PATTERNS IN DENTATE NUCLEUS DURING VISUALLY JAL GUIDED ARM MOVEMENTS. W.A. MacKay, H.C. Kwan, J.T. Murphy and Y.C. Wong*. Dept. of Physiology, Univ. of Toronto, Canada, M5S 1A8.

The aim of this study was to analyze single unit activity in the dentate nucleus during voluntary arm movements in terms of motor output parameters and sensory cues. Microelectrode recordings were made in chronically prepared macaque monkeys trained to perform a cyclic visual tracking task with the right arm. By means of horizontal elbow flexion-extension in a manipulandum, they maintained a cursor on top of a target line displayed on a videomonitor in front of them. The target line moved between two fixed positions at 1 sec. intervals. During the tracking task, controlled ramp torque pulses were delivered to the forearm in either direction. Of 193 ipilateral units studied in the cerebellar nuclei, only 27 were clearly related to the task. No task-related cells have yet been found in the fastigial nucleus, and no nuclear units have been uniquely correlated to movement parameters about a single joint (although several fibers in the overlying white matter have displayed such a correlation). Where task-related neurons occurred, they were clustered together. In the dentate, unit discharge was generally modulated phasically and bidirectionally (either more or less firing) and also was usually responsive to visual motor cues. Task-related units were not responsive to other arm movements such as reaching. Neurons which were directionally selective resembled the "joint position" cells of Thach, but the change in discharge rate associated with a particular joint angle was probably not a direct consequence of somatosensory input. Torque disturbances delivered to the forearm did not produce a directionally selective response, and visual influences were demonstrable, dependent on motor state. Many neurons not classified as task-related did modulate their activity in phase with the elbow movements, but only at the onset of a series of trials (volitionally initiated). Another group of non task-related cells showed a correlation with reaching movements. No dentate cells have yet been found with activity patterns correlated to isolated finger movements. In general, clusters of dentate neurons appear to be involved in the regulation of specific movements which may be classified by functional synergies rather than by discrete joint rotations. Furthermore, the regulation appears to be operative under a finite set of conditions.

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211.3 ALTERATIONS IN THE RESPONSE OF PURKINJE CELLS TO MOSSY FIBERS WHEN AN ASSOCIATED CLIMBING FIBER INPUT IS EVOKED BY PERIPHERAL STIMULI. T. J. Ebner and J. R. Bloedel. Departs. of Neurosurgery and Physiology, University of Minnesota Minneapolis 55455.

Experiments were performed in decerebrate, unanesthetized cats to examine the relationship between the Purkinje cell responses to mossy fiber and climbing fiber inputs responding to the same peripheral stimulus. Purkinje cells identified by the presence of spontaneous complex spikes were recorded in the anterior lobe of the cerebellum using conventional electrophysiological techniques. A natural peripheral input was applied with a displacement controlled vibrator which produced a brief (100 msec) flexion of the ipsilateral wrist. Peristimulus time histograms of the simple and complex spike activity of each cell were constructed during 100 presentations of the peripheral input. The trials used to construct the simple spike histogram were further subdivided based on whether or not a climbing fiber event occurred in response to the peripheral stimulus. Simple spike histograms were then constructed from the trials in each group and normalized based on the number of trials in each histogram. These two histograms were compared to determine whether the simple spike response to the peripheral stimulus was different when a climbing fiber response was also evoked. In many Purkinje cells, the increase or decrease in simple spike activity due to the forepaw flexion was considerably greater when a climbing fiber response occurred. In fact, for some cells a component of the response to the mossy fiber input only occurred when climbing fiber afferent input was present. These results support previous work showing that the change in simple spike activity evoked by a peripheral stimulus can be affected by the occurrence of a climbing fiber input. Specifically, when a climbing fiber input to a Purkinje cell is evoked by a peripheral stimulus, the changes in simple spike activity produced by the same stimulus are often greater and/or different than when no climbing fiber input occurred. This work was supported by NIH Grant #2R01-NS-09447-10.

211.4 EFFECTS OF SPONTANEOUSLY OCCURRING CLIMBING FIBER INPUTS TO ONE PURKINJE CELL ON THE RESPONSES OF NEIGHBORING CELLS TO MOSSY FIBER INPUTS. J. R. Bloedel and T. J. Ebner. Departs. of Neurosurgery and Physiology, University of Minnesota, Minneapolis, 55455.

Recent experiments in our laboratory demonstrated that the magnitude of the excitability change evoked in a Purkinje cell by activating a mossy fiber input is dependent upon the time it occurs after a spontaneous climbing fiber input to the same neuron. The present study was performed to determine if the occurrence of a climbing fiber input to one cell is correlated with changes in the response of neighboring Purkinje cells to a naturally evoked mossy fiber input. The activity of two to four Purkinje cells was simultaneously recorded with glass microelectrodes in the anterior lobe of decerebrate unanesthetized cats. The cells were separated by 150-1000 u in various spatial relationships relative to the direction of parallel fibers. Once each cell of a set was isolated, a natural stimulus consisting of a tap on the dorsum of the forepaw and a flexion of the wrist was applied at various intervals after the occurrence of a spontaneous climbing fiber input to one of the cells. The simple and complex spike responses at each stimulus presentation interval were determined by constructing perievent histograms simultaneously for all cells in the set from 100 consecutive responses to these natural stimuli. An analysis of these results revealed that, in several sets, the response of any cell in the set to the mossy fiber input could be increased when evoked at intervals from 20-60 msec following the climbing fiber input to only one of these neurons, even when the spontaneous climbing fiber inputs of the neurons were not temporally correlated. This observation was not associated with a change in the excitability of the neighboring neurons resulting from the action of the climbing fiber input used to trigger the sweep. Effects of this type were observed between neurons separated by as much as 400 u in the sagittal plane or by 1 mm along the direction of parallel fibers. Based on these observations it is concluded that the occurrence of a climbing fiber input to a given Purkinje cell is associated with changes in the responsiveness of neighboring Purkinje cells to naturally evoked mossy fiber inputs. This work is supported by NIH Grant #2R01-NS 09447-10.

211.5 CLIMBING FIBER INPUT FROM MECHANORECEPTORS TO LOBULE VI OF THE CAT CEREBELLUM. Lee T. Robertson and Ethan Schrank*. Neurol. Sci. Inst., Portland, OR 97210.

Descriptions of cutaneous projections to the cerebellum have traditionally placed input from the face in lobule VI and input from the lower body in lobules IV and V. However, our previous studies of climbing fiber (CF) projections to lobule V have shown a more complex organization, including a connection with the ipsilateral face. This investigation of cutaneously elicited CF responses in the intermediate zone of lobule VI of the cat reveals a more complex topographic organization than shown by previous descriptions, including considerable input from all over the body surface.

The cats were anesthetized with sodium pentobarbital and extracellular recordings were made of single Purkinje cells. The CF responses were elicited by gentle taps to the body and by computer-controlled punctate stimuli. Receptive fields were delineated and latency and force thresholds were measured.

CF responses were isolated in 512 cells, of which 77% were directly elicited by cutaneous stimulation of various body surfaces (40% face, 45% forelimb, 8% midbody, and 7% hindlimb). The 157 receptive fields involving the face were distributed to: the ipsilateral ear (32%), tip of the nose (28%), mandible (25%), cornea (11%) and forehead (11%). This distribution was very different from that observed for lobule V face zone, where only a few fields included the ear, none involved the cornea, and many represented the oral cavity. Input from the forelimb elicited CF responses in lobule VI in 181 cells. The receptive fields of these cells included the shoulder and arm (23%), forearm (41%), and forepaw (36%). More than a third of the receptive fields for the forepaw were bilateral and symmetrical, a condition that was never observed in lobule V. The somatotopic organization for intermediate zone consisted of several elongated patches. Medially, the bilateral forepaw fields were located caudally and the face input was found rostrally; in the middle of the zone input from the lower back, rump, and abdomen were identified; and laterally, another forelimb patch was found rostrally and a small hindlimb area caudally.

These data, when compared with the organization of cutaneous input in lobule V, indicate that the same peripheral information is represented in multiple cortical areas. The different proportional representation of skin surfaces between various lobules does not simply reflect peripheral innervation density, but suggests distinct functional roles for each lobule.

211.6 GENESIS AND MODIFICATION OF THE GEOMETRY OF CNS HYPERSPACE. CEREBELLAR SPACE-TIME METRIC TENSOR AND "MOTOR LEARNING". A. Pellionisz and R. Llinas. Dept. Physiology & Biophysics, New York University Med. Ctr., 550 First Ave., New York 10016.

Understanding CNS function is related, in our view, to defining the properties of the geometry of its hyperspace. Tensor network theory treats such geometry formally (Neurosci.Abst.1978), interprets motor coordination as a covariant-contravariant transformation through a cerebellar (CB) metric tensor (Neurosci. Abst. 1979), and features this metric as furnishing the CNS with an internal space-time geometry (Neurosci.Abst. 1980).

A fundamental question here however, is how such metrics arise in the CNS. The CB is an excellent candidate in which to define such task because the musculoskeletal geometry is innate, and thus the CB metric must be ontogenetically constructed. However, given the curvature of motor hyperspace such metric cannot be constant, i.e. the connectivity matrix must be dependent on the position of the motor vector in this hyperspace. The climbing fiber (CF) system has been considered as actively changing the curvature of the hyperspace by altering the physiological transformation of motor vectors through the CB metric. (Neuroscience 1980. Vol.5. p.1125.)

As for the question of CB involvement in "motor learning" we suggest that the inferior olive provides a covariant CF vector (CFV) that is the inner product of the covariant correction vector (the difference between intention and execution) and the contravariant motor status vector, both being expressed in the non-orthogonal motor execution reference-frame. This "motor adjustment" by CFV is an ongoing function that refines motor performance without invoking synaptic plasticity or anatomical modification of the CB metric. The more general "motor acquisition" of new movements arises in this model as an extracerebellar generation of new intention vectors using the existing CB metric. Finally, "motor adaptation" (e.g. in vestibular compensation: Science, 1975, Vol.190. p.1230) requires for its acquisition and retention the CF system indicating that such "adaptation" involves an alteration of CB nuclear activity. We suggest such alteration to be based on the confluence of covariant CFV (through CF collaterals into the nuclei) and the contravariant CFV that is transformed through the CB corticonuclear network.

Indeed, we conclude that co- and contravariant confluence is the basis for the genesis of epigenetically formed metrics throughout the CNS. (Supported by USPHS grant NS13742 from NINCDS)

212.1 APPETITIVE STIMULI AROUSE THE VENTRAL WHITE CELL TO THE PROLONGED BURSTING AND SPIKE BROADENING THAT DRIVE CYCLIC BUCCAL MASS MOVEMENTS IN PLEUROBRANCHAEA. Martha Ulbrick Gillette*. (SPON: Rhanor Gillette). Dept. of Physiol. & Biophys. and Neural & Behav. Biol. Prog., Univ. of Illinois, Urbana, IL 61801.

It was previously reported that spontaneous bursting in a bilateral pair of neurons, the ventral white cells (VWCs) of Pleurobranchaea, drives rhythmic neural output like that of feeding behavior in the cut roots of the isolated buccal ganglion (Gillette, Gillette and Davis, 1980, J. Neurophysiol.: 43). Further, the ability of VWCs to drive motor output is specifically dependent upon spike broadening during repetitive firing. In isolated ganglia, quiescent VWCs can be stimulated to prolonged bursts and attendant spike broadening by agents which elevate intracellular cyclic AMP, which may be an intrinsic modulator of these neurons (Gillette, Gillette and Davis, 1978, Neurosci. Abst.: 4)

The present study was undertaken to establish the extrinsic activators of the VWCs and how these activators relate to the behavioral output of these neurons. All animals used in the study responded to appetitive stimuli (squid juice or 10% amino acid hydrolysate) with a bite/swallowing sequence. Aversive stimuli (10% ETOH or 1% Haemosol) were assessed by their ability to interrupt ingestion of an appetitive stimulus and induce rejection. Immediately after behavioral testing, a semi-intact preparation (head, buccal mass, esophagus, CNS) was made for intracellular recording from the VWC. In this semi-intact preparation, appetitive and aversive stimuli were observed to produce orienting and withdrawal movements, respectively, in the oral veil. Appetitive substances applied to the mouth stimulate prolonged burst episodes (3-5 min) in the VWC with changes in spike broadening and spike undershoot waveform resembling cyclic AMP-induced bursts. During these bursts, the esophagus shortens and the buccal mass undergoes vigorous cyclic movements. The durations of VWC bursts and interburst intervals are similar to bouts of ingestive activity observed when the whole animal is presented with a long, continuous string of squid (8 ft). The latency from presentation of the appetitive stimulus to response in the VWC depends on the appetitive history of the animal, and thus suggests a role for the VWC in behavioral arousal. When the semi-intact animal is presented with aversive stimuli, the VWC either hyperpolarizes or remains silent near resting potential, depending upon the preparation. These results support a role for the VWCs in the animal's response to food ingestion such that the VWCs command motor network output which initiates or intensifies vigorous swallowing behavior. Supported by NSF Grant BNS-79-18329 to R.G.

212.2 CEREBRAL NEURONS CONSTITUTING A COMMAND SYSTEM FOR LOCOMOTION IN APLYSIA. Steven M. Fredman and Behrus Jahan-Parwar. Worcester Foundation for Exprimental Biology, Shrewsbury, MA 01545.

Our studies have indicated that in Aplysia californica, the various parts of the control system for locomotion are anatomically separated and located in different ganglia. The pedal ganglia contain both the foot motor neurons and the oscillator circuitry which drives pedal waves during locomotion. The cerebral ganglion contains neurons that initiate locomotion. These communicate with the pedal ganglia neurons via the cerebro-pedal (C-P) connectives. Cutting the C-Ps abolishes all locomotor activity (Jahan-Parwar and Fredman, Behav. Neural Biol. 27, 1979). In the present study we have identified neurons in the cerebral ganglion which are part of the "command" system for initiating locomotion. Utilizing an isolated CNS-tentacle preparation, bursting neural activity previously shown to be a correlate of pedal waves (Fredman and Jahan-Parwar, Brain Res. Bull. 5, 1980) was recorded from the cut ends of pedal nerves. The cerebral neurons had a characteristic location within the ganglion and met several criteria for being part of a "command" system. All had axons in the C-P. The neurons exhibited a high degree of decussation with 90% (n=20) having an axon in the contralateral C-P. While none had axons in both C-Ps, bilaterally homologous neurons had common synaptic input. The neurons also exhibited polysynaptic interactions among themselves. The neurons were excited by stimuli which evoked locomotion in vivo and its neural correlates in vitro: Stimulation of the tentacles with food (seaweed) extracts and noxious stimulation of the head and tail. Increased firing by the cerebral neurons preceded both spontaneous and evoked pedal wave bursts. When intracellularly stimulated, each neuron either triggered units in the pedal nerves or increased the firing frequency of spontaneously active units. All could alter the firing of units in nerves both ipsilateral and contralateral to the side to which they projected. The influence of each neuron was not equal. Different cerebral neurons altered the activity of different units in the pedal nerves. More prolonged tonic depolarization of each neuron evoked patterned nerve activity which was a correlate of the pedal wave. This indicates that the individual neurons were sufficient to initiate the pedal wave motor program. While pedal wave bursts could occur when a given neuron was silent, hyperpolarizing a neuron frequently increased the interburst intervals. Our results suggest that the initiation of locomotion results from the collective action of these cerebral neurons on their followers in the pedal ganglia. This work was supported by PHS grant NS 12483 to BJP.

212.3 SOME EVIDENCE FOR SINGLE TRIAL LEARNING IN THE NUDIBRANCH MOLLUSC, AEOLIDIA PAPILLOSA. H.S. Orbach, L.B. Cohen and M.B. Boyle. Dept. of Physiology, Yale University School of Medicine, New Haven, CT 06510.

Previous experiments suggested that the gastropod, A. papillosa, was capable of associative learning using a food-aversion paradigm similar to that developed by Gelperin and collaborators with the land slug Limax. We have now attempted to demonstrate single-trial learning in Aeolidia with the aim of obtaining a learned behavior that could be accomplished by a semi-intact preparation.

Two protocols were used. For both protocols there were between four and seven animals in each of two groups. In the first, each group was exposed in a control trial to a different novel food and allowed to bite for three minutes. The foods were then reversed in the experimental trial, with 10 mM quinine administered after the animals had bit for three minutes. The animals' food preferences were then measured by measuring the time it took the animals to bite the control and experimental foods and by using Y-tube olfactometers. The differences in time to bite the control and experimental foods were computed for each animal. Although there was considerable scatter in the data, in each of three experiments the mean difference after one trial with quinine was greater than the mean difference before quinine. Olfactometry measurements also indicated a preference for the control food in each experiment. In two of the three experiments the p values were less than 0.1; in one, the p value was greater than 0.1. In the second protocol the animals were again divided into two groups. In the control trial one group was exposed to sea-water for ten minutes, the other to sea-water plus a seafood odor (flounder or scallop) for ten minutes. In the experimental trial the groups received reversed stimuli for five minutes, and quinine (2.5-5 mM) was then added for five minutes. The animals preferences for sea-water versus sea-water plus seafood odor were measured using the olfactometers. In the three experiments the Aeolidia that received quinine paired with odor showed a clear relative avoidance of the odor side of the olfactometer. In two of the three experiments the p values were less than 0.1.

Because of the scatter in the data our results must be interpreted cautiously. However, they do suggest the possibility of a robust, one-trial associative learning in Aeolidia that could be used for in vitro cellular analyses.

Supported in part by NIH grant NS08437.

212.4 APLYSIA LEARN THAT A SPECIFIC FOOD IS INEDIBLE. A.J. Susswein, S. Gev* and M. Schwartz*. Dept. of Life Sciences, Bar-Ilan Univ., Ramat-Gan, Israel 52100.

Aplysia were taught that either of two foods was inedible. Strong and stable biting responses are elicited approximately equally well by the green algae Ulva lactuca or by leaves of the vascular plant Hemesocallis fulva. Pieces of these foods were placed in a plastic mesh, allowing Aplysia to taste the food through the holes in the mesh, but preventing Aplysia from consuming the food. Mesh-enclosed food arouses feeding behavior, and elicits repetitive biting and swallowing responses, initially at a rate comparable to that produced by non mesh-enclosed food. By five minutes after onset of feeding, rate of feeding responses is slowed by 61.3 ± 31.0 (S.D.)%. Responses cease in a mean of 22.1 ± 16.7 (S.D.) min. Stimulation of the lips for comparable periods of time with non mesh-enclosed food does not produce a similar decrease of feeding responses, indicating that sensory adaptation or habituation of taste pathways is not occurring. Following training on mesh-enclosed food, animals still respond to the same food when it is not enclosed in the mesh, or to the alternate food even if it is enclosed in mesh. This indicates that the decrease in responsiveness to mesh-enclosed food is not due to fatigue, or to non-specific inhibition of feeding behavior. The data also indicate that Aplysia do not learn to stop responding to a specific taste, or to the texture of the net. These data suggest that Aplysia learn that a specific combination of taste and texture is inedible.

Supported by U.S.-Israel Binational Science Foundation Grant No. 2210.

212.5 ANALYSIS OF THE SYNAPTIC DECREMENT UNDERLYING HABITUATION OF THE GILL-WITHDRAWAL REFLEX IN APLYSIA. J. Byrne Dept. of Physiology, University of Pittsburgh School of Medicine, 15261.

Repeated stimulation of the siphon skin results in habituation of the reflex contractions of the gill (Pinsker et al, 1970). The habituation, in turn, is casually linked to a depression of the EPSPs in motoneurons from mechanoreceptor sensory neurons (SN) (Castellucci et al, 1970; Byrne et al, 1978). The present study was undertaken to examine the kinetics of the depression and its recovery, as a first step in formulating a quantitative model of the release process.

Intracellular recordings were made from sensory neurons and motoneurons in solutions of elevated Ca and Mg to block interneuronal contributions. A SN action potential was elicited with a depolarizing current pulse and the amplitude of the resultant EPSP in the motoneuron was monitored, while ten stimuli at an interstimulus interval (ISI) of 1,3,10,30, or 100 s were presented. To control for the state of arousal of the animal and long-term decrement of the SN synapses, animals were maintained in separate chambers, dissections performed under Mg anesthesia, and only a single decrement run performed on each cell. Each SN was rested 30 m prior to stimulation. The results represent recordings from 47 cells in 29 experiments. At least 9 cells were examined at each ISI. The synaptic decrements vary as a complex function of ISI. At an ISI of 1 s there is a rapid depression which reaches a plateau of 35% of control. In contrast, the depression at an ISI of 100 s is less pronounced showing a gradual decay to 68% of control with the 10th EPSP. Surprisingly, the decrements at ISIs of 3,10 and 30 sec were similar in time course and magnitude and are intermediate between the 1 and 100 s ISIs. There is also a complex relationship between spike interval and the depression of the second of two EPSPs. The 2nd EPSP is reduced to 57% of control with a spike interval of 1 s, 84% with 3s, 81% with 10s, 79% with 30s, and 88% with 100s. Thus, depression of the 2nd of 2 EPSPs or decrement of a train of EPSPs is not a monotonic function of spike interval. Indeed, the data suggest that there may be a slight underlying facilitatory process with short spike intervals. Preliminary results indicate that the recovery of synaptic depression following a train of 10 stimuli is not constant. Shorter spike intervals seem to produce more rapid recovery.

These data are inconsistent with a classical depletion model (Liley & North, 1953) for synaptic depression, and indicate that either a single complex function of time and ISI or multiple functions underlie synaptic depression at the sensory neuron synapse.

212.6 OLFACTORY CHOICE BEHAVIOR OF NORMAL AND MUTANT DROSOPHILA IN A CONFLICT SITUATION IN A SUCCESSIVE CONDITIONING PARADIGM. Yadin Dudai, Dept. of Neurobiology, The Weizmann Institute of Science, Rehovot 76100, Israel.

The behavior of wild-type Drosophila (C-S strain) and of the X-linked olfactory conditioning mutants, dunce^{DB38} (Dudai et al., PNAS 73:1684, 1976) and rutabaga and cabbage (Aceves-Pina and Quinn, Science 206:93, 1979; Tempel and Quinn, Neurosci. Abst. 6:589, 1980) was analyzed in an olfactory conditioning task. Populations of flies were exposed to an odorant, A (e.g., benzaldehyde) associated with an electric shock and later tested for choice between A and another odorant, B (e.g., 3-octanol), in a T-maze. The experiments were controlled for odor-bias and non-associative behavioral modifications. The fraction of normal flies in the non-shock odorant arm minus the fraction in the shock-odorant arm was 0.44±0.06 when measured 30 sec after training. A transient decrease was detected in memory within 2-4 min after training. Memory reached initial values again at about 5-10 min and decayed slowly ($\tau_{1/2}$≈45 min). Memory of dunce^{DB38} was 70% of normal, of rutabaga was 60% of normal, and of cabbage was 40% of normal 30 sec after training. Memory decayed rapidly in all the mutants to ~5% of normal at 7 min. Memory of heterozygotes was intermediate. Successive conditioning experiments were conducted to test whether the mutants store latent memory that cannot evoke a normal choice but may interact with newly acquired information. Flies were exposed to a shock-odorant, were exposed again a few minutes later to the same or another shock-odorant, and were then tested for their choice between A and B, so that the test was performed 30 sec after exposure to the last shock-odorant and 7 min after exposure to the first shock-odorant. Successive conditioning to A increased only slightly the selective avoidance of A in C-S and did not significantly improve immediate memory of the mutants. Conflicting conditioning, i.e., first to B and later to A, resulted in a marked decrease of selective avoidance in C-S and the mutants, except dunce. A shock in the presence of a non-relevant odorant 30 sec or 7 min before testing did not revive lost memory in the mutants or abolish their immediate memory, respectively. Taken together, the results suggest that all the mutants can form short-lived olfactory associations. Behavioral analysis, however, can separate the mutants into different types: rutabaga and cabbage, but not dunce, apparently can behave as though they store information for a longer period than that revealed by straightforward memory tasks, but this information reveals itself only by interaction with newly acquired information in a conflict situation. (Supported by the U.S.-Israel Binational Science Foundation, Jerusalem).

212.7 USE OF A LEARNING PARADIGM TO DEFINE BEHAVIORAL STATE IN AN INSECT. Robin R. Forman and Sasha N. Zill*. Dept. Biol., Univ. of Oregon, Eugene, OR 97403.

In invertebrates, many postural and load compensatory reflexes are variable and can exhibit changes in gain or sign depending upon the behavioral state of the animal (Bassler, Biol.Cyber. 24:47, 1976; DiCaprio & Clarac, J.Exp.Biol. 90:197, 1981). While learning paradigms have been widely used to define behavioral state in vertebrates (Evarts, Science 179:501, 1973; Bizzi, et al., J. Neurophysiol. 41:542, 1978), these paradigms have not been applied to invertebrate preparations. We here report the use of a leg position learning paradigm to investigate the role of an insect leg proprioceptor, the locust metathoracic femoral chordotonal organ, in actively maintained postures.

The femoral chordotonal organ monitors the angle of the femoro-tibial joint. Reflex effects of the chordotonal organ were examined by abruptly displacing the main ligament of the organ. Pulls to the ligament mimicked ligament shortening that accompanies flexions of the tibia, releases after initial pulls mimicked equivalent extensions.

In untrained animals, step displacements of the ligament elicited variable resistance reflexes in motoneurons to tibial muscles, as monitored myographically, that consisted of brief phasic discharges of slow motoneurons.

In learning experiments, locusts were trained to hold the tibia of one hindleg within a narrow (15-20°) range of joint angle in order to avoid heating of the head or mouthparts by a focussed lamp. After training, step displacements of the ligament resulted in prolonged compensatory bursts in leg motoneurons. These bursts were significantly greater in intensity and duration in animals that were trained and actively maintaining a joint position than in untrained animals, animals that had not learned after training, or yoked control preparations. Abrupt releases of the ligament demonstrated substantial hysteresis in motoneuron response. Similar hysteresis is seen in recordings of afferent activity from the femoral chordotonal organ.

These studies demonstrate that the reflex effects of input from the femoral chordotonal organ are consistently increased when an animal is induced to actively maintain a specified posture. We suggest that learning paradigms may be of use in investigations of postural mechanisms in other invertebrate preparations. Supported by NSF grant BNS 75-00463 and NSF grant SPI-7914916.

212.8 ULTRASTRUCTURE AND CONNECTIVITY OF IDENTIFIED NEURONS IN THE FLY'S BRAIN. H.Eckert and K.Meller*. Univ. Bochum, Animal Physiol., D-4630 Bochum, W.-GERMANY

Some 22 motion sensitive neurons in the lobula plate of the fly, Phaenicia, were identified by intracellular dye injection; they are believed to control torque, lift and thrust responses sind their responses to moving stimuli accord with those of the behavioural responses. Some of these cells can be recognized in semithin sections. By alternately cutting serial semi- and ultrathin sections, their synaptic structures were studied.

The axons of the 9 V-cells bifurcate in the lobula plate. The two main dendrites, second and higher order dendritic branches are all postsynaptic to small fibers; the highest density of synapses is found for the smallest dendritic branches. All synaptic structures are characterized by a high number of synaptic vesicles in the presynaptic cell, a presynaptic T-shaped ribbon and a thickening of the postsynaptic membrane. The axons are partially surrounded by 2-14 layers of membranes resembling myelinated axons. Within the protocerebrum, the axons possess pre- as well as postsynaptic structures, the former showing only a few synaptic vesicles. Associated with the V-cells is a VH-cell similar in appearance but possessing an additional dorsal branch which originates at the proximal edge of the lobula plate; it possesses postsynaptic structures at the level of the H-cell-dendrite. The 3 H-cells possess a large dendritic fan which is postsynaptic to small fibers. A second 'axonal arborization' in the central protocerebrum, as well as the axonal endings, possess pre- as well as postsynaptic structures. The axonal arborization is presynaptic to CH-cells. Most synaptic structures are characteristic for chemical synapses; membrane specializations probably indicating electrical synapses were also found. The 2 CH-cells are postsynaptic to H-cells and unidentified cells in the protocerebrum. In the lobula plate, pre- as well as postsynaptic structures were found.

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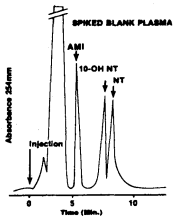
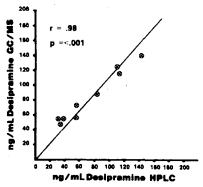
213.1 HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC (HPLC) QUANTITATION OF AMITRIPTYLINE, NORTRIPTYLINE AND 10-HYDROXY-NORTRIPTYLINE IN HUMAN SERUM USING A RADIAL COMPRESSION MODULE. D.M. Martin*, W.R. Dixon*, A.L.C. Pottash and M.S. Gold.
Psychiatric Diagnostic Labs. of America, Summit, N.J. 07901 and Hoffmann La Roche, Nutley, N.J. 07110.

Accurate measurement of plasma tricyclic antidepressant concentrations and their psychoactive metabolites facilitates the effective clinical management of depression. Nortriptyline (NT) is the most widely studied tricyclic, with an established therapeutic response "window" of 50-140 ng/mL. However, in amitriptyline (Ami) treated patients the combined Ami and NT concentrations do not reliably yield a plasma level-response correlation. This may be due to conversion of Ami to both NT and a psychoactive hydroxylated metabolite. This hydroxylated metabolite (10-OH-NT) has been demonstrated to inhibit synaptosomal monoamine reuptake and is capable of reversing the "reserpine" syndrome in rats. This suggests that 10-OH-NT has antidepressant activity and emphasizes the need to include the hydroxylated metabolite when measuring total plasma antidepressant concentrations and correlating them to clinical effects. We report a rapid and sensitive HPLC procedure for the simultaneous measurement of AMI, NT, and 10-OH-NT in clinical plasma samples. The compounds were extracted from 1.0 mL of plasma at pH 10.5 into iso-octane:ether (9:1), the organic phase freeze dumped and taken to dryness under a stream of nitrogen. The reconstituted residue was injected into a HPLC using an isocratic system of 0.3% butylamine in methanol flowing at 1.5 mL/min through a HPLC radial compression module containing a silica gel cartridge. Detection was in the ultra-violet at 254 nm. Ami, 10-OH-NT and NT were readily separated under these conditions (Fig). The limit of sensitivity of the procedure was approximately 25 ng/mL for each compound. Over three hundred patient samples have been analyzed utilizing this technique, yielding a mean day-to-day coefficient of variation of less than 9%. It was noted that steady state 10-OH-NT levels were generally greater than NT levels in the same patient and in some instances it would appear that high levels of 10-OH-NT may be responsible for observed cardiovascular arrhythmias and/or reduced therapeutic response.



213.2 HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC) VERSUS GAS CHROMATOGRAPHY MASS SPECTROSCOPIC ANALYSIS (GC/MS) OF A TRICYCLIC ANTIDEPRESSANT IN HUMAN PLASMA. A.L.C. Pottash, D.M. Martin*, I. Extein, W.Z. Potter*, A.P. Zavodil* and M.S. Gold. Psychiatric Diagnostic Laboratories of America, Summit, N.J. 07901 and Clinical Psychobiology Branch N.I.M.H., Bethesda, Md. and Clinical Research Facilities, Fair Oaks Hospital, Summit, N.J. 07901.

Tricyclic antidepressants (TCA) are the most widely prescribed medications used in the psychopharmacological treatment of major endogenous depression. Steady state plasma concentrations of these compounds vary considerably between subjects treated with the same dose and can be influenced by pharmacokinetic and other factors. This makes therapeutic drug monitoring essential to insure maintenance of adequate plasma concentrations during a medication trial. Analytical techniques for routine monitoring of TCA's which have appeared in the literature are being utilized in the clinical laboratory. Variations in analytical methodology may explain the conflicting findings in some reports on the clinical utility of TCA levels. We have previously reported a HPLC method for monitoring TCA's and have recently completed a double blind study of this technique versus a GC/MS technique. Nine patients on varying doses of imipramine or desipramine were included in the study. The blood samples were drawn and the plasma split into two coded vials. All operators were blind to the code throughout the analysis. The HPLC assay procedure was reported by Martin, et al (Neuroscience Abstract, 1979). The GC/MS assay procedure was reported by Claeys et al (Biomed. Mass. Spec. 3:110, 1976). The results demonstrated a highly significant correlation between the GC/MS and HPLC assays ($r=.98$; $p < .001$; see figure). These data suggest the reported HPLC method compares well with existing GC/MS techniques and has the additional benefit of simplicity of instrumentation and ease of applicability for routine therapeutic monitoring in the research and clinical laboratory.



213.3 NEUROPHARMACOLOGICAL PROFILE OF WELLBUTRIN™ (BUPROPION): A NOVEL ANTIDEPRESSANT WHICH INHIBITS DOPAMINE UPTAKE. B. R. Cooper, K. Viik, T. Uicker; Department of Pharmacology, Wellcome Research Laboratories, Research Triangle Park, North Carolina 27709.

Bupropion, a novel compound with antidepressant effects in man was found to reduce immobility in the Porsolt "Experimental Helplessness" forced swimming antidepressant test like imipramine or amitriptyline. Higher doses produced elevated locomotor activity in an automated open field and produced stereotyped sniffing. When bupropion or desmethylimipramine were given prior to intracisternal injections of 6-hydroxydopamine, bupropion produced a dose-related selective antagonism of the destruction of dopamine (DA) neurons beginning at 25 mg/kg i.p. Under the same conditions, desmethylimipramine produced a dose-related selective antagonism of the destruction of noradrenergic (NE) neurons. Bupropion also antagonized 5HT uptake in vivo at doses of 50 mg/kg i.p. or greater as judged from antagonism of p-Cl-methamphetamine-induced serotonin depletions. Studies of synthesis of ^{3}H-catecholamines from 2,6-^{3}H-tyrosine revealed that low doses (3 mg/kg to 12 mg/kg) decreased ^{3}H-DA formation and increased ^{3}H-NE formation. Higher doses led to an increase in ^{3}H-DA formation. A decrease followed by an increase in DA utilization was also reflected in changes in DOPAC levels in brain. It is felt that these changes in DA systems reflect the consequences of DA uptake inhibition on the activity of DA neurons. Thus low levels of DA uptake inhibition reduce DA neural activity via the dopamine being directed to presynaptic inhibitory autoreceptors while high doses of bupropion effectively prevent reuptake thereby calling into play mechanisms for synthesis of DA. The importance of dopamine neurons for the CNS pharmacology of bupropion was studied in rats with DA neural destruction produced by 6-hydroxydopamine. Destruction of DA neurons antagonized the effects of bupropion in the Porsolt antidepressant test and in tests of locomotor activity. Destruction of NE neurons had no significant effect on the locomotor stimulating effects of bupropion or on its actions in the Porsolt test.

Results indicate the importance of dopamine for the CNS pharmacology of bupropion in rat. Evidence of elevated 5HT and NE neural activity was also obtained after bupropion in rats. Since all three amines are theoretically implicated in human depression, clinical evaluation of the activation of all three monoamine neural systems by bupropion in depressed patients through monitoring DOPAC, 5HIAA, and MHPG·SO_4 levels in CSF is desirable. Such monitoring may provide the data required to determine the mechanisms of action of this drug, as well as provide insights valuable in bridging the gap between the effects of drugs in animal models and in human mental disease.

213.4 NOVEL ANTIDEPRESSANTS AND NEUROLEPTICS: EFFECT ON THE CENTRAL ADRENERGIC SYSTEM. R.L. Winsky*, S.H. Preskorn, R. Glotzbach*, and G. Irwin* (SPON: C.W. Hughes). Dept. of Psychiatry and Pharmacology, Univ. Kansas Medical Center, Kansas City, KS 66103.

Integrity of the blood:brain barrier (BBB) is of vital importance to normal cerebral functioning. The BBB appears to be regulated by central adrenergic neurons and this regulation can be influenced by administration of psychoactive drugs such as tricyclic antidepressants, TCA's (Preskorn et al., JPET 1980). In this series of studies, the effects of experimental antidepressant and neuroleptic drugs on cerebral permeability to water and blood flow (CBF) in the rat were studied. E_W --the cerebral extraction fraction for water--was determined by i.v. administration of a tracer solution of ^{3}H-water and ^{14}C-butanol as previously described. CBF was measured by comparing brain uptake of ^{14}C-butanol in an arterial blood sample withdrawn at a known, uniform rate (Preskorn, et al., Sci. 1981). The drugs tested have varying clinical effects and different neuropharmacological mechanisms. Amitriptyline, a norepinephrine reuptake blocker, causes an increase in E_W at a dose of 0.4mg/ kg i.v. This increase is mediated by the central adrenergic effects of the drug (Preskorn, et al., Neurosci. Abst. 1980). Three drugs (thioridazine, fluphenazine, and an analogue of loxapine) which block postsynaptic dopamine receptors did not alter E_W when given as a single i.v. dose of 0.4mg/kg. Neither did drugs which are predominantly dopamine or serotonin reuptake inhibitors: (a) bupropion and nomifensine, and (b) citalopram and trazodone, respectively. However, two drugs with predominantly central adrenergic mechanisms did alter E_W. Dothiepin--an analogue of amitriptyline--produced an increase in E_W similar to that of amitriptyline and the other TCA's. In contrast, mianserin--a drug having greater binding affinity for both alpha one and two adrenergic receptors than TCA's--reduced E_W.

These results should be interpreted cautiously because only one dose was employed. Yet, they do support the central adrenergic vasoregulatory hypothesis which states that this neuronal system has, as one of its functions, regulation of brain permeability and blood flow. Of the drugs tested, only those with known adrenergic mechanisms of action altered E_W. This in vivo animal model may be a useful adjunct to the biochemical and behavioral approaches used to assess the central effects of experimental drugs. This model is unique in that it reflects the in vivo effect of experimental manipulation on the interaction between the central adrenergic system and the cerebral microcirculation-- an end-organ which it directly innervates.

Supported by Research Scientist Development Award MH-00272 (S.H.P.) and NS-17252 (S.H.P.)

213.5 EFFECTS OF ANTIDEPRESSANTS ON THE CENTRAL CHOLINERGIC NERVOUS SYSTEM. Mark E. Goldman and Carlton K. Erickson, Div. of Pharmacology, College of Pharmacy, Univ. of Texas, Austin, TX 78712

The purpose of this investigation was to study the effects of antidepressants and anticholinergics on the cholinergic system after acute and chronic drug administration. Drugs were first divided into highly potent, moderately potent or weak anticholinergic categories based upon each compound's ability to displace ^{3}H-QNB from synaptosomal membranes. One antidepressant and one non-antidepressant with similar anticholinergic properties were chosen as representative agents of each anticholinergic potency category. In the first study, high affinity choline uptake was used as an indicator of the turnover rate of acetylcholine. Acute administration of amitriptyline or atropine (highly potent anticholinergics) increased choline uptake levels in the hippocampus and striatum. After 30 days pretreatment with either drug, an acute challenge dose no longer altered choline uptake in either region. The tolerance seen after chronic drug administration could not be attributed to hepatic mechanisms since the challenge was still ineffective in altering choline uptake levels in rats treated with SKF-525A (50 mg/kg; 60 min. prior to the challenge). Choline uptake levels were also unchanged following an amitriptyline challenge in rats chronically pretreated with atropine. Imipramine and thioridazine (moderately potent anticholinergics) increased choline uptake only in the striatum. After chronic drug treatment followed by a challenge dose, choline uptake levels were not significantly different from controls. Nomifensine and d-amphetamine (non-potent anticholinergics) are presently being evaluated. In the second study, changes in muscarinic receptor distribution and binding affinity were determined after chronic drug administration and 72 hr. withdrawal. Atropine caused an elevation of receptor density in the cortex, hippocampus and striatum. Amitriptyline elevated muscarinic receptor density only in the cortex. Imipramine, thioridazine, nomifensine and d-amphetamine did not alter muscarinic receptor density in any of the brain regions examined. None of the drugs altered receptor affinities after 72 hr. withdrawal. The results of these studies show that tolerance can occur to the central cholinergic effects of antidepressants. Since the central cholinergic actions of antidepressant drugs matched the central cholinergic actions of anticholinergic drugs lacking clinical antidepressant activity, we conclude that the effects of antidepressants on the central cholinergic nervous system are more closely related to the side effects of these drugs than to their mechanism of therapeutic action. (Supported by funds from the University of Texas.)

213.6 TRAZODONE ENHANCES LOCUS COERULEUS FIRING RATE: EVIDENCE FOR RELATIVE LACK OF α-2 ADRENORECEPTOR INVOLVEMENT. Welch, J. J., Kim, H. K.* and Liebman, J. Res. Dept., Pharma. Div., CIBA-GEIGY Corp., Summit, NJ 07901.

α-2 Adrenoreceptor antagonism has been proposed as a possible mode of antidepressant action, particularly in the case of mianserin. Recently, it has been suggested (Clements-Jewery et al., Neuropharmacol. 19:1165, 1980) that α-2 adrenoreceptor antagonism may also be a property of trazodone, another clinically effective antidepressant. This mechanism was proposed to account for the ability of trazodone to increase the turnover of norepinephrine in rat brain. Using extracellular single unit recording techniques, we have explored this possibility by examining the effects of trazodone and other adrenoreceptor antagonists on noradrenergic locus coeruleus neuronal firing rate.

Intravenous administration of low doses of trazodone (0.5 - 4 mg/kg) enhanced locus coeruleus firing rate dose-dependently. This increase was comparable in magnitude to that following i.v. treatment with yohimbine (0.5 - 2 mg/kg), a known preferential α-2 antagonist. However, the α-1 antagonists, prazosin and WB-4101, were also found to increase locus coeruleus firing rate. Prazosin is believed to be devoid of α-2 blocking activity, but WB-4101 has α-2 as well as α-1 blocking properties.

The comparative ability of these drugs to antagonize clonidine's known suppressant action on locus coeruleus neurons is believed to be mediated through a direct agonist action on α-2 adrenoreceptors. When clonidine was administered by itself in small incremental doses, approximately 6 μg/kg was required to inhibit firing to 50% of baseline rate (ID_{50}). Pretreatment with yohimbine elevated the ID_{50} of clonidine markedly (to 90 μg/kg), presumably reflecting strong α-2 blockade. In contrast, trazodone only partially blocked the effects of clonidine (clonidine ID_{50} = 16 μg/kg), despite its marked effectiveness in elevating locus coeruleus firing rate by itself. Prazosin and WB-4101 also failed to attenuate the effects of clonidine.

These results indicate that trazodone may enhance the synaptic availability of norepinephrine by increasing locus coeruleus impulse flow. These effects, however, are unlikely to be mediated by α-2 noradrenergic receptors. Other factors, including α-1 receptor antagonism, are apparently capable of modulating locus coeruleus neuronal activity.

213.7 TRAZODONE: RECEPTOR BINDING BY A NOVEL ANTIDEPRESSANT. Duncan P. Taylor, Elizabeth M. Ashworth*, Jerry A. Becker*, and Deborah K. Hyslop*. Biologic Research Mead Johnson Pharmaceutical® Division, Evansville, IN 47721.

Trazodone HCl (Desyrel®) is a clinically effective and well-tolerated antidepressant (J. Clin. Psychiat. 40: 390). Its pharmacologic profile is quite different from that of known antidepressants inasmuch as it does not show the type of response exhibited by conventional tricyclic antidepressants in traditional animal test systems. Trazodone has been shown to block serotonin (5-HT) uptake with a high degree of selectivity compared to its potency toward uptake of catecholamines (Psychopharmacol. 63: 99). We have investigated the in vitro interactions of trazodone in a variety of radioreceptor binding systems. Trazodone is a weak inhibitor of binding at sites labeled by ligands for α_2, β, benzodiazepine, dopamine, glutamate, glycine, H_1 and H_2 histamine, and 5-HT_1 sites. In marked contrast to currently available antidepressant drugs, trazodone only weakly inhibits binding at muscarinic cholinergic sites, and this interaction is noncompetitive in nature. Chronic treatment of rats with tricyclic antidepressants leads to decreased in vitro binding at β and 5-HT_2 (labeled with [^{3}H]spiperone) receptors. Chronic oral dosing of trazodone (20 mg/kg, 28 days) leads to a significant loss of 5-HT_2 receptors without change in the number of β-adrenergic receptors. In combination with phenoxybenzamine (10 mg/kg), i.p. administration of 40 mg/kg trazodone resulted in decreased 5-HT_2 receptor binding after only four days of treatment, while neither drug alone had any effect during this time. Changes in β-adrenergic binding were seen only after 11 days' treatment with 100 mg/kg., i.p., trazodone. In this experiment, 5-HT_2 binding was reduced 45% while β-adrenergic binding was reduced 17%. These results suggest that changes in β-adrenergic binding are secondary to changes in 5-HT_2 binding. Trazodone and the tricyclic antidepressants are good inhibitors of 5-HT_2 binding in vitro. It is not yet known if this phenomenon is related to the selective inhibition of 5-HT uptake or if either effect is clinically relevant. In view of the time course of trazodone's action, the selective effect on the number of 5-HT_2 receptor sites following chronic administration may represent the mechanism of antidepressant action.

213.8 LITHIUM ISOTOPES: DETERMINATION OF ABUNDANCES IN PLASMA AND ERYTHROCYTES. K.W. Lieberman, *P. Karczmar* and P.E. Stokes. Division of Psychobiology, Department of Psychiatry, New York Hospital-Cornell Medical Center, New York, New York 10021.

Lithium (Li) is used in the treatment of the manic phase of manic depressive illness, but the reasons for its therapeutic efficacy remain unclear. Naturally occurring lithium (Li-N) which is used as the chloride salt in the treatment of manic patients is composed of 2 stable isotopes; lithium-7 (Li-7) is the major component (92.58%) and lithium-6 (Li-6) the minor component (7.42%). Results have been reported suggesting the 2 isotopes of Li have dissimilar membrane transport, behavioral and toxicological properties. Work up to this time has involved the use only of isotopically pure (99%+) Li-6 and Li-7 in the chloride form. Experiments that used mixtures of Li-6 and Li-7 were precluded because of the difficulties inherent in the methodology. An expensive and highly complex technique, mass spectroscopy, is the usual approach and for our purposes this was impractical. On the basis of the work of Brost. et al. (Anal. Chem. 51, 1512, 1979) we developed a method for the simultaneous determination of the isotopic abundances of Li-6 and Li-7 in plasma and erythrocytes that utilizes atomic absorption spectrophotometry, an economical and convenient technique. The analytical lines for the 2 Li isotopes are the resonance doublet at 670.8 nm. Determination of the Li isotopic composition in plasma and erythrocytes is based upon the method used when several compounds that are analyzed simultaneously possess overlapping ultraviolet or visible spectra. Absorbance measurements are carried out using Li-6 and Li-7 hollow cathode lamps. At a total Li concentration of 1.3 ppm and a Li-6 abundance of 7.42% the relative error in the determination ranged from 2-5%. For a total Li concentration of 1.0 and 1.5 ppm and a Li-6 abundance of 7.42% the relative now was larger, 5-10%. This technique will now enable us to do experiments with mixtures of Li isotopes so that we may further investigate to what degree biological systems differentiate between Li-6 and Li-7.

213.9 SHORT-TERM LITHIUM ENHANCES 5-HT NEUROTRANSMISSION IN RATS ADMINISTERED CHRONIC ANTIDEPRESSANT TREATMENTS. C. de Montigny, A.-T. Tan and G. Caillé*, Départ. de Psychiatrie et de Pharmacologie, Univ. de Montréal, Montréal, Québec, Canada H3C 3J7.

Lithium induces a rapid relief of depression in patients under treatment with but not responding to tricyclic antidepressant drugs (TCA) (de Montigny et al., Brit. J. Psychiat., 138: 252, 1981). The same observation has now been made in subjects having failed to improve with electroconvulsive shock therapy (ECT) (unpublished observations). It was postulated that, in these patients, the effect of lithium might be due to an enhancement of the efficacy of the 5-HT system superimposed to the sensitization of postsynaptic receptors already induced by TCA or ECT. This hypothesis was put to the test using the "5-HT syndrome" model in the rat.

Male-Sprague Dawley rats (200-250 g) received daily injections of imipramine (IMI) (5 mg/kg, i.p.) or saline (0.5 cc, i.p.) or were given ECT (150 V, 10 ms pulses at 50 Hz for 1 s) three times weekly for two weeks. Half of the animals in each group were also given lithium carbonate (27 mEq/g of food) during the last two days of the treatment period. Lithium blood levels ranged from 0.6 to 1.1 mEq/L. On the 15th day, all rats were challenged with tranylcypromine (TCP) (20 mg/kg, i.p.) and L-tryptophan (L-TP) (50 mg/kg, i.p.). The intensity of the 5-HT syndrome was rated 75 min later according to the criteria of Jacobs (Life Sci., 19, 777, 1976) by two evaluators unaware of the treatment.

In rats treated with IMI or ECT, the 5-HT syndrome produced by TCP and L-TP was slightly enhanced as compared with saline controls. In these controls lithium itself produced a marked increase of the syndrome, as already reported by Grahame-Smith and Green (Brit. J. Pharmacol., 52: 19, 1979). An even greater increase was observed when lithium was added to the IMI or ECT treatments. Notably, sustained myoclonia could be observed only in animals having received both antidepressant and lithium pretreatments.

In the last few years, several groups of investigators have demonstrated that chronic administration of TCA or ECT in the rat results in an increased responsiveness of forebrain neurons to 5-HT. The present data suggest that lithium addition to these antidepressant treatments can further enhance the synaptic efficacy of the 5-HT system. This finding is consistent with the hypothesis that lithium might exert its rapid antidepressant effect in TCA- or ECT-nonresponding patients by augmenting the action of the 5-HT system on postsynaptic neurons already sensitized to 5-HT. (Supported by MRC Grant MA-6444 and a CRSQ fellowship to C. de M.).

213.10 CHRONIC LITHIUM TREATMENT MODIFIES THE RESPONSES TO PAIRED PULSE STIMULATION IN THE IN VITRO RAT HIPPOCAMPUS. P.C. Rinaldi, G. Barrionuevo, M.E. Rosnowska*, K. Davila*, S. Tewari*, and G. Lynch. Dept. of Psychobiology and Dept. of Psychiatry and Human Behavior, Univ. of Ca., Irvine, Ca. 92717.

While lithium is presently employed in the treatment of a variety of psychiatric disorders and chronic alcoholism, little is known of its mechanisms of action at the level of the neuron. We have investigated this question by measuring several parameters of synaptic transmission in in vitro slices of hippocampus taken from rats chronically treated with lithium.

Li_2CO_3 was given to male rats intragastrically for 4 weeks (150 mg/Kg/day) producing a stable mean serum lithium level of 1.2 mEq/l. Controls received an equal volume of water in the same manner. After hippocampal slices were prepared, bipolar stimulation electrodes were placed both in the Schaffer commissural projections to the apical dendrites of CA1 pyramidal cells and in the alveus. Extracellular activity was recorded in either the s. pyramidalis or s. radiatum.

The response of neurons to the second of two paired pulses allowed us to examine both neural facilitation and inhibition under well-defined circumstances.

To examine "facilitation", the conditioning and test stimuli were delivered through a single electrode and recordings were made of population dendritic and population spike responses. Facilitation was calculated by plotting the mean amplitude of the second response in the pair both as a percentage of the control responses and as a function of interpulse interval. To measure "feedback inhibition", we antidromically activated the recurrent collaterals of the CA1 pyramidal cells and tested for the effects of this on the population spike elicited by subsequent stimulation of the Schaffer commissural projection. "Feedforward inhibition" was tested using two different Shaffer-commissural inputs. Inhibition was measured as the mean percent decrease in amplitude of the population spike recorded from the the test input.

Li rats exhibited enhanced facilitation as measured in percent increase in amplitude and shift in time course. Conversely the time course of feedback and feedforward inhibition for Li rats was typically shortened. Implications for the mechanism of Li action will be discussed.

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213.11 **BEHAVIORAL ACTIONS OF LITHIUM IN AGED MURICIDAL RATS.** **P. Broderick† and V. Lynch*, St. John's University, Jamaica, New York, 11714.**

We previously published data (Pharmacologist 1978) which reported that lithium inhibited muricidal (mouse killing) behavior in isolated young (2 month old) male, Long Evans rats. These data report that lower doses of lithium are required to produce this same behavioral change in their older counterparts.

Male, Long Evans rats (600-700 gms), approximately one year old, were housed in individual cages, without bedding, in our animal care facility. Food and water were available ad lib. Albino mice (20 gms) were placed in each rat's cage and rats which repeatedly killed mice within a fifteen minute period were selected for the study. Then rats were treated intraperitoneally and acutely with lithium chloride (0.01-10 meq/kg). Testing for the inhibition effect of muricidal behavior took place one hour after drug administration, in the evening. Any rat which did not exhibit muricidal behavior while an albino mouse was present in the rat's cage for one half hour was considered to have the killer response blocked. An adjunct test, rotarod performance, which is typically used to determine the presence of ataxia, was completed concomitantly, in the same dose response range of lithium, to determine whether or not the response to kill had disappeared due to a motor disorder by lithium.

The results showed that the ED50 for the inhibition of muricidal behavior in these rats was 0.25 meq/kg, while the ED50 for any impairment observed via rotarod performance was 7.7 meq/kg. Saline and distilled water vehicle injections produced no effect on muricidal behavior. The dose of lithium chloride needed to block the killer response in older animals was four-fold less than that needed to produce the same behavioral change in younger rats.

Therapeutically, this study is of interest since comparison of the effects of lithium on young vs old rats appears to pattern itself after the clinical response of lithium in young vs elderly patients. Elderly patients may require lower doses of lithium due to lower renal lithium clearance (Schou, 1967), and this influence of lithium on kidney function can be shown in rats (Thomsen, JPET, 199, 1976). Further research will specify whether renal clearance is a factor in this particular animal model.

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214.1 TRIGEMINAL MEDIATION OF AN OROMOTOR FRACTIONAL ANTICIPATORY GOAL RESPONSE. M. Jacquin* & H. Enfiejian* (SPON: H.P. Zeigler). Dept. of Anatomy, CMDNJ-Rutgers Medical School, Piscataway, N.J. 08854.

As well as their role in reinforcing feeding and drinking, orosensory stimuli (S) have another function which is essentially motivational. The fact that a reinforcing S can also function as an incentive poses theoretical problems. How does experience which coincides with the termination of behavior come to contribute to the initiation of the response (R) in a different situation? Learning theorists have proposed that incentive motivation arises through classical conditioning of a goal R to the S in the reinforcement situation. When the primary reinforcer is absent, a fractional anticipatory goal R is theoretically necessary for initiation and maintenance of operant behavior (Hull, 1943); e.g., in the absence of food or water in the lever pressing or paw reaching situation, anticipatory jaw-related movements and their concomitant somatosensory feedback may be necessary to bind these operant sequences together, resulting in eventual delivery of reinforcement. To test this hypothesis rats were subjected to either trigeminal motor root sections (V) or hypoglossal nerve sections (XII), eliminating jaw or tongue movements, respectively. Rats from each group provided pre- and postoperative data on either time spent feeding and drinking, meal frequency and duration in a simple home cage operant paw reaching paradigm, food or water-reinforced lever pressing, or lateral hypothalamic (LH) stimulation-reinforced lever pressing. Verification of the central projections of sectioned nerves by horseradish peroxidase histochemistry was carried out in a separate group to ensure their proper identification.

V or XII did not impair responsiveness to food or water in the home cage; in fact compensatory hyperresponsiveness was the rule. However, V produced marked reductions in meal frequency and duration in the operant paw reaching situation, while XII did not disrupt meal patterns. In the food- or water-reinforced lever pressing situation, rats with V performed at a level far below that of extinction/sham operated controls and rats with XII. The operant deficits produced by V are likely specific to food or water reinforcement since rats with V lever pressed for LH electrical stimulation at or above preoperative rates. Neither "motor deficit" preparation (V or XII) was capable of ingesting significant amounts of food or water, yet their R rates were strikingly different. How do these two preparations differ? V eliminates pre-ingestive jaw movements while XII does not. The operant deficits seen after V may, therefore, reflect a functional deafferentation produced by removal of somatosensory inputs which normally occur as feedback from jaw-related prefeeding or fractional anticipatory goal R's.

214.2 ONE-TRIAL PASSIVE AVOIDANCE LEARNING IN THALAMIC RATS. M. Pritzel*, M. Joosten*, I. Fix*, H. Mutawi* and J. P. Huston (SPON: M. Terman). Inst. of Psychology III, University of Düsseldorf, Düsseldorf, FRG.

A series of experiments was designed to investigate the possibility of passive avoidance learning in thalamic rats and to search for possible neuronal mechanisms underlying task performance. After bilateral removal of the telencephalon rats were subjected to an up-hill response test. In this test they are placed on a slanted surface with the body oriented downhill and the latency for turning up-hill is recorded.

The thalamic rats exhibited the up-hill response reliably and with a latency comparable to that of unlesioned control animals. To test for passive avoidance learning, the up-hill response was punished by an electrical tail shock. The up-hill response latencies were measured after response-contingent shock, response-noncontingent shock and conditions without shock. Only the response-contingent shock group showed a significant increase in the latency to emit the up-hill response on a testing trial 2 hrs later. The results suggest that thalamic rats, like normal control animals, are capable of learning the one-trial up-hill passive avoidance task. It was concluded that the necessary neuronal mechanisms for learning of the task must be represented at a subtelencephalic level.

We also investigated neuronal systems which might be critically involved in the mediation of the up-hill response by interfering with the vestibular-occulomotor information processing. The up-hill avoidance test was still performed by rats with bilateral lesions of the vestibular nuclei and/or whiskers trimmed and/or eyes occluded. It was found that a combination of vestibular lesions and occlusion of the eyes was necessary to prevent learning of the up-hill avoidance task. Thus, learning of the up-hill passive avoidance task does not depend on an intact telencephalon. As long as the animals can rely on a critical amount of sensory feedback, they are able to master the avoidance task on a diencephalic-mesencephalic level.

214.3 Trimethyltin Neurotoxicity: A Potential Neurobiological Tool For Learning and Memory. C.F. Mactutus, J.J. Valdes, J.S. Young, and Z. Annau. Dept. Environ. Hlth. Sci. The Johns Hopkins University, Baltimore, MD 21205.

The widespread CNS edema produced by triethyltin stands in marked contrast to the relatively specific neuronal degeneration of the hippocampus and limbic system produced by trimethyltin (TMT) (Am. J. Path., 97: 59-82, 1979). The present study assessed the neurotoxicity of TMT administered on a chronic dosing regimen, but over a different exposure route, shorter intervals, and lower doses than previously reported, as well as the utility of this preparation as a tool in studying the neurobiology of learning and memory.

Adult Long-Evans hooded rats received 3 i.p. injections, one/week, of either 2, 3, or 4 mg/kg TMT or ETOH vehicle (ns=12). Two-way avoidance performance, typically facilitated by hippocampal lesions, was assessed starting 24 hr after the last injection and continuing over 4 days, 25 trials/day. Four randomly selected rats from each group were sacrificed for neuropathological examination.

A significant body weight loss, observed only for the 4 mg/kg group, often culminated in death. In the remaining groups avoidance performance was facilitated following TMT exposure. Of particular interest was a dose related increase in response perseveration (i.e., avoiding in the same direction) in the TMT exposed rats. Across groups initial shock escape latencies were similar suggesting no alteration of motivation or pain thresholds. Initial adaptation responding was also comparable among groups indicating no alterations in activity levels, however, an increase in intertrial crossings (reactivity) was found in the 3-, but not 2-, mg/kg TMT groups. Preliminary data indicates similar changes in shuttle performance when TMT is administered after acquisition training but prior to a retention test.

Neuropathological examination confirmed extensive neuronal degeneration within hippocampal cell fields CA3 and CA4, moderate damage in CA1, and a sparing of the CA3a field. Neuronal alterations within the pyriform cortex and an enlargement of the 4th ventricle were also noted.

In summary, TMT produces relatively specific neuronal damage and does so at doses lower than initially suspected. Moreover, that low doses of TMT alter avoidance performance in the absence of changes in emotionality indicates the potential of this preparation for studying the neurobiology of learning and memory.

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214.4 TRIMETHYLTIN, A SELECTIVE HIPPOCAMPAL NEUROTOXICANT, IMPAIRS LEARNING AND MEMORY OF A RADIAL ARM MAZE TASK. Thomas J. Walsh* Diane B. Miller* and Robert S. Dyer (Spon; D. E. Weil). Neurotoxicology Division, US Environmental Protection Agency, Research Triangle Park, NC 27711.

Trimethyltin (TMT) is a neurotoxic organometal which selectively damages the limbic system, in particular the hippocampus (Brown et al., Am J Path, 1979). Systemically administered TMT preferentially destroys pyramidal neurons in the CA3 and CA4 cell fields of the hippocampal formation. The presumed hippocampal involvement in behavioral and neural plasticity suggests that TMT may produce long term functional impairments of learning and memory. Indeed, memory loss was a symptom in two reported cases of accidental human exposure to TMT (Fortemps et al., Int Arch Occup Environ Hlth, 1978).

We examined the effects of acute TMT administration on the acquisition and retention of a radial arm maze task. In the first experiment, adult male hooded rats were intubated with either saline, 5, 6 or 7 mg/kg TMT 21 days prior to training in an automated radial arm maze. TMT-treated rats took significantly longer to enter and eat in all eight arms (i.e. acquire the task) and exhibited impaired selection accuracy, reflected in a tendency to repeatedly enter previously selected arms. In the second experiment untreated rats were trained for three weeks in the maze to achieve stable performance. Following this training period the animals were intubated with either saline or 6 mg/kg TMT and allowed 14 days before retention of the task was evaluated. Saline-treated rats retained the task, whereas the TMT-treated animals required extensive retaining, and exhibited perseverative responding throughout the period of testing. During testing, activity did not consistently differ between groups, and TMT-treated rats did consume all food pellets within any given test session. Therefore it is unlikely that the impairments in maze performance were secondary to changes in either locomotor activity or motivation. Rather, these data argue that TMT impairs learning and retention of the task. This study demonstrates that damage to specific hippocampal cell fields produces long-term deficits in learning and memory processes.

214.5 ACQUISITION AND EXTINCTION OF AN OPERANT RESPONSE FOLLOWING SELECTIVE DAMAGE TO ROSTRAL HIPPOCAMPAL PROJECTIONS IN RATS. T. A. Silverhart*, B. Osborne, and J. Seggie. Dept. of Psychology, Middlebury College, Middlebury, VT 05753 and Dept. of Neuroscience, McMaster University, Hamilton, Ontario, Canada.

It is well established that hippocampal lesions or total fornix transections result in an increase in resistance to extinction following continuous reinforcement. Although the rostral projections of the hippocampus are heterogeneous, few studies have examined these functionally. Recently, Gray (Gray, J. A., Handbook of Psychopharmacology, New York: Plenum Press, 1977) proposed a model to account for the extinction deficit following hippocampal damage that utilizes these differences. The present study examined the role of the rostral projections during the transition to extinction and tested Gray's model of separate functions for different portions of the fimbria-fornix. The response to the transition to extinction of an operant lever press response was examined in rats with total fimbria-fornix transections (n = 8), medial fornix lesions (n = 11), fimbria lesions (n = 7) and operated controls (n = 7). The measure of total lever presses was supplemented by making a detailed analysis of behavior (frequency, duration, sequencing, and topography) and by measuring plasma corticosterone levels.

Analysis of total lever press responses showed that all four groups acquired the response at the same rate and that total fimbria fornix transections increased resistance to extinction. Rats with medial or fimbria damage responded in extinction at intermediate levels but at levels not significantly higher than controls. The detailed analysis examined food related behaviors (lever press and food cup check bouts and bout durations), trips away from food cup and lever, trip duration and topography shifts as indexed by biting lever presses. The frequencies of lever press bouts for total and medial groups were significantly higher than control or fimbria groups. For frequency of food cup bouts, totals were higher than all groups, and medials were higher than fimbria or control levels. When compared to controls or partial transected groups, the total group exhibited altered sequencing with short duration trips away interspersed with food behaviors. All groups except totals exhibited topography shifts while neither medial, fimbria or total groups reacted to extinction with the normal corticosterone elevations.

Gray's model suggests that the medial group would respond to the transition to extinction like totals and that the fimbria group would respond like controls. The present results do not support this model. A possible explanation in terms of additive effect for damage to separate regions is offered.

214.6 PERFORMANCE DEFICITS INDUCED BY HIPPOCAMPAL DISCONNECTIONS ON REFERENCE-MEMORY TASKS. R. Pico*[1,3], L.K. Gerbrandt[1,2] and J.L. Davis[3]. 1) Dept. Psychology, Calif. St. Univ., Northridge, CA 91324, 2) Neurosciences Res. Progr., Mass. Instit. Tech., 165 Allandale St., Boston, MA 02130, 3) Psychobiol. Lab., V.A. Hospital, Sepulveda, CA 91343.

Ideally, hypotheses accounting for the behavioral effects of hippocampal disconnections will be explicitly applicable to all experimental procedures where permanent performance deficits are found after damage to hippocampal connections. This series of experiments was designed to explore some of the potential limits of applicability of the working-memory and place-mapping hypotheses of hippocampal functioning (Olton, D.S. et al., Behav. Br. Sci., 2:313, 1979; O'Keefe, J.O. and Nadel, L., Behav. Br. Sci. 2:487, 1979).

Fimbria/fornix connections were electrolytically lesioned in 7 rats (FFX) and sham lesioned in 7 rats (SHC). In the first phase of the experiment, rats were trained to obtain food pellets at the end of each of the 8 arms of a radial maze on the basis of 4 topologically invariant extramaze cues that were aligned at the end of 4 of the maze arms. These cues were rotated independently from all other cues. On half of the arms, rats were rewarded after every choice to that topologically defined arm (reference-memory arms). On other arms, they were rewarded only on their initial choice (working-memory arms). FFX rats were compared to SHCs in their probabilities of visiting previously unchosen arms relative to topological cues. FFXs performed near chance levels both on working-memory and on reference-memory arms, whereas SHCs had high, stable probabilities of visiting unchosen arms on either section of the maze. Subsequent tests were run to determine whether rats could discriminate working and reference memory arms.

In the second phase of the experiment, prior reference-memory arms were again rebaited after each choice, but prior working-memory arms were continually unbaited, turning these also into reference-memory arms. The effects of random deletions of 1, 2, or 3 cues within the topological set were then tested. Finally, the effects of topological randomization of cues were tested. These and additional results will be presented.

214.7 HIPPOCAMPAL LESIONS DISRUPT DISCRIMINATION REVERSAL LEARNING OF THE RABBIT NICTITATING MEMBRANE RESPONSE. W.B. Orr* and T.W. Berger (SPON: A. Caggiula). Psychobiology Program, Dept. of Psychology and Dept. of Psychiatry, University of Pittsburgh Pittsburgh, PA 15260.

The effect of hippocampal lesions on reversal of a two-tone discrimination task was measured using classical conditioning of the rabbit nictitating membrane response. Three different groups of New Zealand white rabbits were conditioned. One group received bilateral aspiration lesions of the hippocampus and the parietal neocortex overlying the dorsal hippocampus; a second group received bilateral aspiration lesions of just the parietal neocortex; a third group (operated controls) was anesthetized and received a midline incision. All surgical procedures were performed prior to conditioning, and animals in all groups (including operated controls) were chronically implanted with a headstage used to hold devices for transduction of nictitating membrane movement during conditioning. Animals were allowed 2-3 weeks for recovery. Training for all groups consisted of two-tone discrimination conditioning using either a 1K Hz or a 10K Hz tone (counterbalanced) as the CS+ or the CS-, respectively. Corneal airpuff served as the UCS. A 750 msec ISI was used, with a 20, 30 or 40 min. ITI (average: 30 min). A total of 96 CS+ and 96 CS- trials were given each day (pseudo-random sequence), the last half of which were used to determine criterion behavioral performance. After reaching a conditioned response (CR) rate of $\geq$ 85% to the CS+ and $\leq$ 15% to the CS-, the CS+ and CS- were reversed. Reversal learning was terminated when animals reached a CR rate of $\leq$ 90% to the new CS+ (the previous CS-) and < 50% to the new CS- (the previous CS+), or when 56 days (8 weeks) of training were completed.

Results showed that animals in all three groups -- hippocampal lesion, cortical lesion and operated control -- learned the initial discrimination at the same rate. On the average, animals required approximately 4 days to reach the initial discrimination criteria. In contrast, rabbits with hippocampal lesions required significantly longer than either cortically lesioned or operated control animals to reach criterion for reversal learning. While rabbits in the latter two groups typically completed reversal learning in 14 days, animals with hippocampal damage maintained consistently high rates of responding to the CS-, with some animals failing to meet the reversal criteria within 56 days.

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214.8 FOOD DEPRIVATION DIFFERENTIATES SEPTAL AND HIPPOCAMPAL LESION-INDUCED EXPLORATORY PATTERNS. S.D. Berry and D.A. Yutzey. Dept. of Psychology, Miami Univ., Oxford, OH 45056 and Dept of Psychology, Univ. of Connecticut, Storrs, CT 06268.

Among the many behavioral effects of septal or hippocampal lesions, alterations in reactivity to novel or biologically significant stimuli are observed frequently. However, in some conditioning experiments, it is difficult to separate the effects of lesions on learning from the interaction of lesions with motivational variables. This study, therefore, examined the impact of a strong motivational variable (food deprivation) on the patterning of exploratory behavior following lesions of either septum or hippocampus.

Twenty-six male, hooded rats, 90-122 days of age, were tested for amount and patterning of exploratory behavior in an octagonal runway under dim illumination. The lesion groups were: Septal (N=9), Hippocampal (N=5), Electrolytic Control (N=5), Cortical Control (N=3), and Normal (N=4). Two weeks after surgery, behavioral testing was initiated, with each subject allowed to explore the runway for two 3 minute sessions per week for a total of 12 weeks. After 2 weeks of testing under ad libitum feeding conditions, a 23 hr deprivation schedule was begun, with food intake restricted to produce a 10% drop in body weight each week. During this phase, tests were run at 90, 80, 70, and 60% of pre-deprivation weight. During a "refeeding" phase, the rats were put on a 22 hr deprivation schedule, and their weights were allowed to increase 10% per week back to the initial levels. Ad libitum feeding was allowed during the last 2 weeks. During each testing session, records were made of the latency to start locomotion, amount of locomotion, reversals of direction in the runway, and rearing.

Initially, both septals and hippocampals were hyperactive, although their latencies to start were longer. They also ran significantly farther than controls before reversing their direction of locomotion. Under deprivation, both groups, although still hyperactive, approximated the control group in the distance run before reversing. During the "refeeding" phase, the septal group immediately (although still at 60% body weight) resumed its lesion-induced exploratory pattern, while the hippocampal group remained similar to controls for the remainder of the experiment.

These data are consistent with studies showing differences between septals and hippocampals in the permanence of lesion-induced disruptions of learning, but extend them to include unlearned responses to motivational conditions.

214.9 THE EFFECTS OF HOME CONTEXTUAL CUES ON SPONTANEOUS ALTERNATION AND CONDITIONED PLACE AVERSION IN RATS WITH SEPTAL OR HIPPOCAMPAL LESIONS. T. Wigal*, C. R. Goodlett*, S. B. Eisenberg*, N. E. Spear, J. H. Hannigan, P. J. Donovick, R. G. Burright* and R. L. Isaacson. Department of Psychology, State University of New York at Binghamton, Binghamton, NY 13901.

Spontaneous alternation deficits typically found in rats with septal lesions have been alleviated by the presence of home contextual cues. This series of experiments examined the influence of home litter shavings on rats with septal or hippocampal damage on both a conditioned place aversion and a spontaneous alternation task.

Sixty-four Sprague-Dawley rats were given either bilateral electrolytic septal lesions or control surgery. Total aspiration lesions of the hippocampus, cortical lesions or sham control surgery were performed on 48 additional rats. All subjects were lesioned at 60 days of age and handled for one week postoperatively before undergoing place aversion conditioning. Training consisted of 12 inescapable shock presentations on a VI schedule in the black compartment and an equal exposure to the white compartment without shock. Rats of each lesion type were trained and tested with either clean shavings or 7-9 day old home shavings placed beneath the apparatus. At test, rats were placed in either the black (unsafe) or the white (safe) side, and the latency to cross was recorded. No shocks were delivered during testing.

Regardless of placement, septals, in the presence of home shavings, did not cross into the opposite compartment. This freezing behavior was not seen in septals tested over clean shavings, although both groups showed avoidance deficits relative to controls. The hippocampectomized rats did not exhibit consistent freezing behavior in either shavings condition.

Spontaneous alternation, tested 1-2 months postoperatively, indicated that septals, as seen previously, showed reliable alternation when tested over home shavings, but not when tested over clean shavings. Hippocampal subjects did not show response alternation in either shavings condition.

The results of these studies suggest that rats with septal lesions are more responsive to home contextual cues than hippocampal animals. The modification of septal deficits by the stimulus conditions of the task is consistent with the proposed role of the septum in sensory integration. Behavioral changes following hippocampal damage were less modifiable by these environmental manipulations.

214.10 POSTERODORSAL SEPTUM AND GO-NOGO DISCRIMINATION IN RATS. G.N.O. Brito and G.J. Thomas, Center for Brain Research, Univ. of Rochester Sch. of Med., Rochester, NY 14642.

The effects of lesions in the posterodorsal septum on the performance of a successive GO-NOGO discrimination task in a double runway were investigated in this study. Rats were trained preoperatively for 11 sessions [6 S+ (GO) and 6 S- (NOGO) trials per day according to a balanced sequence, 1 session/day] to perform GO-NOGO discrimination in a double runway, in which the two alleys, placed side-by-side, were made sensorially distinct (at least visually and tactually) from each other. Rats were reinforced (2 Noyes pellets of 97 mg each) for running in the S+ alley, and they were not reinforced for running in the S- alley. The rats were then divided into 2 matched groups. One group (N=12) received small bilateral stereotaxically guided electrolytic lesions in the posterodorsal septum (1 ma. for 10 sec) and the other group (N=9) served as operated control. After postoperative behavioral testing, lesions were evaluated from celloidin-embedded, cresyl-violet stained histological material.

The results showed that rats with posterodorsal septal lesions, when tested 2 wk postoperatively, ran significantly more slowly on the S+ trials and faster on the S- trials on the first postoperative session compared with the last preoperative session. Control rats were unaffected by the control surgery and the 2 wk-recovery period. Although the septal group did not differ from the control group in sums of median speeds on S+ trials across 7 postoperative sessions, septal rats ran significantly faster than control rats on the S- trials.

The results are consistent with the internal inhibition hypothesis of septo-hippocampal function, i.e., rats with septal lesions might be more distractible than normal rats and so they are more likely to disregard the availability of crucial cues for the successful performance of GO-NOGO discrimination. However, the results are not consistent with the working memory theory of septo-hippocampal function because septal lesions impaired performance of GO-NOGO discrimination, and this task involves reference, but not working, memory processes.

214.11 THE EFFECTS OF MAMMILLARY BODY LESIONS ON SPATIAL VERSES OBJECT REVERSAL LEARNING IN MONKEYS. E.J. Holmes, S. Jacobson, B.M. Stein* and N. Butters. Lab. for Neurosurg. Neurobehav. Res., Boston VA Med. Ctr., Boston, MA 02130.

Despite numerous experimental studies, the specific roles of the mammillary nuclei in learning and memory remain unclear. Recent investigations in monkeys have revealed that the fornix and hippocampus are important for spatial reversal learning (i.e., periodically reversing responses to one of two paired response sites), but not for object reversal learning (i.e., periodically reversing responses to one of two choice objects). We thought it would be of interest to extend these findings on reversal learning by examining the effects of bilateral mammillary body (MB) lesions (in monkeys) on the acquisition of spatial and object reversal tasks.

The mammillary nuclei were ablated using a sub-temporal, micro-surgical approach; that is, the left temporal lobe was gently elevated and the ipsilateral 3rd cranial nerve transected to reveal the base of the hypothalamus. The mammillary nuclei were located by utilizing the origin of the pituitary stalk and the median eminence as neuronal landmarks. The operated control subjects received unilateral 3rd cranial nerve transections alone.

The results of the spatial reversal task showed that although the experimental and control animals did not differ in the initial acquisition of a position habit, the monkeys with MB lesions were markedly impaired in reversal learning relative to the operated control subjects. In the object reversal task, however, the subjects with MB lesions and control subjects were found to be the same, not only in initial learning (i.e., learning to discriminate between the two objects), but also in learning to reverse their responses between the two objects. Moreover, a lack of impairment by the subjects with MB lesions in the object reversal task was obtained even though the control animals found this task to be more difficult. Thus, as with fornix and hippocampal ablations, the impairment in attaining the spatial reversal task by monkeys with MB lesions does not appear to simply reflect an inability or deficit to reverse acquired responses, but rather an impairment in adapting to the spatial requirements of the task.

214.12 Delayed Extinction of a Classically Conditioned Response in the Rabbit Induced by Locus Coeruleus Lesions: Involvement of the Neocortex and the Hipppocampus. David A. McCormick* and Richard F. Thompson. Dept. Psychology, Stanford University, Bldg. 420, Jordan Hall, Stanford, CA 94305.

Recent investigations have shown that extinction of instrumental conditioned behavior in rats can be prolonged through selective depletions of neocortical and hippocampal NE. In addition it has long been known that hippocampal lesions result in a marked extinction deficit. Previous studies in this laboratory have shown that hippocampal unit activity increases during conditioning before behavioral learning takes place. Likewise more recent investigations have shown that the hippocampal unit activity seen in the trained animal decreases during extinction with a faster initial time course than does the behavior. It is thus hypothesized that the extinction deficit seen in NE depleted animals could be an expression of a dysfunction of the hippocampus and related structures.

Multiple unit recordings were taken from the CA1 field of the hippocampus in normal and lesioned animals during behavioral training and extinction. The training paradigm consisted of a tone CS paired with a corneal air puff UCS to elicit NM extension. After a criterion of 8 conditioned responses out of 9 consecutive trials, the animals were given one additional day of training (117 trials) and were then extinguished with 4 days of unpaired training (104 tone alone, 104 air puff alone trials per day). After training was complete each brain was dissected into 12 parts: (left and right) anterior half of cortex, posterior half of cortex, hippocampus, caudate, hypothalamus, and cerebellum. The NE levels in each of the parts were analyzed using a LCEC system. The multiple unit activity from the hippocampus will be analyzed and its relation to extinction discussed.

Animals with bilateral electrolytic lesions of the locus coeruleus (LC) were found to show a marked extinction deficit. This deficit was found to be significantly correlated with each of the total hippocampal, anterior cortical and posterior cortical NE levels. However, this deficit was not significantly correlated with caudate, hypothalamic or cerebellar depletions. These results suggest that the hippocampus and neocortex are involved in the extinction deficit following lesions of the LC NE system in classical conditioning.

214.13 CONDITIONED FLAVOR AVERSION: DISRUPTION OF ODOR-TASTE POTENTIATION BY REVERSIBLE AMYGDALA LESION. F. Bermudez-Rattoni*, K.W. Rusiniak*, and J. Garcia*. (SPON: J.P.Lieb). Depts. Psychol. and Psychiat., Univ. Calif., Los Angeles, CA 90024.

When odor and taste are presented in compound and followed by delayed LiCl, taste potentiates odor; odor becomes as strong as taste. Taste may "index" or "gate" odor into feeding (emetic) mechanisms (Palmerino, C.C., Rusiniak, K.W., & Garcia, J., Science, 208: 753, 1980). The amygdala receives both odor and taste afferents. Therefore, we infused procaine (i.c.) to disrupt amygdala function during odor + taste - LiCl pairing and then tested odor and taste components. Rats implanted with bilateral 25 ga. cannulae in the amygdala received 3µL of 10% procaine infused over 3 min., 15 min. before the odor-taste CS (n=10), 0-min. after the CS (n=10) or 1-min prior to US (n=10). Controls (n=9) received normal acquisition and procaine prior to water on a control day. Almond odor and .1% saccharin were the CSs, 190 mg/kg LiCl i.g. was the US. Water consumption averaged 1000 licks; procaine treatment did not suppress water consumption. There was a time dependent effect on odor aversions [$F(3,35) = 7.5$, $p<.01$] and taste aversions [$F(3,35) = 5.01$, $p<.01$]. Pre-CS amygdala lesion disrupted odor ($p<.05$) but not taste, indicating a role for the amygdala in odor and/or odor-taste integration. Post CS or pre-US procaine facilitated both odor and taste ($ps<.05$), indicating that procaine did not disrupt the illness US; facilitation may be due to non-specific aversive stress. These preliminary data suggest the amygdala is involved with the integration of odor and taste during illness-induced aversions, disrupting either odor perception, odor-taste pairing, or odor-illness associations. (Research supported by NIH NS11618, AA03513, HD05958, and Conacyt 24142).

	CONTROL	PRE CS	POST CS	PRE US
ODOR TESTS:	220 ± 100	550 ± 120↑	30 ± 20↓	20 ± 20↓
TASTE TESTS:	325 ± 50	370 ± 70	120 ± 75↓	175 ± 70↓

Licks ($\bar{X}$ ± SE) on test trials. ↓↑ = $p<.05$ change relative to control.

214.14 SELF-STIMULATION OF THE CAUDATE-PUTAMEN IN THE RAT. A MAPPING STUDY. R.A. Prado-Alcalá and R.A. Wise. Physiol. Dept., Sch. Med., Natl. Univ. México, México 20, D.F. and Psychol. Dept., Concordia Univ., Montreal, P.Q., Canada H3G 1M8.

Brain stimulation reward is closely associated with dopaminergic elements in the ventral tegmental area. High pressing rates and low thresholds for intracranial self-stimulation (ICSS) are seen when electrodes are placed in the layer of dopamine (DA) cells in the nigral and ventral tegmental regions (1). ICSS is also readily obtained in most DA terminal fields (e.g., septal area and nucleus accumbens). However, there has been some controversy regarding the rewarding properties of electrical stimulation of the caudate-putamen nucleus (CPU), which has the greatest DA inervation in the forebrain. Several authors have reported that the CPU does not support ICSS (2), while others have described ICSS from the medial aspect of this structure (3) or from some other regions of the CPU (4).

In the present experiment we conducted a systematic mapping involving the anterior-posterior, dorsal-ventral, and medial-lateral regions of the CPU, using a moveable electrode to test 4-12 sites in a vertical penetration (250 um steps) in each animal. Rate of responding was assessed at currents ranging from 60 uA down in 2 uA steps to the lowest current that would sustain responding (threshold). In addition, we determined the relative density of DA (as revealed by glyoxylic acid-induced fluorescence histochemistry) surrounding the stimulation sites.

ICSS was observed in more than 80% of the sites tested, although there was not a clear correlation between DA density and pressing rates or thresholds. The highest pressing rates (40-60/min) were seen with the most medial placements, and progressively lower rates were seen as the electrode tips stimulated the tissue of the middle and lateral aspects of the CPU. Peak ICSS rates were found to be inversely related to thresholds.

These results indicate that all regions of the CPU are capable of supporting ICSS, and that the most rewarding stimulation is obtained from the medial aspect of this structure.

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214.15 HYPOTHALAMIC SELF-STIMULATION IN RATS WITH ONE HEMISPHERE ISOLATED ANTERIOR TO THE MIDBRAIN AND THE OTHER HEMISPHERE DEVOID OF THE TELENCEPHALON. J. P. Huston, M. Pritzel* and W. Buscher*. Institute of Psychology III, Univ. of Düsseldorf, Düsseldorf, FRG.

In rats the telencephalon of one hemisphere of the brain was removed. Furthermore, the other hemisphere was isolated from the parts posterior to the diencephalon by a precollicular transversal cut. In this preparation self-stimulation was tested via electrodes implanted in the lateral hypothalamus - medial forebrain bundle (LH-MFB) area of both hemispheres before and after the lesions. Self-stimulation by lever pressing in a Skinner-box was still exhibited with stimulation delivered to either hemisphere. This was the case following either a unilateral detelencephalization, a unilateral precollicular transversal cut, or a combination of both operations. Lever-pressing underwent extinction when reinforcement was withheld.

If, as is likely, the complex response of lever-pressing is dependent on telencephalic structures, it must have been generated by the hemisphere with the intact forebrain. This forebrain, however, being isolated from its brain stem, can function only via the remaining diencephalic interhemispheric connections to the contralateral detelencephalized hemisphere.

To investigate possible interhemispheric pathways in this preparation, the tracer horseradish peroxidase was injected into the LH-MFB region of one hemisphere and its trace of migration to the contralateral hemisphere was examined. The results revealed that the thalamic commissure most likely transmitts information from the LH-MFB to the contralateral part of the brain. In addition, the supraoptic decussation as well as diffusely organized interdiencephalic connections provide possible pathways for interhemispheric intercourse.

214.16 BRAIN STEM ELEMENTS ESSENTIAL FOR THE CLASSICALLY CONDITIONED NICTITATING MEMBRANE RESPONSE OF RABBIT. J. E. Desmond*, N. E. Berthier, and J. W. Moore. Dept. of Psychology, Univ. Mass., Amherst, Mass. 01003

Unilateral radio-frequency lesions of the dorsolateral pons produced a loss of conditioned extension of the nictitating membrane response in the ipsilateral eye. Conditioned stimuli (CSs) included auditory, visual, and tactile modalities, and the unconditioned stimulus (US) was electrostimulation of the facial region near the eye. Lesioned animals could be classified into those with profoundly disrupted conditioned responding (n = 9) and those with but slight disruption of conditioned responding (n = 12). Disrupted animals gave conditioned responses on less than 5% of trials; nondisrupted animals and sham-lesioned controls (n = 6) gave conditioned responses on over 90% of trials, on the average. The two grouping did not overlap. Unconditioned responding in both eyes was unaffected by lesions.

Two types of experiments were employed. Animals received lesions either before training (acquisition) or after training (retention). Lesions in the critical zone produced comparable results, i.e., little or no acquisition of ipsilateral conditioned responses in the first case and virtually complete loss of conditioning in the second case. Conditioning of the contralateral response was unimpaired in both instances.

Histological analysis from disrupted and nondisrupted cases indicated that the parabrachial region of the dorsolateral pons contains elements essential for conditioned responding in this preparation. This region of the brain stem is approximately 2-4 mm rostral of the accessory abducens nucleus, the nucleus containing motoneurons principally responsible for defensive eye ball retraction (e.g., Berthier, N. E., and Moore, J. W. Neurosci., 6: 427, 1980). Extension of the nictitating membrane in rabbit is due to eye ball retraction, and the relevant muscles are the retractor bulbi muscles innervated by the 6th cranial nerve (e.g., Berthier, N. E., and Moore, J. W. Physiol. Behav., 24: 931-937, 1980). The anatomical interconnections between the critical region of the dorsolateral pons and the relevant motoneurons are under investigation.

214.17 The effects of local cooling in the temporal lobe of monkeys on delayed-match-to-sample. James A. Horel and Dorothy E. Pytko*. Dept. of Anatomy, Upstate Med. Ctr., Syracuse, NY 13210.

Five monkeys were trained on a delayed-match-to-sample task. The animals faced 3 rear-projection screens. Four hundred colored slides of objects were used as stimuli. A sample stimulus was projected to the center screen and the animal was rewarded with a squirt of juice to its mouth for pressing this screen 10 times. The sample stimulus was then extinguished and a delay of 0, 15, 30 or 45 sec (randomly selected) was begun. At the end of the delay a stimulus that matched the sample and one that did not appeared at the two side panels and if the animal pressed the panel that matched the sample it was rewarded.

After the animals had learned the task to better than 90% correct at all delays, they were operated. One temporal lobe was removed and guide tubes were placed over the intact temporal lobe. A steel nut was also affixed to the skull to attach a cryoprobe holder. A Benita cryogenic probe was used to make reversible functional lesions. Freon was injected into the expansion chamber of the tip. A vacuum jacket provided insulation for the shaft. A thermocouple soldered to the metal tip permitted continuous monitoring of tip temperature. The tip was 4mm long and 1mm in diameter. An insulated wire used for marking tip position was also attached to the probe.

During testing the cryogenic probe was lowered to position through one of the guide tubes and the animal was run on the task for 20 trials. The temperature of the metal tip was then lowered to -6°C and the animal was run for another 20 trials. Alternating blocks of 20 control and experimental trials continued until the animal was run 160 trials. The probe position was then marked for the Prussian-Blue reaction with the marking wire and the probe was removed.

At most placements, performance during cooling was identical to that during control trials. At some placements, there was a clear drop in performance at all delays during cooling, at others only the long delays were affected and two placements not in the temporal lobe produced a drop in performance at only the short delays. Most of the loci where the cold disrupted performance were in the anterior 9mm of IT. Only two placements out of 13 produced an effect in IT posterior to this area, disrupting performance at all delays. But all 12 placements in the anterior tip of IT produced a clear deficit during cooling. Five of these anterior placements produced an effect at all delays and 7 produced deficits concentrated at the long delays. The behavioral consequences of the cooling were completely reversible. Where the cold produced an effect, performance returned to normal when the cooling was stopped. Supported by NSF Grant BNS 80-40301

214.18 LATERALIZATION OF STORAGE OF VISUAL INFORMATION IN CATS WITH UNILATERAL SUPRASYLVIAN LESIONS.* Franco Lepore, Maurice Ptito, Serge Couture, Dom Miceli and Maryse Lassonde. Laboratoire de Psychologie, Univ. de Montréal, C.P. 6128, Montréal, P.Q. and Laboratoire de Neuropsychologie Expérimentale, Univ. du Québec, C.P. 500, Trois-Rivières, P.Q., Canada.

It has been postulated that the lateral suprasylvian area is important in learning and transfer of visual pattern discriminations. It is not clear, however, whether the storage of the engram occurs primarely within the lateral suprasylvian area and is then transmitted to the homologous contralateral region via the corpus callosum. The aim of the present study was to investigate the involvement of lateral suprasylvian area in both storage and transfer of visual information. Six adult cats were used in the experiment. Under deep general anasthesia, they underwent sectioning of the optic chiasma and unilateral destruction of the lateral suprasylvian area (LSSA). After an adequat recuperation period, they were trained monocularly on a variety of visual pattern discrimination tasks in a two-choice "thompson" box. Training on different pattern discriminations was performed with the eye ipsilateral to the LSSA lesion and the contralateral eye (intact side). In this part of the experiment no transfer capacity was assessed. Once performance criteria were achieved (90% correct, 3 sessions in a row) for all tasks and with each hemisphere (intact and lesioned), the cats were subjected to a split of the corpus callosum. They were then tested on the same tasks with the contralateral eye for transfer of visual information to the untrained hemisphere. In the analysis of data, 3 criteria were considered: the number of errors during the first 40 trials (IP), the number of errors to final criterion (EC) and the index of transfer derived from Murdock's formula. The results showed that the learning capacity of the lesioned hemisphere (LSSA lesion) is identical to that of the intact hemisphere suggesting that the lateral suprasylvian area is not as essential to learning as generally reported. Moreover, when we looked for the mnesic trace in the naive (untrained) hemisphere (contralateral to the previously trained eye for a given pattern), the visual pattern discrimination acquired with the intact hemisphere first was poorly represented in the lesioned hemisphere. Contrastly, the acquisition of the task with the lesioned hemisphere first was transferred normally to the intact side. These results suggest that the lateral suprasylvian area is involved in a storage mechanism for visual input transmitted via the corpus callosum.

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214.19 FOREBRAIN SEROTONIN DEPLETION AND THE ATTENUATION OF THE LATENT INHIBITION EFFECT. David Berry, Joan F. Lorden, and Edward J. Rickert.* Dept. of Psychology, University of Alabama in Birmingham, Birmingham, Alabama 35294.

Forebrain serotonin (5HT) and norepinephrine (NE) have been implicated in processes underlying an animal's ability to ignore stimulus information which has no signal value. For example, in the latent inhibition (LI) paradigm, animals are pre-exposed to a stimulus (A) in the absence of reinforcement. In normal animals, pre-exposure retards learning if A is later used to signal reinforcement. Animals with raphe (Solomon et al., 1979) or dorsal noradrenergic bundle lesions (Mason & Lin, 1980) show an attenuation of the LI effect. These experiments suggest that similar deficits result from NE and 5HT depletion. However, using the blocking paradigm, Lorden et al., (1980) were able to distinguish between the effects of NE and 5HT depletion. In this procedure animals receive pretraining trials in which stimulus A predicts reinforcement followed by trials in which a compound stimulus (A + a new stimulus, B) predicts reinforcement. When tested to B alone, normal rats and rats with raphe lesions showed no evidence of learning to B; however, NE-depleted rats were unable to ignore this redundant or irrelevant cue.

In order to clarify the role of forebrain 5HT and NE in the LI effect, rats with 6-hydroxydopamine lesions of the dorsal NE bundle or 5, 7-dihydroxytryptamine lesions of the dorsal and median raphe nuclei were compared to vehicle-treated controls. Rats trained to make an operant response were pre-exposed to a stimulus which was then paired with footshock. Vehicle-treated and NE-depleted rats displayed the LI effect and failed to suppress operant responding in the presence of the cue paired with shock. 5HT-depleted animals, however, showed an attenuation of the LI effect. In a control condition in which there was no prior experience with the stimulus, all groups responded similarly. Selective depletion of NE or 5HT was verified by fluorometric assays. These results support the view that 5HT depletion, like NE depletion, impairs an animal's ability to exclude irrelevant stimulus information, but suggest that different mechanisms account for the deficits in NE and 5HT-depleted animals.

A second experiment examined the effects of NE and 5HT depletion in a paradigm which combined features of LI and blocking. Rats were pre-exposed to a stimulus (A) which was later presented in compound with a novel stimulus (B). The A/B compound predicted footshock and rats were tested in extinction for their response to B. Normal and NE-depleted rats behaved similarly; however, the response suppression of the 5HT-depleted group was attenuated in the presence of B. Serotonin but not NE-depletion appears to result in an inhibitory deficit. (Supported by NSF Grant BNS 80-14675).

214.20 ELECTROLYTIC LESION OF ENTORHINAL CORTEX CAUSES SEIZURES. R. M. Dasheiff and J. O. McNamara. Departments of Medicine (Neurology) and Pharmacology, Duke University; and Epilepsy Center, VA Medical Center; Durham, NC 27705

Destructive lesions of the entorhinal cortex (EC) are routinely employed to define anatomical pathways and study such diverse processes as neuronal plasticity and learning. We have consistently observed, and electrographically recorded, seizures in rats with electrolytically induced destruction of EC. Adult male Sprague-Dawley rats had EC lesions as described by other investigators, Brain Res. Bull. 2:31, 1977, and Brain Res., 131:241, 1977; controls were sham operated. Recording electrodes were placed bilaterally into the septal dentate gyrus of the hippocampus in all rats. The EEGs were interpreted by JOM who was blind to the protocol. Evidence of epileptiform activity (EA) was found in 19 of 20 EC lesioned rats as defined by either clinically observed seizures, electrographic seizures or spontaneous interictal spikes (SIS). No control rats had seizures and only 1 of 25 displayed SIS. The clinical seizures resembled the limbic seizures induced by kindling or kainic acid. Some of these behaviors are difficult to differentiate from normal rat behavior, or subject to misinterpretation as "fits of imbalance". However, electrographic recordings confirmed the true nature of these behaviors as seizures. The seizures could be suppressed by phenobarbital (PhB). The EA was intermittent but extremely frequent. It occurred as early as 8 hrs after lesion, peaking at 48-72 hrs, and had remitted by day nine. Right, left or bilateral EC lesions were equally effective in inducing EA. To test anatomic specificity further, other lesions were made in the septum, hippocampus, or frontal cortex without EA. Amygdala lesions induced SIS only. Biochemical changes of muscarinic cholinergic receptors due to these seizures is discussed by us in another abstract in this volume. Neuronal rearrangment (septal sprouting induced by EC lesion) occurred in the presence and absence (blocked by PhB) of seizures. The mechanism underlying these seizures following the lesion is unclear, but could result from a convulsant agent produced by the lesioning technique for which the EC is extremely susceptible. Alternately, the acute and massive deafferentation of EC input to other limbic structures could result in disinhibition and epileptogenesis. These seizures represent a potentially confounding variable for the interpretation of experiments involving electrolytic lesions of EC.

214.21 LESIONS OF THE STRIA TERMINALIS ATTENUATE THE EFFECT OF AMYGDALOID STIMULATION ON *RETENTION OF AVOIDANCE RESPONSES.*
K.C. Liang* and James L. McGaugh, Department of Psychobiology, University of California, Irvine, 92717, U.S.A.

Considerable evidence indicates that posttrial electrical stimulation of the amygdala affects retention of learned responses. These experiments were designed to determine whether the effect of amygdaloid stimulation on memory is blocked by transecting the efferent pathways of the amygdala: the stria terminalis (ST) and/or the ventral amygdalofugal pathway (VAF).

Bipolar electrodes were implanted bilaterally into the basolateral/basomedial nuclei of the amygdala of male ARS Sprague-Dawley rats (60 days old). Half of these rats also received bilateral radiofrequency lesions of the ST (ST-) or knife-cut transections of the VAF (VAF-), while others received sham operations on either pathway (ST+ or VAF+). Two weeks after surgery, animals were trained and, 24 hours later, tested in a one-trial inhibitory avoidance task (1 mA, 2 sec footshock). Two weeks later, they were trained and tested in an 8-trial one-way active avoidance task (640 μA footshock). Immediately after the training, half of the rats received amygdaloid stimulation (AS) (100 Hz, 0.2 ms pulses, 50 μA/electrode, 30 sec), while others served as implanted controls (IC). The 24 hour retention measures are shown in the table. In the ST+ rats, amygdaloid stimulation impaired retention in both the inhibitory and active avoidance tasks. Lesions of the ST did not significantly affect retention of the IC rats. However, the ST lesions blocked the impairing effect of amygdaloid stimulation in both tasks. In the VAF+ rats, amygdaloid stimulation impaired retention in the inhibitory avoidance task. After transecting the VAF, both the IC and AS rats had poor retention. In the active avoidance task, the retention of the IC/VAF+ rats was rather poor and was enhanced by amygdaloid stimulation. Transecting the VAF altered neither the retention of the IC rats nor the enhancing effect of amygdaloid stimulation. The present findings suggest that the memory modulatory effect of amygdaloid stimulation in rats is mediated through the output influences of the ST.

	ST+	ST-	VAF+	VAF-
Median Retention Latencies (sec) in the Inhibitory Avoidance Task				
IC	366.8	298.9	246.4	21.2[a]
AS	27.7[a]	276.3	45.1[c]	45.1[a]
Mean Difference Avoidance Scores in the Active Avoidance Task				
IC	2.92	2.67	1.13	1.38
AS	1.08[b]	2.79	2.56[c]	2.57[c]

a. $p<.01$; b. $p<.02$; c. $p<.05$ different from IC/ST+ or IC/VAF+

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215.1 PHYSIOLOGICAL AND BEHAVIORAL COUNTERREGULATORY RESPONSES TO INSULIN-INDUCED HYPOGLYCEMIA. P. Christopher Coburn* and Edward M. Stricker. (Sponsor: I. Hanin) Department of Psychology, University of Pittsburgh, Pittsburgh, PA 15260.

In intact rats deprived of food for 90 minutes before testing and during the test itself, iv injection of 2U/kg of regular insulin was found to induce a rapid reduction in blood glucose to 50-60 mg/100 ml followed by a gradual recovery to basal levels of 100-120 mg/100 ml within the 4-hr period of observation. After iv injection of 4U/kg, blood glucose remained depressed longer and some rats succumbed to hypoglycemic shock. Higher doses invariably led to hypoglycemic shock. In contrast, when food was made available during the test intact rats were able to tolerate iv doses of 10U/kg or higher. These observations draw attention to the ability of the rat to consume food, and its gastrointestinal tract to deliver the ingested calories, so as to adequately compensate for acute glucoregulatory challenges.

Food may be necessary to deal with marked insulin-induced hypoglycemia but it is not sufficient. Adrenal enucleated rats that had been deprived of food for 90 minutes before testing often do not tolerate an iv injection of 2U/kg insulin, even when food is available during the test. However, these animals were much more tolerant of insulin when it was delivered subcutaneously, so that its impact on blood glucose was not so rapid. Under such circumstances, adrenal enucleated rats were able to tolerate doses of insulin as high as 20U/kg when food was available. Also, they had no difficulty tolerating an iv dose of 2U/kg insulin in the absence of food when rats were tested 3 weeks rather than 3 days after enucleation, by which time the sympathetic response to insulin-induced hypoglycemia had improved so much that near normal glucose regulation occurred despite the absence of adrenal medullary secretions.

These observations emphasize the importance of integrated physiological and behavioral responses to insulin-induced hypoglycemia, and provide another example of the joint roles of physiology and behavior in the maintenance of homeostasis. Supported by NIMH grant MH-25140.

215.2 OBESITY AS A DELAYED CONSEQUENCE OF VIRAL INFECTION IN MICE D. R. Buskirk, M. J. Lyons and J. B. Zabriskie, Laboratory of Microbiology and Immunology, The Rockefeller University, New York, NY 10021

Previous work in our laboratory has shown that 20-30% of a strain of NCS mice infected at 4-5 weeks of age with canine distemper virus (CDV) develop obesity 3 to 4 months after recovering from the acute infection (M. J. Lyons et al., manuscript in preparation). The virus used in these studies had been maintained by serial passage in vivo in suckling mouse brain, and is highly virulent for mice. We report now that canine distemper virus maintained exclusively in vitro in Vero green monkey kidney cells is capable of eliciting obesity in mice with a latency of 3 to 4 months after injection. In addition, obesity can occur as a delayed symptom when the mice are injected with fewer than 100 plaque forming units of this virus, a dose incapable of causing noticeable symptoms of acute infection in juvenile mice. Obesity has been observed to follow intraperitoneal as well as intracranial injection of CDV, but preliminary results suggest that intracranial injection is more effective.

Histopathological sections of mouse tissue revealed adipose tissue directly comparable to that observed in genetically obese mice, or in mice affected by the gold thioglucose technique. Brain sections of these animals revealed no signs of inflammation, and immunofluorescence has not revealed the presence of viral antigens in the brains studied. The hypothalamic nuclei of the obese mice show no remarkable pathological changes. Studies of the endocrine function of these animals and more detailed pathological observations are in progress.

Supported by a grant from the Multiple Sclerosis Society.

215.3 GLYCEROL EFFECTS ON HUMAN FEEDING, J. Grinker, The Rockefeller University, N.Y., N.Y., and F. Bellisle*-lab de Prof. J. leMagnen, Neurophysiologie Sensorielle et Comportementale, College de France, Paris, France.

Glycerol has been proposed as a humoral signal reflecting the state of fat storage. Plasma glycerol levels and turnover rate are proportional to adipocyte size, total mass of adipose tissue and duration of short-term fasting. In animal studies, subcutaneous doses of glycerol produced decreased body weight (Wirtshafter and Davis, Science, 1977). In studies in our laboratory, both subcutaneous (160 mg kg/day via Alzet minipump) and oral administration (1g/kg/day) of glycerol but not equicaloric glucose produced temporary decrements in food intake and body weight gain of lean and obese rats. (Grinker et al., Brain Res. Bull., 1980). Within 3 to 4 days of beginning treatment, recovery in food intake and body weight was observed. Obese human inpatients maintained on 600 kcal liquid formula/day showed no differential response to glycerol or glucose as the primary carbohydrate in the diet. Outpatients on 1000 kcal/day with glycerol supplements failed to show greater diet adherence or reduced hunger compared with equicalorie glucose supplements (Leibel et al, Metabolism, 1980). These human studies investigated the effects of glycerol on perceived hunger and weight loss when caloric intake was already restricted in contrast to animal experiments using freely feeding rats.

We now report that oral administration (15 gram in 30 ml) of glycerol or equicaloric glucose failed to reduce subsequent food intake or alter the microstructure of feeding in normal weight volunteers. Four subjects received 6 trials of a citrus flavored preload (grapefruit juice adulterated with saccharin, glucose or glycerol)(Latin Sq. Design) one hour prior to test sandwich meals. The dose of glycerol is sufficient to produce marked elevations in plasma glycerol (Broyer et al., Le Nouvelle Presse Medicale, 1978). Total food intake was unaffected by glycerol or glucose administration. The microstructure of feeding (rate/food unit, number of swallows, number of chews) while changing over the course of the feeding bout was also unchanged by glycerol or glucose administration. These results make it highly unlikely that glycerol, or glycerol in relation to FFA levels (Grinker et al., 1980) is a specific satiety signal.

215.4 CARBOHYDRATE REGULATION IN RATS. C.L. Theall*, J.J. Wurtman, and R.J. Wurtman. Lab. of Neuroendocrine Regulation, Massachusetts Institute of Technology, Cambridge, MA 02139.

Previous research in this laboratory has suggested that rats select between two percentages of carbohydrate in the diet so as to maintain a constant percentage of carbohydrate in terms of total food consumption. It is thought that carbohydrate intake may trigger metabolic mechanisms that feed back on the rat's consummatory behavior, perhaps by affecting amino acid and neurotransmitter metabolism. The present experiments were designed to investigate this regulation in more depth.

Isocaloric agar-based diets were utilized in the present experiments. Six diet pairs ranged from 7.5 vs 17.5% to 27.5 vs 37.5% carbohydrate (wet weight percentages). One group of rats chose exclusively from dextrin-based diets, another group from sucrose-based diets. Each rat ate from one of the six diet pairs for one 10 day period followed by a different diet pair for each of two successive 10 day periods. The measurement for each diet pair was the percent of carbohydrate consumed versus the total grams of food consumed. Twenty Sprague-Dawley male rats were used in each experiment.

In Experiment I, 25-day-old rats weighed 69-85 g. Diet pair means for dextrin-fed and sucrose-fed animals were plotted as observed percent carbohydrate versus the expected percent carbohydrate if rats were randomly choosing carbohydrate. Linear regression analysis demonstrated that the dextrin-fed rats randomly selected whereas the sucrose-fed rats regulated carbohydrate intake. There was no difference between the regression lines for sucrose and dextrin. Due to a possible ceiling effect of the 27.5 vs 37.5% diet pair, these data were omitted in a second analysis. Both dextrin and sucrose lines differed from random selection. The mean percent carbohydrate consumed was found to be 20.3 and 22.5% for dextrin- and sucrose-fed rats, respectively.

Experiment II tested older animals weighing 225-260 g. Food was presented as in Experiment I. The results showed that the dextrin line differed both in slope and intercept. The dextrin and sucrose lines were not different from each other. The mean percent carbohydrate consumed was found to be 24.2 and 23.9% for dextrin- and sucrose-fed animals, respectively.

In conclusion, carbohydrate regulation is apparent in both dextrin-fed and sucrose-fed rats. The regulation is independent of the sweetness of the carbohydrate when either dextrin or sucrose are offered as the sole carbohydrate source. The overall mean percent carbohydrate consumed was found to be 22.7% of total food intake.

215.5 DIURNAL FEEDING AND THE INGESTIVE RESPONSES FOLLOWING GLUCOSTATIC AND OSMOTIC CHALLENGES ARE UNALTERED BY HEPATIC VAGOTOMY. Michael G. Tordoff*, Jill Hopfenbeck* and Donald Novin. Dept. Psychology and Brain Research Institute, UCLA, Los Angeles, CA 90024.

The hepatic branch of the vagus nerve was transected in 10 male and 10 female Long Evans rats. Sham surgery was given to 9 males and 9 females. All subjects were maintained on lab chow in a vivarium with 12:12 hr illumination (lights on at 0500). The following ingestive behaviors were observed:

Postoperative Body Weight. Daily body weight readings following surgery showed that hepatic vagotomized (HV) rats gained weight at the same rate as controls (SHM).

Diurnal Ingestion. Commencing 10 days postsurgery, food and water intakes during the light and dark periods were recorded for 3 days. Although males ingested more than females, there were no differences between same-sex HV and SHM rats in daytime or nighttime ingestion,or in day:night ratios.

Insulin. Commencing 20 days postsurgery, all rats were given injections of insulin (4, 8, 12 U/kg, IP). Each rat received, in counterbalanced order, each dose of insulin twice, with 3 days between injections. Food and water intakes were recorded every hour for 6 hr and at 24 hr postinjection. Insulin significantly increased ingestion in all groups, and although males ate and drank more than females, there were no differences between same-sex HV and SHM rats.

2-Deoxy-D-Glucose. Commencing 54 days postsurgery and using the same technique as for insulin injections, all rats were administered 2DG (125, 250, 500 mg/kg, IP). Each dose was given only once. 2DG caused significant increases in ingestion that were generally dose-related. No differences between same-sex HV and SHM rats were found.

Sodium Chloride. Commencing 85 days postsurgery, rats were given NaCl (10 ml/kg: 0.5, 1.0, 2.0 M), with 3 days between injections. Water consumption was recorded for the first 4 hr and 24 hr postinjection. Dose-related increases in drinking were found but no differences between same-sex HV and SHM rats were present.

Polyethylene Glycol (PG). On Days 102 and 106 postsurgery, rats received either PG (10 ml/kg: 30% w/v, SC) or saline. PG produced a long-lasting increase in drinking, but at no time was there a difference between same-sex HV and SHM rats.

Although several explanations for these results exist, they support the hypothesis that the hepatic vagus contains only a small fraction of the total complement of hepatic metabolic and osmotic afferents. (Supported by NS07687).

215.6 PREFEEDING SERUM GLUCOSE AND DIURNAL SERUM CORTICOSTERONE LEVELS VARY WITH BODY WEIGHT IN CHRONICALLY FOOD RESTRICTED RATS. John P. Heybach* & Peter C. Boyle* Central Research Department, General Foods Corp., White Plains, NY 10625 SPON: DOROTHY CHOU.

Thirty male S/D rats (310-330g), housed individually and maintained at 21°C with a 12h photoperiod (lights on 6am), were given ad lib access to tap water and powdered chow (Wayne) in food cups. Following a 14d acclimatization period rats were placed on a feeding regimen with food cups presented daily at 4pm and removed the next morning at 9am. Body weight (BW) and food consumption (corrected for spillage) were recorded daily at 9-10am. On days 7-35 of this regimen 2 groups (n=10/grp) were presented with 80% and 60%, respectively, of the weight (calculated daily) of control group consumption (n=10). On day 36 rats from each group (n=5/grp) were rapidly sacrificed by decapitation at either 9am or 4pm (just prior to daily food presentation). Trunk blood was collected and serum aliquots were used to determine circulating glucose (G) and corticosterone (B) levels. The mean BW across the three groups diverged over the 28d restriction regimen while within group food consumption remained relatively constant. At both 9am and 4pm circulating B levels were increased significantly across groups as body weight decreased. Circulating G levels did not differ across groups at 9am. However, at 4pm (just prior to daily food presentation) fasting serum G levels were decreased across the restricted groups as a direct function of the decrease in body weight. These results are consistent with a glucostatic theory of appetite and body weight regulation and demonstrate that prefeeding, fasting serum G levels covary with body weight during chronic food restriction. The data also suggest a degree of specificity of the lowered serum G levels with respect to the time immediately preceeding daily presentation of food that is not apparent in circulating glucocorticoid levels, which varied inversely with body weight across groups at both times of day.

		Control	80% Food Restriction	60% Food Restriction
BW $\bar{x}$ g		405 ± 4.6	345 ± 6.1	302 ± 6.3
Serum G	9am	135.5 ± 1.4	136.0 ± 2.1	138.0 ± 1.9
$\bar{x}$ mg/dl	4pm	142.6 ± 3.3	135.3 ± 1.8	127.4 ± 3.5
Serum B	9am	9.06 ± 0.8	22.0 ± 2.6	26.1 ± 3.6
$\bar{x}$ ug%	4pm	24.01 ± 2.5	34.9 ± 3.1	38.6 ± 2.3

215.7 MULTIPLE REGRESSION ANALYSIS OF SHORT-TERM AND LONG-TERM FOOD INTAKE IN INTACT AND SUBDIAPHRAGMATICALLY VAGOTOMIZED RABBITS. Paula J. Geiselman, James R. Martin, and Donald Novin. Dept. Psychol. and Brain Res. Instit., UCLA, Los Angeles, CA 90024; Dept. Behav. Sci., Swiss Fed. Instit., Zurich, Switzerland.

Individual differences in meal-patterning parameters and total food intake were modeled in intact and vagotomized rabbits with multiple regression analyses. During short-term periods (each of the four 6-hr periods during a 12:12 light/dark cycle) as well as long-term periods (24-hr measurements), satiety ratio (intermeal interval following meal/meal size) provided the best single-variable predictor of food intake, but the combination of meal frequency and meal size provided a significantly better prediction than satiety ratio alone in intact rabbits. These results suggest that satiety ratio, meal frequency, and meal size are functions of both short-term and long-term signals in intact rabbits.

In subdiaphragmatically vagotomized rabbits, satiety ratio was no longer the best single predictor of food intake during either short-term or long-term measurement periods, indicating that satiety ratio is not controlled by short-term signals in vagotomized rabbits. Following vagotomy, meal frequency provided the best single-variable predictor of short-term food intake, but the combination of meal frequency and meal size provided a significantly better prediction than meal frequency alone. The long-term analysis suggests that control of food intake during this time interval is more complex in vagotomized rabbits: A combination of several measures provided nonsignificant prediction of total food intake accounting for only 31 percent of the variance.

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215.8 PREGASTRIC STIMULI ARE SUFFICIENT FOR THE HEDONIC VALUE OF A MEAL IN THE RAT. W. Van Vort* and G.P. Smith.(SPON: J.A. Sechzer). Dept. Psychiatry, Cornell Univ. Med. College and E.W. Bourne Behavioral Research Lab., New York Hosp., White Plains, NY 10605.

We previously reported that rats prefer a meal that is really fed to 60 min of sham feeding (Neuroscience Abst. 6:179.11, 1980). To clarify the contribution of postingestional elements of real feeding for the development of this preference, we investigated the preference for sham or real feeding when duration of feeding and volume of intake were similar or equal. Male Sprague-Dawley rats weighing about 350g were implanted with chronic gastric cannulas (GF). After recovering from surgery, rats were adapted to a 17h food deprivation schedule. At the end of a deprivation period rats were offered sweet milk. On alternate days the rats really ate the milk (GF closed) or sham fed the milk (GF open). For each GF condition, the diet was marked with a specific flavor (almond or vanilla) and offered at a specific location at the front of the cage (left or right) for each rat. Preference testing, conducted after a series of training tests, consisted of giving rats (GF closed) simultaneous access to both test diets in their usual locations for a 4 min period. Two groups of rats were tested. In the first group (n=9), the duration of sham or real feeding during training tests was limited to 20 min. On real feeding days rats ate 16.8±0.5 ml. On sham feeding days rats ate 38.9±0.7 ml and did not display satiety. After 15 real and 15 sham feeding trials, rats did not develop a preference. To evaluate the hedonic effect of volume ingested, the volume sham fed by the second group (n=12) was limited to the volume ingested on the previous real feeding day (15.9±0.2 ml). After 19 real and 19 sham feeding trials, the rats did not develop a significant preference.

Thus, under our conditions, when rats really feed and sham feed the same volume or for the same duration they do not prefer real feeding. Our results suggest that (1) food stimuli acting at pregastric sites are sufficient to elicit the reward of a meal in the rat; and (2) when sham feeding is prolonged for 60 min these stimuli become less rewarding.

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215.9 AN ANALYSIS OF MEAL PATTERNS IN THE GENETICALLY OBESE MOUSE(ob/ob). **A.J.Strohmayer and G.P.Smith.Dept. Psychiatry,Cornell Univ.Med.Coll.,Manhasset,N.Y.11030**

The genetic obesity of the ob/ob mouse is characterized by hyperphagia.To determine which characteristics of feeding behavior(meal size,frequency or intermeal interval(IMI)),contribute to the hyperphagia we recorded 24 hr meal patterns under conditions of unlimited access to liquid food(EC116) and tap water.The mice used were 7-8 weeks old and in the dynamic phase of weight gain.

Obese male mice ate larger meals(*P<.05,see table) than lean males in the light and dark.Obese males also ate less frequent meals(P<.05)than lean males in the dark but not in the light.Obese females showed similar abnormalities;they ate larger(P<.05)less frequent(P<.05)meals than lean females in the dark but not in the light.

GENOTYPE	PERIOD	MEAL#	MEAL SIZE(ml)	IMI(min)
MALE				
LEAN(+/+)				
	LIGHT	13.8	0.16	64.2
	DARK	34.6	0.18	20.9
OBESE(ob/ob)				
	LIGHT	11.3	0.21*	65.3
	DARK	25.5*	0.26*	33.6
FEMALE				
LEAN(+/+)				
	LIGHT	13.6	0.22	50.1
	DARK	23.4	0.25	32.8
OBESE(ob/ob)				
	LIGHT	10.4	0.28	88.6
	DARK	14.0*	0.49*	43.4

Since this pattern of feeding has been shown to produce weight gain and increased body fat,(Fabry 1967), the hyperphagia of the obese mouse contributes to the development of obesity through:1)an increased caloric intake and 2)an obeseogenic pattern of feeding.(supported by NIMH#MH15455)

215.10 ANALYSIS OF AMINO ACID PATTERNS AND REGIONAL BRAIN SEROTONIN LEVELS IN THE GENETICALLY OBESE ZUCKER RAT. J. A. Finkelstein, W. T. Chance and J. E. Fischer*. Dept. Anatomy, N. E. Ohio Univs. Col. Med., Rootstown, OH 44272 and Dept. Surg., Univ. Cincinnati Med. Ctr., Cincinnati, OH 45267.

The genetically obese Zucker rat has been suggested as an appropriate model of juvenile onset obesity. Levels of norepinephrine are different in certain brain nuclei of the obese rats, but no data on regional serotonergic activity have been reported. Since CNS serotonin (5-HT) has been suggested as a mediator of satiety, we fluorometrically assayed brain 5-HT, tryptophan (Trp) and 5-hydroxyindoleacetic acid (5-HIAA) in the following regions: cortex (C), hippocampus (HI), corpus striatum (CS), hypothalmus (HYPO), remaining telencephalon-diencephalon (TEL-DI), mesencephalon (MES), pons-medulla and cerebellum (CB). In addition, amino acid levels were determined in plasma and cerebral cortex. For seven days prior to sacrifice, food intake was monitored in eight male genetically obese (fa/fa) and eight lean littermate (Fa/-) Zucker rats (10-12 weeks of age). Food intake and body weight were significantly elevated in fa/fa rats during this period. Although total plasma Trp was slightly elevated (non-significant) in fa/fa rats, free (unbound) Trp was significantly decreased. Other significant differences between fa/fa rats and their lean littermates were observed. Plasma levels of proline, alanine, valine, isoleucine, leucine, phenylalanine, threonine and methionine were elevated in fa/fa rats, while aspartic acid, glutamine, glycine and lysine were decreased. Brain serine, glycine, alanine, and valine were also elevated in fa/fa rats; glutamate, methionine, isoleucine, tyrosine and phenylalanine were decreased. Analysis of indole activity in brain regions revealed decreased Trp in CS, HI, C, HYPO and TEL-DI, decreased 5-HT in MES and CB, as well as decreased 5-HIAA in TEL-DI. The branched-chain amino acids leucine, isoleucine and valine compete with free Trp, tyrosine and phenylalanine for transport across the blood-brain barrier. Thus, the combination of decreased free Trp and elevated branched-chain amino acids in plasma apparently results in reduced levels of brain Trp. Similarly, the aromatic amino acids tyrosine and phenylalanine were also reduced in the brains of fa/fa rats. This reduction in brain Trp may be reflected as decreased 5-HT in MES and decreased 5-HIAA in TEL-DI. Whether these biochemical changes are the result or the cause of the hyperphagia in fa/fa rats remains to be determined.

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215.11 CHRONIC INTRAVENTRICULAR INSULIN INFUSIONS REDUCE FOOD INTAKE AND BODY WEIGHT IN RATS. Deborah J. Brief* and John D. Davis, Dept. of Psychology, Univ. of Illinois, Chicago, IL 60680.

Insulin receptors and an insulin-like peptide have been identified in regions of the rat brain thought to be involved in the control of food intake and body weight. Direct application of insulin to hypothalamic cells modifies cell activity and reduces short-term food intake, while injections of insulin antibodies increase food intake. One study (Woods et al., 1979) has shown that chronic daily infusions of insulin into the lateral ventricle produce a reduction of body weight in baboons. The purpose of the present study was to examine the effects of chronic intraventricular insulin infusions on body weight regulation in the rat.

Guide cannulas terminating in the third ventricle were implanted in male albino rats adapted to a 12 hour light/dark cycle. Following recovery from surgery, food, water and body weight measurements were taken twice daily, at 9 am and 6 pm. After food intake on P.J. Noyes pellets appeared stable for 7 days, a permanent cannula which also terminated in the third ventricle and which was attached by polyethylene tubing to an Alzet minipump (implanted subcutaneously on the back), was cemented in place. The animals were infused with either a control solution (Ringer) or insulin (.5, 1 mU in Ringer) at a constant rate of 1 μl/hour for 7 days.

Only infusions of the higher dose of insulin (1 mU) produced a sustained reduction in body weight. By day 7 of the treatment period there was an average 16% reduction in body weight among the animals infused with 1 mU/day insulin, compared to approximately a 4% reduction in animals infused with Ringer or .5 mU/day insulin. The reduction in food intake occurred during the night portion of the diurnal cycle. The fact that day food intake was not affected in the 1 mU group suggests that the animals were not suffering from a toxic effect of insulin. Unlike the Ringer or .5 mU animals, which decreased body weight on the first night of infusion and began to recover during the infusion period, the 1 mU group continued to lose weight throughout the infusion, although the rate of loss decreased after the 4th day.

These results support the notion that brain insulin is involved in central regulation of body weight, possibly because it may modulate activity of cells or uptake of metabolites in the area surrounding the third ventricle which is involved in this function.

Woods, S. et al., Nature, 1979, 282, 503-505.

215.12 EFFECTS OF VAGAL ASSOCIATED GASTRIC AFFERENT STIMULATION ON MESENCEPHALIC AND HYPOTHALAMIC NEURONAL ACTIVITY. F. C. Barone, M. J. Wayner and I. Zarco de Coronado*. Brain Research Lab., Syracuse Univ., 601 University Ave., Syracuse, NY 13210 and Depto de Fisiologia, Facultad de Medicina, U.N.A.M., Mexico.

Male rats were anesthesized with urethane and prepared for hypothalamic and mesencephalic single neuron recording. A gastric balloon was filled with 10-15 ml of water, 20-30 sec, and/or the whole left cervical vagus nerve was stimulated with multiple pulses or pulse trains of 0.5 msec pulse duration at 1-40 Hz, while simultaneously monitoring mesencephalic central gray (CG) unit activity. Data were collected from only those animals which displayed a constant bradycardia effect at a stable threshold to vagus stimulation and only those animals which did not exhibit any gastric pathology following the experiments were used for the analysis. Most CG neurons examined increased discharge frequency during vagus nerve stimulation and gastric distension. The magnitude of change in CG neuronal unit activity was directly related to the intensity of gastric or electrical stimulation. Since lateral hypothalamic (LH) neurons are also affected by gastric distension and vagus stimulation (Barone et al., Brain Res. Bull. 4: 267-282, 1979; Barone et al., Brain Res. Bull. 4: 381-389, 1979) and neurons in the CG project to and terminate on LH neurons (Barone et al., Brain Res. Bull. 7, in press), it was hypothesized that the CG mediates gastric afferent input to the LH. Therefore, recordings were obtained from the LH and neurons were tested for the effects of gastric distension and vagus stimulation. In addition, the effects of CG electrical stimulation, single pulses of 0-500 μA through a small concentric bipolar electrode, were determined. If all three stimuli affected LH unit activity in a similar manner, a series of small DC electrolytic lesions were made in the CG. Neuronal responses to gastric distension and vagus and CG electrical stimulation were then evaluated again. In addition, seven barrel electrodes were utilized to determine the sensitivity of these hypothalamic neurons to catecholamines and some endogenous opiates. Results indicate that small lesions confined within the CG attenuate the response of LH neurons to gastric afferent stimulation. (Supported by NIH Grant NINCDS USPHS No. 13543.)

215.13 RAT BRAINSTEM RESPONSES TO GASTRIC SIGNALS OF DISTENSION AND NUTRIENT AS EXAMINED WITH [^{14}C]2-DEOXY-D-GLUCOSE. W.G. Young, T.D. Tom*, and J.A. Deutsch. Departments of Neurosciences and Psychology, University of California, San Diego, La Jolla, CA 92093.

We have shown that the stomach of the rat regulates food ingestion based on two mechanisms of distension and nutrient content (Deutsch, Gonzalez, and Young, Br. Res. Bull. 5, suppl. 4, 55-57, 1980). The distension system depends on the integrity of the vagus nerve projecting from the stomach (Gonzalez and Deutsch, Soc. Neurosci. Abstr. 6, 519, 1980). The nutrient system has been further defined in terms of dietary and gastric interactions (Deutsch and Gonzalez, Soc. Neurosci. Abstr. 6, 528, 1980). This study presents evidence that specific areas in the brainstem do respond to the nutrient signals produced solely by the stomach when food is ingested.

Two groups of subdiaphragmatically vagotomized (VAG) and sham-operated (SHAM) anesthetized rats were injected with 2-^{14}C-deoxy-D-glucose (2-DG) and subjected individually to one of three conditions: gastric infusion with 5 ml of Vivonex standard diet, gastric infusion of 5 ml of saline, or no infusion. Emptying of the infusate from the stomach was arrested with previously described techniques (Young and Deutsch, J. Neurosci. Meth. 3, 377-384, 1981). 2-DG was preferentially taken up into the general area of and surrounding the nucleus of the solitary tract (NST) of SHAM rats infused with saline as compared to the SHAM controls. Additionally in the nutrient infused rats, the ventral aspect of the brainstem just lateral to the corticospinal tract also took up 2-DG. Presently, the specific areas have not yet been identified, but the uptake corresponds with the appearance of the lateral reticular nucleus (LRN) at the caudal end and the nucleus ambiguus (NA) at the more rostral end. However, when the vagus was transected in the VAG rats, saline infusion no longer produced an uptake by NTS area. Vagal transection, on the other hand, did not affect uptake in the area surrounding the LRN or the NA when nutrient was infused and the NTS response was still not present. We conclude that the vagus carries the distension messages from the stomach to the general area of the NTS, most likely the NTS specifically. Gastric nutrient messages project to the ventral lateral aspect of the brainstem surrounding the area of the LRN and the NA, and these are not vagally mediated. However, the vagus may still carry nutrient messages to the NTS that are not presently distinguishable from the accompanying distension.

215.14 ELIMINATION OF HYPOTHALAMIC HYPERPHAGIA AND OBESITY BY ADRENALECTOMY IN RATS. B. K. Bruce*, B. M. King, G. R. Phelps* and M. C. Veitia*. Department of Psychology, University of New Orleans, New Orleans, Louisiana 70122.

Two previous investigations into the effects of adrenalectomy (ADX) on ventromedial hypothalamic (VMH) hyperphagia and obesity have produced incongruent results. One study concluded that adrenalectomy produces a significant depression of hyperphagia and obesity (Mook, Fisher, and Durr, Horm. Behav., 6, 65-79, 1975). The other study reported that adrenalectomy had no effect (York and Bray, Endocrinology, 90, 885-894, 1972), but failed to include a sham ADX-VMH control group with which to compare the weight gain in ADX-VMH animals. The sequence of the surgeries differed in these two studies.

In order to reassess the role of adrenal hormones in hypothalamic hyperphagia and obesity, VMH or sham lesions were performed either 15 days prior to or after ADX or sham adrenalectomy (SADX). Body weight and food intake were recorded for 30 days after the second surgery. Adrenalectomy both reversed and prevented the development of hyperphagia and obesity in VMH-lesioned rats. The SADX-VMH group displayed a mean weight gain of 180 g in 30 days after the lesion, but the ADX-VMH animals did not significantly differ from the sham-lesioned groups in body weight or food intake. Animals with VMH lesions continued to display weight gains after SADX (240 g/45 days), while adrenalectomy in obese VMH animals eliminated all excess weight gain, and decreased food intake to below the level of all control groups. Adrenalectomy had no effect on body weight or food intake of animals with sham VMH lesions. As adrenal demedullation has been found to have no effect on hypothalamic obesity (Mook et al., 1975), it was concluded that adrenocortical secretions are necessary for the development and maintenance of VMH hyperphagia and obesity.

215.15 FEEDING AND DRINKING RESPONSES OF RATS WITH KAINIC ACID INFUSIONS INTO THE DORSOMEDIAL HYPOTHALAMUS. L.L. Bellinger, L.L. Bernardis and F.E. Williams*. Department of Physiology, Baylor College of Dentistry, Dallas, Tx 75246 and V.A. Medical Center, Buffalo, N.Y. 14215.

Male Sprague-Dawley (150gm) rats received bilateral infusions of Kainic Acid (KA) into the Dorsomedial Hypothalamus using the coordinates: AP,+4.2mm; Lat.,0.5mm, Depth 2.4mm above ear bar zero. After being anesthetized with Diabutal the rats were infused with 1µg of KA in 0.5µl saline. The infusions were conducted over 2.5 min. and the cannula left in place another 2.0 min. Saline infused rats served as controls (C). Following surgery the rats were given rat chow and water ad lib. The KA rats (n=9) were severely aphagic and adipsic. Mean weekly chow intakes (gms) for four weeks post surgery for the KA rats were 1.4±1.0, 2.6±1.4, 3.4±2.0 and 8.3±2.6 while the C (n=12) intakes were 15.3±0.5, 19.5±0.4, 20.5±0.1 and 19.0±0.4, all P<0.001. During this period the KA rats would also not eat a high fat diet but did eat a baked chow mix that contained 30% sucrose. At experiments end (37 days) three rats still would not eat chow. Mean weekly water intakes (ml) for four weeks post surgery for the KA rats were 2.4±1.2, 3.9±1.8, 7.8±2.9 and 12.6±3.8 while C intakes were 26.8±1.0, 35.5±2.0, 38.5±2.0 and 38.6±2.0, all P<0.001. During this time some but not all of the KA rats would drink some 30% sucrose water. At experiments end all rats were drinking water or sucrose water. All rats were injected with 2-deoxy-d-glucose starting four weeks post surgery. The C rats elevated their three hour food intake (gm) over saline infusions at both the 200 mg/kg (2.5±0.2 vs 4.2±0.5, P<0.01) and 400 mg/kg dose (0.5±0.1 vs 4.1±0.6, P<0.01) while the KA rats did not elevate their food intake over saline injections at either dose (2.0±0.4 vs 1.1±0.5; 0.4±0.1 vs 0.7±0.4). When food deprived for 24 hr and then refed both C and KA consumed a similar amount of food in the first hour of refeeding (expressed as % of baseline intake); however the 24 hr intake of the KA rats was significantly greater than C (314±13.1% vs 181±8.0%, P<0.001). During food deprivation the KA and C rats consumed a similar amount of water (expressed as % of baseline intake). The KA rats weighed significantly less than C six days post surgery and by experiments end were also significantly lighter (256±6.8 vs 314.8±3.9, P<0.001) and also demonstrated reduced linear growth (Δnaso-anal length 24.1±3.2mm vs 35.5±1.5mm, P<0.01).

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215.16 CORRELATION BETWEEN HYPOTHALAMIC KNIFE-CUT AND DIETARY OBESITIES. Richard M. Gold. University of Massachusetts, Amherst, Massachusetts 01003.

Parasagittal hypothalamic knife-cuts in rats reliably produce excessive food intake, weight gain, and adiposity. However, the magnitude of these excesses varies considerably both within and between studies. Previous work has shown that several variables can greatly alter the rate of this weight gain. These include the strain, sex, and age of the rats, the palatability, fat content, and accessibility of the diet, and the availability of exercise. Considerable inter-rat differences remain even after these factors are held constant, and have generally been attributed to variability in the placement of the knife-cuts.

We have recently, however, found extreme and reproducible inter-rat variability in susceptibility to dietary obesity in response to junk foods obtained from supermarkets. This difference surely cannot be attributed to surgical variables, as no surgery is involved. We attribute this variability in susceptibility to obesity to inter-rat genetic differences. This genetic basis has been confirmed by selective breeding. This finding raises the possibility that variability in response to obesifying hypothalamic knife cuts is also due to inter-rat genetic differences.

Twenty individually housed adult female rats received a supermarket diet consisting of sweetened milk, cookies, and peanut butter for 22 days. Twenty rats from the same shipment served as chow fed controls. As predicted, the weight gains of the supermarket diet fed rats were extremely variable (mean 3.01g/day variance 22.17g) as compared with the chow fed controls (mean 1.58g/day variance 1.58g). During subsequent weight stabilization for 14 days on chow diet 61% of the excess weight was lost. Subsequently, under rigidly controlled conditions 3x3 mm bilateral parasagittal hypothalamic knife-cuts were made in the 7 most dietary-obesity prone, 7 least dietary-obesity prone (i.e., lean prone), and 12 former pellet-fed control rats. The remaining rats were sham operated. The formerly obesity-prone rats did not respond more vigorously to the knife cuts than did the formerly lean-prone rats, and there was surprisingly little variability in response to knife cuts amongst the former pellet-fed controls. Since genetic predisposition to dietary obesity does not predict the magnitude of the response to obesifying hypothalamic knife cuts, we return to the working hypothesis that, given standardized subjects, diet, etc., differences in weight gain in response to hypothalamic knife cuts are indeed due to individual differences in the disruption of hypothalamic neurocircuitry, the identification of which remains to be determined.

215.17 VENTROMEDIAL HYPOTHALAMIC LESIONS PRODUCE EXAGGERATED SHAM FEEDING. J.E. Cox* and G.P. Smith. (SPON: D.N. Lorenz). Dept. Psychiatry, Cornell Univ. Med. Coll. and E.W. Bourne Behavioral Lab., New York Hosp., White Plains, NY 10605.

Altered reactivity to gustatory stimuli may be an important factor in the hyperphagia that follows lesions of the ventromedial hypothalamus (VMH). We used sham feeding to directly assess lesion-produced changes in reactions to oropharyngeal food stimulation, without the confounding influences of stimulation at subsequent preabsorptive and postabsorptive sites.

Female Sprague-Dawley rats (approximately 300g) that had previously sustained electrolytic lesions of the VMH or sham brain surgery were implanted with gastric cannulas. VMH rats were then reduced to control body weights, after which all rats were adapted to eating a sweet milk diet following 3.5 h food deprivation. For sham feeding, gastric cannulas were opened and ingested food drained immediately and completely through collecting tubes. Testing comprised alternating days of normal and sham feeding, and 3 tests were performed for each condition.

During 1 h tests with gastric cannulas closed (normal feeding), VMH rats (n=6) overate, consuming 16.3 ± 1.5 ml (mean ± SEM) of milk diet, compared to 5.7 ± 0.5 ml for nonlesioned rats (n=6). Both groups increased their intake during sham feeding. Controls ate 13.0 ± 2.0 ml, an increase of 130% over normal feeding. By comparison, the increase by VMH rats was markedly exaggerated: VMH rats sham fed a mean of 57.0 ± 4.1 ml, an elevation of 250% over baseline.

The exaggeration of sham feeding by VMH rats is highlighted by preliminary observations of that shown by genetically obese and hyperphagic Zucker rats. After 3.5 h food deprivation, female Zucker rats (n=2) consumed 13.2 ± 0.2 ml of milk diet during 1 h tests of normal feeding and 24.2 ± 1.8 ml during sham feeding. Lean siblings (n=2) ingested 6.5 ± 0.8 ml and 11.5 ± 0.2 ml during normal and sham feeding, respectively. In contrast to VMH rats, Zucker rats increased their intake during sham feeding no more than nonobese littermates; both of these latter groups increased consumption 80% over normal feeding. Thus, though Zucker rats overate during normal feeding almost as much as VMH rats, they sham fed less than half as much.

The dramatic enhancement of sham feeding in VMH rats points to a disturbance in reactivity to oropharyngeal stimulation as a primary factor in overeating after hypothalamic lesions. Comparison with the sham feeding by hyperphagic Zucker rats suggests the specificity of such a disturbance for hypothalamic hyperphagia.

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215.18 HYPOTHALAMIC OBESITY IN THE WEANLING RAT: DIETARY SELF SELECTION, RESPONSE TO STARVATION AND HIGH-PROTEIN DIET. L.L. Bernardis, R. Luboshitzky* and L.L. Bellinger, VA Med. Ctr. and SUNY at Buffalo, NY 14215 and Dept. Physiol., Baylor Coll. Dent. Dallas, TX 75246.

Weanling Sprague-Dawley rats received bilateral electrolytic lesions in the ventromedial hypothalamus (VMNL rats); sham-operated animals served as controls (CON). For two weeks after the operation the rats were fed lab chow. VMNL rats weighed as much as the CON but their Lee Index was higher ($p<0.01$). Caloric intake (CI) was normal but the VMNL rats laid down less ($p<0.05$) body weight (BW) and deposited more fat ($p<0.001$) for the CI. For the next 21 days all rats received three equicaloric (4.03 kcal/gm) diets of different macronutrient content. CI from all three diets was similar in both groups but the VMNL rats selected more ($p<0.02$) of the high-carbohydrate and less ($p<0.05$) of the high-protein diet. Intake from the high-fat diet was slightly but not statistically significantly higher in the VMNL rats. In terms of macronutrient intake the VMNL rats ingested more ($p<0.01$) carbohydrate (CHO) and fat ($p<0.05$) and the same amount of protein (PRO) as the CON. However, in percent of total macronutrient intake, the VMNL rats ate less ($p<0.01$) CHO and PRO ($p<0.01$) than the CON; fat intake was comparable. During a two-day fats the VMNL rats lost less BW and fat (both $p<0.01$) but regained both BW ($p<0.01$) and Lee Index ($p<0.05$) slower than the CON. During the subsequent refeeding period there was no difference in dietary selection between the two groups but in terms of actual nutrient intake the VMNL rats ate less PRO. During the following nine days all rats were fed a high-PRO diet (43.2%). Both VMNL rats and CON increased their CI over that of the previous periods but both groups ate similar amounts. BW gains were similar in both groups but the Lee Index remained higher ($p<0.02$) in the VMNL rats. During the next 31 days all rats were again fed lab chow. VMNL rats ate less ($p<0.01$) calories than CON, showed normal BW gains but retained their higher ($p<0.05$) Lee Index. They utilized CI similarly for BW gain but poorer ($p<0.001$) for fat deposition. At sacrifice, plasma glucose and total protein were similar in the two groups but white (epididymal) fat pads were heavier ($p<0.001$) and contained more fat ($p<0.001$) and less ($p<0.05$) protein than CON. It is concluded that the weanling VMNL rat selects more CHO diet and less PRO diet than CON but that in terms of actual percent macronutrient intake the VMNL rats ingest less of both CHO and PRO. It thus shows a pattern different from that of the mature rat with hypothalamic obesity.

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216.1 SLOW POTENTIAL RESPONSES FROM RAT FRONTAL CORTEX TO A SINGLE PULSE CUE PRECEDING REWARDING MEDIAL FOREBRAIN BUNDLE STIMULATION: EFFECT OF AMPHETAMINE. J.H. Pirch, T.C. Napier and M.J. Corbus*. Department of Pharmacology and Therapeutics, Texas Tech University Health Sciences Center, Lubbock, TX 79430

We previously demonstrated, in the rat frontal cortex, negative slow potential (SP) responses to an auditory cue (click) which preceded rewarding medial forebrain bundle (MFB) stimulation (Pirch, Soc. Neurosci. Abst. 6: #274.4, 1980). This study was conducted to: 1) determine whether a single electrical pulse to the MFB can serve as a cue, as reflected by the development of SP responses to the pulse when it is paired with the train of rewarding stimulation; and 2) examine the effect of d-amphetamine on these SP responses. SP responses were recorded bilaterally from the frontal cortex of rats with permanently implanted silver-silver chloride electrodes. Trials were presented at variable intervals of 15 to 50 seconds and the interval between the single pulse and onset of the rewarding train was 2 seconds. The cue stimulus was a single 0.5 msec monophasic square wave pulse of the same current intensity as rewarding stimulation (100 Hz, 0.5 sec train). Appropriate current strength was determined by prior testing for self-stimulation. The single pulse by itself failed to evoke an SP response but after repeated pairing, large negative SP responses developed which were bilaterally equal. Training to the point of maximum SP response amplitudes generally required more trials with the pulse cue than with the auditory cue. Amphetamine (0.125, 0.25 and 0.5 mg/kg, SC) produced a dose-related depression of the SP response to the pulse cue, an effect comparable to that observed using an auditory cue with either food or MFB stimulation reinforcement. The results indicate that frontal cortex SP responses which develop in anticipation of a meaningful event are primarily independent of the type of cue which evokes the responses. Furthermore, amphetamine consistently suppresses these SP responses, regardless of the type of cue or reinforcement employed. (Supported by USPHS MH29653 and by the Tarbox Parkinson's Disease Institute at Texas Tech University Health Sciences Center).

216.2 BRAINSTEM AND BASAL FOREBRAIN PROJECTIONS TO THE CEREBRAL CORTEX IN THE RAT. C.B. Saper. Dept. of Neurology, New York Hosp.-Cornell Univ. Med. Ctr., New York, NY 10021

Several brainstem and basal forebrain cell groups which have defined neurotransmitters and which project to the cerebral cortex have been implicated in control of cognitive function. A systematic mapping of brainstem and basal forebrain afferents to the cerebral cortex has therefore been undertaken to identify any additional such cell groups which might contribute to cortical regulation. Injections of wheat germ agglutinin-conjugated-horseradish peroxidase solution were placed into a variety of cortical sites, and the distribution of retrogradely labeled neurons stained by the TMB method was mapped.

Several cell groups were found to project topographically to all cortical areas investigated: 1) the noradrenergic locus coeruleus, 2) the serotonergic dorsal and superior central raphe nuclei, 3) a group of ventral tegmental neurons, at least some of which are dopaminergic and 4) the cholinergic magnocellular nucleus of the basal forebrain. Neurons in the area of the A1 catecholamine cell group also projected to the medial frontal cortex.

A major cortical projection system from the hypothalamus and dorsal pontine tegmentum was also delineated. A hypothalamic cell group was found, extending from the lateral hypothalamus at the premammillary level caudally and medially through the supramammillary area, which projected topographically upon all of the cortical areas studied. The more medial parts of this cell group appeared to project primarily to the medial frontal and cingulate cortex and the hippocampus, while more lateral neurons projected to the lateral wall of the hemisphere. The tuberomammillary nucleus appeared to have an extensive bilateral cortical projection.

In the pons, a group of neurons extending medially from the parabrachial nucleus across the central gray matter at that level was also found to project topographically in a medial-to-lateral fashion upon the cerebral cortex. The fibers from the pontocortical projection appeared to traverse the hypothalamus in a topographic distribution, passing through the hypothalamic areas which project to the same parts of the cortex. Evidence from other sources indicates that these pontine and hypothalamic cell groups are topographically reciprocally connected. This suggests that there may be a major system of topographically organized direct and relayed projections to the cerebral cortex from the dorsal pons and hypothalamus. The role of such a system remains conjectural but earlier physiological studies suggest that it may play a part in alerting and arousal mechanisms.

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216.3 ROLE OF THE ANTEROMEDIAL CORTEX IN ORIENTING BEHAVIOR AND VISUALLY GUIDED LOCOMOTION OF THE RAT. T.M. Barth,*S.M.Parker* and H.M.Sinnamon. Lab. of Neuropsychology,Wesleyan University, Middletown,CT 06457

Unilateral damage to the frontal cortex projection areas of the dorsomedial thalamus produces multimodal contralateral neglect in man and monkeys. In rats damage to the homologous area, the anteromedial cortex (AMC), reportedly produced a type of visual neglect in a Y-maze. In this study we made unilateral lesions in the AMC to study its role in orientation to stimuli of different modalities and in the process by which orienting is used to guide locomotor-approach.

Simple orienting behavior was tested by presenting visual, auditory,olfactory and tactile stimuli on each side of the subject. Visually guided locomotion was examined in a cross maze in which a rat shuttled from pole to pole to obtain milk from an illuminated bottle under three testing conditions. In one condition, the bottle cues were visible to the rat at both the start and choice point, and thus visual information was continuously available to guide locomotion. In the second condition, cues in the left and right arms were available only at the choice point and not at the start point. In the final condition, the bottle cues were exposed only at the start point but not at the choice point,and thus the rat had to remember the location of the cue to guide locomotion.

Ten Sprague-Dawley male rats were pretested in the simple orienting test and pretrained in the three cross maze conditions. Six animals received unilateral AMC lesions and four served as unoperated controls. Animals with medial lesions made significantly more errors than controls when the cue was on the side contralateral to the lesion in all three cross maze conditions. Rats with lesions had greatest difficulty when the cues had to be remembered. In addition, animals with lesions made few errors in any condition when the cues were in the anterior field and on the side ipsilateral to lesions. There was no difference in simple orienting behavior between the experimental and control groups.

These data suggest unilateral AMC lesions produce a deficit that is similar to the neglect syndrome displayed by primates with damage in the homologous region. This deficit cannot be viewed as a simple sensory deficit because animals with lesions can orient to the contralateral side. This deficit is likewise not the manifestation of a motor bias (i.e.,circling) because subjects can approach cues in the anterior field. Currently smaller lesions of the AMC are being made in order to delineate the exact regions involved in these processes.

216.4 MODIFICATION OF ACOUSTIC STARTLE REFLEX AMPLITUDE BY BACKGROUND LEVEL AND PRESTIMULI IN TWO STRAINS OF MICE. J.E. Storm*, K.L. Hulebak* and L.D. Fechter. (SPON: M. Bleecker). Div. Env. Toxicology, Dept. Env. Health Sciences, The Johns Hopkins University, Baltimore, Maryland 21205.

The general properties of reflex modulation by both tonic background stimuli and punctate prestimuli has been demonstrated to apply to several reflex behaviors including the eyeblink elicited by an airpuff or tap to the forehead, and the startle reflex elicited by noise or electric shock. Such modulation occurs in several species including man (adults & infants), rats and rabbits, and is believed to be characteristic of reflex behaviors in general. For example, the amplitude of the acoustic startle reflex can be maximized when the response is elicited by a sudden intense burst of noise presented against a white noise background level in the neighborhood of 75dBA. Furthermore, the acoustic startle response can be inhibited when a brief white noise prestimulus, which by itself does not elicit startle, precedes the startle-eliciting stimulus by approximately 40-100msec.

In the present study, the amplitude of startle following a 10 kHz 120 dB SPL tone of 30 msec duration (incorporating a 5 msec rise and decay time) in adult ICR strain laboratory mice (Blue Spruce Farms) (n=8) was compared to that of adult grasshopper mice Onychomys torridus (n=8) in the presence of background white noise levels of 60, 75 and 90 dBA. Both species of mice showed maximum amplitude of startle in the presence of 75 dBA, and a marked reduction of amplitude in the presence of 90 dBA background level.

However, in a separate study with the same startle tone parameters, modification of the startle response amplitude by a white noise prestimulus was markedly different in the two species. In the presence of a 70 dBA background noise level, prestimuli of 80 dBA or 85 dBA were presented 0, 10, 40, 160 and 500 msec prior to the startle tone. Presentation of the prepulses 10 or 40 msec prior to the startle tone significantly inhibited the startle response of ICR strain mice (n=25), whereas prepulses did not inhibit the startle response of Onychomys torridus (n=28). The failure of preliminary stimuli to inhibit the acoustic startle reflex under such conditions is unique among all species studied to date. We are currently investigating the efficacy of light and pure tone prestimuli on inhibition of this reflex in order to evaluate the use of Onychomys torridus for further study of the neuroanatomical and physiological basis of reflex modulation.

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216.5 HIPPOCAMPAL SUSTAINED POTENTIAL SHIFTS. John Fowler[a] and Lauren K. Gerbrandt. (SPON: Joseph Arezzo) Dept. of Psychology, California State University, Northridge, Ca. and Neuroscience Research Program, MIT, Boston, Ma. 02116

The neocortical arousal response consists of low voltage fast activity and can be elicited by novel stimulation of sensory modalities as well as by electrical stimulation of the reticular formation (Moruzzi and Magoun EEG J 1:455, 1949). The hippocampal arousal response is characterized by low frequency (2.5-13 Hz., depending on species), high amplitude synchronous waves and typically occurs simultaneously with the neocortical arousal response (Green and Arduini J. Neurophysiol 17:533, 1954). Because neocortical and hippocampal arousal responses have independently been associated with facilitory memory effects, one may speculate that potential field changes common to both arousal processes exist. During either the reticulo-cortical desynchronized EEG pattern or the highly synchronous hippocampal arousal pattern, negative sustained potential shifts typically develop over neocortex (Arduini et al. Arch. Ital. Biol. 95:137, 1957). Recent studies indicate these arousal concurrent sustained potential shifts can be recorded within the hippocampus during periods of hippocampal rhythmical slow activity (RSA) (Gerbrandt and Fowler Prog. in Brain Res. 154:109, 1980). In the studies reported here, sustained potential shifts and RSA were recorded from the hippocampus of locally anesthetized, curarized rats. Somatosensory stimulation was used to induce sustained potential shifts from a non-aroused irregular EEG background. Both laminar and reverse laminar profiles were recorded in order to assess the possible effects of tissue damage caused by the advancing electrode tip. Neither the form nor the amplitudes were appreciably different when forward and reverse profiles were compared at the same depth. The mean and maximal sustained potential shifts as well as the amplitude 3 seconds after stimulus onset were recorded at different depths. While there were minor variations in amplitude, the profiles were similar for all three measures. These shifts were correlated with the corresponding RSA amplitude profile. The two profiles were similar with the RSA amplitude being higher at all points prior to the "null" region. At the "null" point the sustained potential shifts were larger and continued to be so with the exception of the point 500 micrometers below the "null". Spontaneous sustained potential shifts were observed but were always concurrent with bursts of RSA. In several experiments an attempt was made to dissociate hippocampal sustained potential shifts and RSA by transecting the dorsal fornix. Information from the fornix transection experiments indicate that sustained potential shifts and RSA can be dissociated.

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216.6 NALOXONE AUGMENTS ELECTROPHYSIOLOGICAL MEASURES OF SELECTIVE ATTENTION IN MAN. A. Arnsten, D.S. Segal, H. Neville and S. Hillyard. Psychiatry Dept., UCSD Med. Sch., La Jolla, CA 92093, Salk Inst., San Diego, CA 92138, Neurosci. Dept., UCSD Med. Sch., La Jolla, CA 92093.

In previous studies of rat behavior we observed that low doses of the opiate antagonist naloxone (NAL) increased the average time an animal spent per contact with stimuli in a novel environment (Arnsten, A. and Segal, D.S., Life Sci. 25: 1035, 1979). These findings suggested that NAL might enhance stimulus-directed behavior by altering attentional mechanisms, particularly since the opiate agonist morphine is reported to diminish the ability to concentrate in humans (Lewis, M.E. et al., Science 211: 1166, 1981). The pattern of NAL binding in primate cortex has also been interpreted as suggesting an opioid role in attentional processes (Jaffe, J.H. and Martin, W.R., in Pharmacol. Basis of Therapeutics, 6th Ed., p. 494, 1980). We examined the hypothesis that NAL might increase selective attention in humans utilizing an auditory event-related potential paradigm developed by Hillyard and associates (Science 182: 177, 1973; J. Exp. Psychol. Human Perception Performance 2: 313, 1976). Auditory event-related potentials were recorded from subjects who listened selectively to sequences of discrete tones, all of the same frequency, delivered to one of three perceived spatial locations: left ear, right ear, and center of the head (binaural). Subjects were instructed to detect occasional target tones of a slightly longer duration at the attended location. In this paradigm, the measure of selective attention was the amplitude of a broad negative component of the event-related potential to the tones when they were attended relative to when they were inattended (the "attention effect"). Typically, tones at the center of the head exhibited the smallest attention effect and were the least discriminable, while a larger attention effect was observed for the left and right tones. Naloxone (2 mg, IV, doubleblind, crossover design) significantly increased the amplitude of the attention effect to the left and right tones, but not to the center tones, as well as improving some aspects of behavioral performance. These findings indicate that NAL enhances electrophysiological signs of selective attention for more easily discriminable channels of stimuli, suggesting a role for endogenous opioid systems in early stimulus filtering (Treisman, A., Psychol. Rev. 76: 282, 1969).

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216.7 SELECTIVE ATTENTION-INDUCED CENTRIFUGAL INFLUENCES ON THE HUMAN RETINA. Robert G. Eason, Marta Oakley* and Lynn Flowers*, Dept. of Psychology, Univ. of N. Carolina, Greensboro, N. C. 27412.

It is known that about 10% of the mammals' optic nerve fibers, including humans, are of central origin (R. B. Livingston, Sen. proc., percept. & beh., Raven Press, 1978). Following Cajal's early description, a significant number of studies have provided data supporting their existence (Hasselt, Van P., Ophthal. Res., 1973, 4, 298-320). Yet their functional significance is not well understood; also, some workers are still skeptical of their existence in adults.

In 1969, Eason, Harter, and White (Physiol. & Beh. v. 4) demonstrated that the instruction to selectively attend (and respond) to flashes presented peripherally in the left and right visual fields results in larger visual evoked potentials (VEPs) over occipital cortex when the flashes in a given field are attended than when they are not. Since relatively early components of the VEPs were affected, it was postulated that cortically induced centrifugal activity associated with "stimulus set" differentially altered the inflow of relevant and irrelevant information at precortical synaptic relays. This neural filtering would permit relevant information to have a greater impact on arrival at visual cortex than irrelevant information.

Since centrifugal fibers project to the retina, cortically induced centrifugal influences produced by selective attention instructions should be measurable at the retina if the above hypothesis has validity. Detection of attention-induced changes in retinal activity not only would provide further evidence for the existence of functionally active centrifugal fibers in adult humans, but would show one aspect of their functional significance.

Using a paradigm similar to the 1969 study, averaged ERGs and scalp VEPs (over occipital cortex) were recorded from 16 young adults during peripheral stimulation of the right and left visual fields. The right eye only was used. The leading edge of the b-wave of the ERG was significantly greater ($p < .01$) to attended than to unattended flashes. Also, the earliest measurable component of the VEP (onset latency, 40-50 msec.; peak latency, 80-90 msec.) was significantly greater to attended flashes ($p < .05$). These results support the hypothesis that voluntary sustained selective attention involves the cortical induction of centrifugal neural activity which selectively filters certain dimensions of relevant and irrelevant information prior to its arrival at cerebral cortex. The results provide further support for the existence of functionally active centrifugal fibers projecting to the retina in adult humans.

216.8 NEURAL DETERMINANTS OF THE HEART RATE ORIENTING RESPONSE IN NEONATAL RATS. V. Haroutunian and B. A. Campbell. Dept. of Psychology, Princeton Univ., Princeton, NJ 08544.

Neonatal rats and human infants both respond to novel, mild sensory stimuli with phasic bradycardia (BR). This BR response has been interpreted as indexing orienting and stimulus processing and is termed the heart rate orienting response (HR-OR). Three experiments investigated the neural origins of the HR-OR.

Presentation of a novel auditory stimulus evoked pronounced BR in 19-20 day-old rats. This response was mediated by vagal efferents to the heart. Two stage bilateral vagotomy abolished the HR-OR. The right and left vagi were able to exert this inhibitory influence on cardiac rhythmicity relatively independently of each other since unilateral vagotomy was without effect on the HR-OR. Pharmacological blocakde of peripheral muscarinic receptors by low (.1 mg/kg) and intermediate (.5-2 mg/kg) doses of methyl scopolamine showed that the response was mediated through peripheral cholinergic systems. Peripheral sympathectomy by guanethedine (45 mg/kg/day X 15 days), which led to 70% depletion of cardiac norepinephrine, had little or no effect on the response. The relatively small influence of the sympathetic NS (SNS) upon the HR-OR was further shown by the negligible effect of propranolol on evoked cardiac responses, even though tonic heart rate decreased significantly in response to the beta adrenergic receptor blockade. These findings show that the HR-OR is almost exclusively mediated by vagal cholinergic efferents to the heart with the SNS playing a relatively minor role. The generality of these results was further shown by methyl scopolamine blockade of the HR-OR evoked by olfactory stimuli (isoamyl acetate) in 6-day-old neonates.

The influence of vagal cholinergic efferents upon phasic cardiac responses also extends to pain-evoked tachycardia (TA). Following presentation of the auditory stimulus in the experiments described above, each rat received one (.5 sec 1 ma) foot shock and the heart rate response was noted. Shock evoked TA in control, guanethedine, propoanolol, and unilaterally vagotomized rats. This TA response was blocked by methyl scopolamine and bilateral vagotomy. These findings show that both the TA and BR evoked by psychologically relevant stimuli are under direct neural control, and that this control is exerted through cholinergic vagal efferents to the heart.

216.9 PSYCHOPHYSIOLOGIC PERSONALITY ASSESSMENT ON THE BASIS OF CARDIO-RESPIRATORY ORIENTING RESPONSES. S.A. Corson, E.O'L Corson, and R.M. Andrysco, Dept. of Psychiatry, Academic Faculty of Early and Middle Childhood Education, Ohio State University, and Psychophysiologic Stress Research Center, 285 West Kenworth Rd., Columbus, OH 43214 USA.

We previously described stable constitutional (genetic) differences in psychophysiologic reactions of dogs exposed to repeated psychologically stressful situations induced by Pavlovian conditioning techniques with electrocutaneous reinforcement. Some dogs (e.g., beagles) showed high psychophysiologic adaptation (HA dogs). Other dogs (e.g., wirehair fox terriers, border collies, English shepherds) developed persistent, marked, almost inextinguishable reactions to the entire Pavlovian aversive room complex: tachycardia, polypnea, profuse salivation, vasopressin release, high energy metabolism, and high urinary catecholamines (low adaptation or LA dogs). Analysis of the development and extinction of orienting reflexes (O.R.) in these two types of dogs indicates that the LA dogs exhibit higher frequency and more intense, persistent, and highly fluctuating (poorly modulated) cardiac and respiratory O.R.s than those exhibited by the HA dogs, which show rapid O.R. habituation and good modulation of cardiac and respiratory functions. These observations suggest that the degree of modulation and the dynamics of development and extinction of orienting reflexes might serve as a basis for predicting the type and persistence of physiologic reactions an individual may exhibit to psychosocial stressors and his/her possible susceptibility to psychovisceral (cardiac and respiratory) pathology. Supported in part by NIH grant HL 20861 and The Ohio State University Graduate School Biomedical Sciences Support Grant.

217.1 ONLY ESTERS PROTECT THE OLFACTORY MUCOSA FROM INHIBITION BY THE VAPOROUS ALKYLATING AGENT, ETHYL BROMOACETATE. Rollie Schafer and Darrell W. Criswell. Department of Biological Sciences, North Texas State University, Denton, TX 76203

The vaporous alkylating agent, ethyl bromoacetate (EBA) inhibits the ability of the frog nose to respond to all odorants except the aliphatic amines (Criswell, D.W. et al., Science, 210:425, 1980). As a part of this study and continuing studies with other chemically active odorants, we have attempted to produce specific inhibition using the technique first reported by Getchell and Gesteland (PNAS, 69:1494, 1972). In this technique, the nasal mucosa is flooded with a solution containing a "protecting" odorant, then treated simultaneously with an inhibitory agent such as the protein-specific sulfhydryl reagent, N-ethyl maleimide (NEM). The expectation is that the odorant, present in excess, will occupy most of its receptor sites on the receptor neurons and protect them from interacting with the reagent.

We found that only isoamyl acetate and a few closly-related esters were capable of protecting the olfactory mucosa from the inhibiting effects of EBA applied in the vapor phase. Other odorants such as isoamyl sulfide, isoamyl alcohol, etc. did not protect. By contrast, isoamyl acetate served as a universal protector. It protected responses to itself and a wide variety of odorants from many different odorant classes.

Getchell and Gesteland and other experimenters who have since replicated their work all used ethyl-n-butyrate or similar esters to protect. Delaleu and Holley (Chemical Senses and Flavour, 5:205, 1980) used vaporous NEM to inhibit olfactory responses and also demonstrated protection by isoamyl acetate in the vapor phase. Thus, there is ample evidence that some esters protect, both in the liquid and vapor phases. But why is protection conferred only by esters? Perhaps protection involves some chemical or electrical effect other than simply occupying a receptor site which would otherwise react with the inhibitor. Experiments are underway to determine whether the protecting effect depends on depolarization of receptor cells rather than a site-blocking action of the protecting molecule.

217.2 HIGH RESOLUTION 2-DEOXYGLUCOSE AUTORADIOGRAPHY IN THE OLFACTORY EPITHELIUM AND BULB. Doron Lancet*, Charles A. Greer, John S. Kauer and Gordon M. Shepherd. Section of Neuroanatomy, Yale Univ. School of Medicine, New Haven, CT 06510.

We have employed high resolution 2-deoxyglucose (2DG) autoradiography to analyse cellular localization of odor-elicited activity in the vertebrate olfactory epithelium and olfactory bulb. Animals were injected with 3H- or 14C-2DG and exposed for odor stimulation to isoamyl acetate vapors at 10^{-2} to 10^{-1} saturation. The tissue underwent freeze substitution in acetone followed by plastic embedding. Sections cut at 1-4um were processed for nuclear emulsion autoradiography and examined for cellular localization of silver grains (cf. Sejnowski et al., Nature 287: 449, '80).

In the olfactory neuroepithelium of the mouse and tiger salamander increased 2DG uptake was found in the superficial supranuclear zone (not including the mucus layer) and in the basal layer. The latter uptake was mostly in the form of clusters of grains interspersed among basal cell nuclei; one possibility is that these clusters are related to exiting fascicles of receptor axons. In addition, thin, radially oriented grain clusters were seen between receptor cell nuclei. In all laminae the uptake was differentially distributed across the epithelial sheet suggesting spatial heterogeneity of odor-related activity. Uptake was high in the cells of Bowman's glands whereas it was low in the respiratory epithelium.

In the olfactory bulb of the rat and the salamander high 2DG uptake was seen in some regions of the glomerular layer. Individual glomeruli with uptake higher or lower than the tissue background could be clearly seen, suggesting the glomerulus as a unit of activity. Uptake in periglomerular borders often differed markedly from that of the adjacent glomerular neuropil. Of special interest were small grain clusters over individual periglomerular, mitral and especially granule cell nuclei. Immediately neighboring cell nuclei displayed background activity.

The results in the rat olfactory bulb confirm the work of Stewart et al. (J. Comp. Neurol. 185: 715,'79) using the Sokoloff method, and extend the analysis to the cellular level. The results in the olfactory epithelium show that the 2DG technique can be successfully applied to this peripheral tissue, and provide a basis for further analysis of olfactory receptor mechanisms. The data obtained at these two levels in the olfactory pathway provide insight into the neuronal correlates of olfactory processing.

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217.3 IMMUNOFLUORESCENT LOCALIZATION OF PUTATIVE NEUROTRANSMITTERS IN OLFACTORY PATHWAYS. R. Jacobson*, R. Elde. University of Minnesota Medical School, Minneapolis, MN 55455.

The olfactory system has well defined connections with limbic, diencephalic and brainstem structures which are enriched with currently known putative neurotransmitters. This study extends the current knowledge of neurotransmitter localization at the level of the olfactory bulb (OB), olfactory nerve (ON), and centrifugal pathways.

Male rats (150-200 grams) were perfused with ice cold buffered 4% paraformaldehyde 2 days after intraventricular colchicine (50 µg), or 1 week after right OB transection at the junction with olfactory nuclei, or without manipulation. 10 µm sagittal cryostat sections were thaw mounted onto gel coated slides and processed with antisera to cholecystokinin octapeptide (CCK), somatostatin (SOM), substance P (SP)**, alpha melanocyte-stimulating hormone (α-MSH), serotonin (5-HT), and insulin using the method of indirect immunfluorescence.

Although the dipeptide carnosine is the only neurotransmitter candidate presently identified in ON, SP and CCK immunoreactive fibers were clearly seen in the ON and the glomerular layer of OB. Numerous α-MSH, SOM and 5-HT fibers were distributed throughout the OB and olfactory nuclei. Clusters of 5-HT fibers occurred in glomeruli. After OB transection, 5-HT, α-MSH, SOM and CCK immunoreactivity increased caudal to the cut with diminished staining rostrally, except for SOM which increased on both sides of the transection. CCK and SOM cell bodies in ipsilateral olfactory nuclei and SOM cell bodies in olfactory bulbs were revealed with either OB transection or colchicine treatment. Although OB contains among the highest concentrations of insulin and insulin receptors in brain, no specific insulin immunoreactivity was detected.

These data demonstrated ascending CCK and SOM olfactory pathways originating from olfactory nuclei. 5-HT afferents have been shown to be from raphe nuclei. We speculate that α-MSH may originate from recently described α-MSH cell bodies in lateral hypothalamus which is known to project to contralateral OB. The origin of CCK and SP in ON and the histochemical localization of insulin within OB are currently unknown.

**Kindly provided by Raymond Ho, Ohio State University.

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217.4 PICROTOXIN SLOWS OLFACTORY BULB INDUCED WAVES. Wm. B. Forbes. Worcester Foundation for Experimental Biol., Shrewsbury, MA 01545.

The effects of a convulsive dose of picrotoxin on oscillatory slow-wave activity recorded in the main olfactory bulb (MOB) in five adult male Sprague-Dawley rats was studied. Side-by-side bipolar stainless steel electrodes constructed of 250 µ wire having a vertical tip separation of 1.5 mm were chronically implanted across the mitral body layer in the ventral portion of the MOB unilaterally. Sniffing was monitored via a thermocouple held in a chronically implanted nasal cannula. At least ten days following surgery sniffing and MOB E_G were monitored prior to and following a 2 mg/kg i.p. dose of picrotoxin. This dose induced at least one motor convulsion with a latency of 10 - 15 min in all animals studied. EEG changes were quantified using power spectral analysis and digital filtering. Spectra were computed having a Niquist frequency of 256 Hz with 0.5 Hz resolution.

A frequency component centered at 60 - 90 Hz was reliably observed in all animals studied. Comparison of filtered MOB activity with sniffing verified that this component comprised inhalation-induced spindles; viz. induced waves. Following picrotoxin administration, a time-dependent slowing of the induced wave component was observed such that the peak frequency of this component was reduced by 10 - 20% immediately prior to the motor convulsion. For several minutes following the termination of the convulsion, the absolute amplitude of the induced waves was dramatically enhanced. This enhancement was also seen following successive convulsions, when they occurred.

These results are interpretted as reflective of a depressive effect of picrotoxin on inhibitory transmission at the granule-to-mitral cell synapse. The data are consistent with the hypothesis that lowering the gain of the negative feedback loop between populations of MOB output neurons and presumed GABAergic granule cells decreases output neuron damping and thereby lowers the frequency of induced wave oscillations.

217.5 ORTHODROMIC RESPONSE PROPERTIES OF OLFACTORY BULB OUTPUT NEURONS IN THE RAT CORRELATE WITH AXONAL PROJECTION PATTERNS.
Stephen P. Schneider* and John W. Scott. Department of Anatomy, Emory University School of Medicine, Atlanta, GA 30322.

Anatomical experiments and our recent electrophysiological studies have shown that olfactory bulb (OB) output neurons, the mitral and tufted cells, can be distinguished by their efferent projections. We now report on an investigation of differences in orthodromic response properties between these cell types.

In this study, three populations of OB units were classified on the basis of antidromic activation: OT units were activated from the olfactory tubercle (OT) but not from the posterior piriform cortex (pPC); pPC-OT units were activated from both regions; and LOT units were activated only by stimulation of the lateral olfactory tract (LOT) at the level of the olfactory peduncle. The orthodromic responses of antidromically identified units were studied during stimulation of the olfactory nerve layer (ONL) at different sites using a linear array of seven bipolar electrodes.

Stimulation of the olfactory afferents excited 95% of the LOT and 96% of the OT units but only 59% of the pPC-OT units. The pPC-OT units also tended to have a higher threshold for excitation than the other unit types. The pPC-OT units responded to orthodromic stimulation with single spikes, even at suprathreshold stimulus currents; whereas 80% of LOT and 38% of OT units showed multiple spikes at suprathreshold currents. The LOT and OT units responded with significantly shorter latencies to orthodromic stimuli than did the pPC-OT units. Finally, LOT and OT units were excited by stimulation of a significantly greater number of ONL sites than pPC-OT units.

These data suggest that OB neurons projecting to anterior olfactory regions, such as the anterior olfactory nuclei, anterior piriform cortex, and the OT, are more responsive and may be excited from a larger region of the olfactory mucosa than those cells projecting to the pPC. Based on anatomical studies and our previous electrophysiological results, we conclude that a predominance of the cells belonging to the first category are tufted cells and those of the second are mostly mitral cells. Our data are consistent with the hypothesis that mitral and tufted cells function differently in the processing of primary olfactory information and that these differences correlate with axonal projection patterns.

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217.6 ELECTROPHYSIOLOGICAL EVIDENCE FOR VAGAL INFLUENCES UPON THE NEURONAL ACTIVITY OF THE OLFACTORY BULB.
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Previous experiments performed in our laboratory suggest that the sympathetic part of the autonomic nervous system exerts a modulatory influence upon the olfactory structures. This study was undertaken to test whether or not the parasympathetic also contributes to modulate the olfactory system. The results were obtained from rats (280-320 g body weight). The rats were anesthetized with chloral hydrate (460 mg/kg). The neuronal activity of the ipsilateral or contralateral olfactory bulb was recorded, using a micropipette with a 3 μ diameter tip, during vagal stimulation was verified by its bradycardia effects and heart rate was continuously monitored. Data were collected from 11 cells in 8 animals, 36.3% of the population (4 neurones) showed a significant increase in their frequency and the other 63.3% (7 neurones) showed a decrease in the discharge frequency. We observed that the neuronal activity was recorded from a narrow band of 1500 μ wide. These results suggest that the autonomic nervous system exerts a very important influence upon the olfactory system.

217.7 ULTRASTRUCTURAL CHARACTERIZATION OF SYNAPTIC TERMINALS OF OLFACTORY BULB AFFERENT FIBERS AND ASSOCIATION FIBERS IN THE PIRIFORM CORTEX OF THE OPOSSUM.
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As part of an ongoing analysis of the neuronal circuitry in the olfactory cortex, electron microscopic autoradiography has been used to characterize synaptic terminals of olfactory bulb (OB) afferent fibers in layer Ia and terminals of intracortical association fibers in layers Ib, II, and III of the opossum piriform cortex. Injections of tritiated amino acids for study of association terminals were made in the rostral part of the piriform cortex and labeled terminals studied in the caudal part (>5mm from injection sites). Injections in the olfactory bulb were carefully placed to avoid involvement of the anterior olfactory nucleus.

The most distinctive features of OB terminals are the presence of evenly-spaced round vesicles, an overall light appearance, asymmetrical synaptic contacts that are convex outward, and an astrocytic glial wrap. No labeled terminals with these features have been observed deeper in the cortex than approximately 50 μm below the center of the layer Ia-Ib boundary. OB terminals usually contact 1-3 dendritic spines, although occasional contacts onto dendritic shafts are observed. These results indicate that terminals of OB fibers in the opossum are very similar to those in the rat (Westrum, Z. Zellforsch., 98:157, 1969) and mouse (Caviness, et al., Br. Res. 134:13, 1977).

The most distinctive features of association fiber terminals are the presence of tightly-packed round vesicles, an overall dark appearance, and straight, asymmetrical synaptic contacts with dark post-synaptic densities. Contacts onto single dendritic spines are most numerous, although occasional contacts onto more than one spine or onto dendritic shafts are present. Small vesicles are often found in dendritic spines contacted by both association and OB terminals but these do not appear to be synaptic vesicles. Terminals with pleomorphic vesicles and symmetrical contacts that are found at all depths in the cortex do not appear to be labeled at greater than chance levels from either anterior piriform cortex or olfactory bulb injections.

Preliminary measurements of vesicle size distributions in OB and association terminals with a computer-coupled graphics tablet indicate that they are very similar and cannot be used to distinguish these two terminal populations. However, due to their very different ultrastructural features, very few OB terminals could be mistaken for association terminals and vice versa, even though considerable morphological variation is present in both populations.

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217.8 AN EXAMINATION OF COUPLING BETWEEN EEGS OF TWO OLFACTORY STRUCTURES.
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This study deals with the relation between the olfactory bulb (OB) and olfactory cortex (OC), comparing the EEGs recorded from multiple sites in each structure. The relation between small bulbar and cortical areas is examined by measuring the shared frequency and phase between their EEGs. The question is asked whether all pairs of bulbar and cortical local areas are actively coordinated (coupled), or whether some pairs show a greater degree of coupling than others. The test of coupling is whether the relation of bulbo-cortical phase to shared frequency is consistent with bulbar driving of OC.

Neural activity in the OB is manifested as a 35 to 85 c/sec sinusoidal burst in the EEG. Bulbar activity is relayed to the OC by pulse activity in mitral cells, whose axons leave the OB to form the lateral olfactory tract (LOT) which terminates in the OC. Pulse activity on axons in the LOT is synchronous with the bulbar EEG. The density of pulses in the LOT provides oscillatory input to cortical pyramidal cells at the same frequency as the bulbar burst. During states of behavioral arousal, similar bursts occur in OC. Anatomically, the axonal projection from OB to OC is diffuse; i.e. small bulbar areas project to almost the entire OC, and small cortical areas receive fibers from all parts of the OB.

Tungsten microelectrodes were chronically implanted at 9 sites in the OB of 10 rabbits. A 10 by 6 rectangular electrode array (300 micron stainless steel wires embedded in dental acrylic at 0.8 mm spacing) was placed epidurally on the surface of the OC. EEGs from all bulbar and cortical electrode sites were simultaneously recorded onto magnetic tape using a 64 channel A-D converter. For selected bulbar and cortical sites, 50 burst pairs were analyzed for shared frequency and phase.

Results demonstrated that for some pairs, the relation between phase and frequency was well-behaved and consistent with bulbar driving of OC. For other pairs, phase was distributed over the whole range of 0 to 360 degrees. These results were interpreted to mean that, although the anatomical projection from OB to OC is diffuse, coupling occurs between the activities of only certain select regions. A possible explanation is that in any animal, only a fraction of the possible synaptic pathways between OB and OC are functionally operative. Supported by MH06686.

217.9 CONNECTIONS OF THE MAIN AND ACCESSORY OLFACTORY BULBS IN THE TOAD, BUFO MARINUS. S. Jiménez-Dietsch and E. Kicliter. Lab. of Neurobiology and Dept. of Anatomy, Univ. of Puerto Rico Sch. of Med., San Juan, P.R. 00936.

In anuran (tailless) amphibians olfactory projections have been studied only in the frogs Rana pipiens and Rana catesbeiana. In order to ascertain whether the results found in these species are representative of anurans we have studied the projections of the main and accessory olfactory bulbs of the toad, Bufo marinus, with an anterograde degeneration technique. Small electrolytic lesions were placed in either the main or accessory olfactory bulbs of 24 B. marinus. After survival periods of 3-10 days the animals were re-anesthetized and perfused intracardially with 10% formalin. Sections of the brains were stained with the Ebbesson-Heimer stain and the locations of anterograde degeneration were determined. After main olfactory bulb (MOB) lesions degeneration was traced caudally through the lateral olfactory tract along the periphery of the lateral pallium to the caudal pole of the hemisphere. Degeneration after MOB lesions was also traced in the medial olfactory tract which distributes to rostral portions of the medial and lateral septal nuclei, rostral medial pallium, and to the nucleus of the diagonal band. After accessory olfactory bulb lesions degeneration was traced through the postolfactory eminence, the striatum, nucleus accumbens and nucleus of the diagonal band to pars lateralis of the amygdala. These results are in general agreement with the results of previous studies of Rana except that previous investigators have not reported a projection of the accessory olfactory bulbs to the striatum. This result suggests that the striatum and pars lateralis of the amygdala may be part of the same cell group. (Supported by PHS grant NS-07464).

217.10 THE EFFECT OF INTRANASAL ZINC SULFATE TREATMENT ON SUCKLING BEHAVIOR AND ODOR-INDUCED ACTIVITY IN THE NEONATAL RAT OLFACTORY BULB. William B. Stewart, Charles A. Greer and Martin H. Teicher, Sec. Neuroanatomy, Neurosurgery and Gross Anatomy, Yale Univ. Sch. Med., New Haven, CT 06510.

We have correlated the behavioral effects of zinc sulfate ($ZnSO_4$) treatment with odor-induced 2-deoxyglucose (2DG) uptake in the developing rat olfactory bulb.

Eight - nine day old rat pups received intranasal irrigation with either 0.9% NaCl, 1% $ZnSO_4$, or 5% $ZnSO_4$. One and 5 days following treatment the rats were tested for suckling behavior and for vibrational responses to the anesthetized dam and a heating pad. Six days following treatment the pups were injected with 200μCi/kg of ^{14}C-2DG and placed, in groups of 3, in an odor chamber where they were exposed to a 10^{-1} flow dilution of amyl acetate. The brains were then processed for autoradiography.

At 1 day following treatment none of the $ZnSO_4$ pups attached to the dam's nipple, while 89% of the saline pups attached within 5 min. At 5 days none of the 5% pups attached while 67% and 100% of the 1% $ZnSO_4$ and saline pups, respectively, attached. On the vibrational tests of maternally directed behavior, similar results were obtained. At one day following treatment neither of the $ZnSO_4$ groups could discriminate between the dam's ventrum, dorsum, or a heating pad. At 5 days post treatment, the 1% group had recovered to control levels while the 5% group was still unable to differentiate. On day 13 postnatal, the saline control pups had gained 5.5g, the 1% $ZnSO_4$ pups had gained 3g and the 5% pups had lost 2.7g.

The 2DG results may be summarized as follows. The saline pups had large regions of increased focal 2DG activity in the glomerular layer of the olfactory bulb. In marked contrast, the 5% $ZnSO_4$ pups did not have distinct focal 2DG activity. The 1% pups had 2DG foci in the glomerular layer but to a lesser extent than the saline control pups. The olfactory bulbs of both the 1% and 5% pups were smaller than the saline pups. This was particularly evident in the nerve and glomerular layers.

These results are consistent with those of Singh et. al. (Physiol. & Behav. 17: 373, 1976) who demonstrated deficits in suckling behavior following 5% $ZnSO_4$. Our results extend these findings demonstrating that 1% treatments produce only temporary deficits. Also, the 2DG results on the functional organization within the olfactory bulb correlate well with the behavioral findings. Overall, the combined use of behavioral and 2DG methods provides a powerful strategy for assessing functional capacity and recovery of function in sensory systems.

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217.11 CONTACT IS IMPORTANT FOR VOMERONASAL-MEDIATED MALE ATTRACTION TO FEMALE HAMSTER VAGINAL SECRETION. J.B. Powers, M. Bergondy* and L. Bopeley*. Dept. Psychology, Vanderbilt Univ., Nashville, TN 37240.

The olfactory (OLF) and vomeronasal (VN) systems are critical for mediating the male hamster's response to chemosensory cues which stimulate copulation. These 2 systems may play different, but complementary roles in producing these behavioral effects. Specifically, the OLF system may respond to distal (volatile) cues, whereas the VN system may require proximal (non-volatile) cues for its activation.

To test the hypothesis that the VN system is not adequately stimulated by volatile odorants, 16 male hamsters were pretested to assess their relative attraction to 1 of 2 anesthetized conspecific stimulus Ss. These were an OVX female treated with E and P, and a gonadally intact male. Tests were conducted in a box with copper screen flooring below which (5cm) could be inserted 2 drawers containing the stimulus Ss. Tests lasted 2 min and the time each test S spent sniffing the floor over either of the 2 stimulus Ss was recorded.

Preference for the stimulus female was robust as evidenced by the different amounts of time spent investigating over the 2 Ss (40 vs 3.6 secs). Test males were then given intranasal zinc sulfate (ZS) to produce an OLF deafferentation or were given saline (SAL) as a control procedure. In behavior tests 2 days later SAL Ss maintained their preference for females (31 vs 2.9 secs) whereas the ZS Ss did not (3.6 vs 2.6 secs). Thus under these conditions males with only the VN system functional (ZS) did not persist in their response to chemosensory cues.

To evaluate these results further, we used 2 anesthetized, castrate hamsters as stimulus Ss, 1 of which was swabbed with female hamster vaginal secretion (FHVS). Two tests were given over successive weeks with the experimental condition of contact vs no contact with the stimulus Ss being counterbalanced among the test males which received either ZS or SAL 2 days before each of the 2 weekly tests. Among the SAL Ss, preference for the FHVS-swabbed castrate was evident independent of whether contact with the stimulus Ss was or was not permitted. In contrast, the males with only the VN system functional (ZS) exhibited a comparable preference only when contact was permitted.

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217.12 OLFACTORY ADAPTATION WITHOUT DISCRIMINATION. Thomas Hellman Morton*, Howard Eichenbaum, and Suzanne Corkin (SPON: Stephan L. Chorover). Dept. Chem., Univ. Calif., Riverside, CA 92506; Dept. Biol., Wellesley Coll., Wellesley, MA 02181; and Dept. Psychol., Mass. Inst. Tech., Cambridge, MA 02139.

The patient H.M. has experienced global anterograde amnesia subsequent to a bilateral resection in the medial temporal zone 28 years ago; his deficits and spared abilities have been well-studied. Eichenbaum, Potter, and Corkin have shown that H.M. has normal olfactory sensitivity but is unable to discriminate among odors. We present here further documentation of H.M.'s inability to distinguish odors from one another, as well as evidence that he exhibited normal olfactory adaptation with the odorants n-butanol (BuOH) and β-phenethyl alcohol (φEtOH).

In signal-detection tasks, H.M. scored significantly above chance in distinguishing 10 mM aqueous BuOH from blank and 3.3 mM φEtOH from blank, when the odorants were presented in equilibrium sniffers; his values of d' lay in the range from 1 to 2, within normal limits. However, he could not discriminate the two odors in a series of triangle tests. In this procedure, a weak sample was matched to either a weak or a strong choice, one of which was the same odor as the sample. Given a weak φEtOH sample, he could match it perfectly to the same stimulus paired with strong BuOH, but he scored at chance in matching to strong φEtOH paired with weak BuOH. He scored at chance in any task that required matching to a weak BuOH sample.

Preadaptation to BuOH significantly impaired H.M.'s detection of BuOH versus blank, but did not affect his detection of φEtOH; similarly, preadaptation to φEtOH impaired his detection of φEtOH versus blank, but did not affect his detection of BuOH. We conclude therefore that:

(1) H.M. adapts to odor quality and not to odor intensity alone.
(2) Odor differentiation is not requisite for olfactory adaptation.
(3) A central olfactory discrimination mechanism is not necessary for adaptation.

This work was supported by grants NS 17109, MH 24433, and RR 00088.

217.13 TASTE RESPONSES OF THE GLOSSOPHARYNGEAL AND VAGAL NERVES IN THE CATFISH. Jagmeet S. Kanwal* and John Caprio. (SPON: G.M. Strain). Dept. of Zoology and Physiology, Louisiana State University, Baton Rouge, LA 70803.

Electrophysiological investigation has revealed that the glossopharyngeal (IXth) and vagal (Xth) nerves of the channel catfish, Ictalurus punctatus, contain taste fibers that respond to amino acids and classical taste stimuli. The IXth nerve innervates taste buds on the ventral surface of the oral cavity and first gill arch. Branches of the vagus innervate the 2nd, 3rd and 4th gill arches and the pharynx. Electrophysiological reports of IXth and Xth nerve taste responses in fish are few in number, and recent studies involving amino acid taste sensitivity have involved primarily facial (VII) nerve recordings. The objectives of the present study were to determine the response properties and chemospecificities of the IXth and Xth nerves and to compare these with similar data obtained from the facial taste system in the same species. Taste buds innervated by facial nerves are highly sensitive to amino acids, especially L-alanine and L-arginine.

In vivo glossopharyngeal and vagal nerve recordings were obtained with a monopolar Pt.-Ir. hook electrode from the cut peripheral end of the nerves in the region of the branchial musculature adjacent to the cranium. The nerves responded phasically to mechanical stimulation of the oral and pharyngeal epithelium; tactile stimuli consisted of pulsing water flow and gentle stroking of the epithelium. Additionally, spontaneous, tonically active units with response frequencies dependent upon the position of the gill arches were observed in the majority of nerve bundles teased from the IXth and Xth nerves. Since this activity obscurred the responses to chemical stimuli, taste recordings were obtained from nerve bundles showing little or no tonic activity. Chemical stimuli were injected into a constant flow of water bathing the oral epithelium. Integrated multiunit activity showed L-alanine and L-arginine to be the most stimulatory amino acids tested with thresholds below 10^{-6}M; thresholds for other amino acids tested were higher ($> 10^{-5}$M). Also, L-proline was highly stimulatory at concentrations $\geq$ 1mM. Reciprocal cross-adaptation studies indicate the presence of relatively independent receptor mechanisms for L-arginine, L-alanine and L-proline. Individually identified units responded best to either L-arginine or L-alanine. The integrated phasic taste responses to amino acids were positively accelerating functions of log molar concentration of the stimulus. Thus with respect to amino acids, the facial, glossopharyngeal and vagal taste systems have strikingly similar chemical response profiles.

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217.14 INHIBITION OF IXth NERVE TASTE RESPONSES INVOLVES CHOLINERGIC EFFERENT FIBERS. E. Outwater* and B. Oakley, Dept. of Physiol. Univ. Calif. Sch. Med., San Francisco, CA 94143 and Dept. of Zoology, Neuroscience Lab. Bldg., Univ. of Mich. Ann Arbor, MI 48109.

Summated taste responses to stimulation of the tongue were recorded from the IXth nerve of gerbils (Meriones unguiculatus) anesthetized with ketamine HCl (330 mg/kg bw im). Acute administration of d-Tubocurarine produced a dose-dependent increase in the magnitude of the summated taste responses to 0.3M NH_4Cl (e.g., +25% with 20 mg/kg bw ip, n=9). Within 5 min d-Tubocurarine (dTC) produced a statistically significant enhancement of taste responses which lasted for more than one hour. Treatment with dTC also caused a significant increase in the time from response onset to peak (from 2.6 to 6.6 sec). Intraperitoneal injection of an equal volume of 0.9% NaCl had no effect. The use of other nicotinic anticholinergic agents [alpha bungarotoxin (0.6 mg/kg), succinylcholine (14 mg/kg), and gallamine triethiodide (200 mg/kg)] also enhanced taste responses recorded from the intact IXth nerve. Enhancement of taste responses was prevented by transection of the IXth nerve before injection of dTC. This result argues against systemic pharmacological reactions or general vascular reactions which should have been evident even after IXth nerve transection. It is likely that dTC acted peripherally to reduce efferent inhibition by the IXth nerve, since impulse activity in the proximal stump of the transected IXth nerve was unaffected by dTC.

To test the suggestion that the IXth nerve contains cholinergic efferent fibers which innervate the vallate papilla, histochemical staining for acetylcholinesterase (AChE) was carried out. The vallate papilla contained specific cholinesterase that was eliminated by bilateral section of the IXth nerve. Butyrylcholinesterase (BChE) was only weakly present. The AChE reaction was unaffected by a specific inhibitor of BChE (tetraisopropylpyrophosphoramide). Our working hypothesis is that the IXth nerve contains cholinergic efferents that can tonically inhibit taste fiber responses.

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217.15 IONIC EVENTS OF ELECTRIC TASTE. M. Scott Herness*.(SPON: L. M. Beidler). Dept. of Biology, Florida State University, Tallahassee, FLA 32304

The phenomenon of electric taste has been known for almost two hundred years since Volta's early discovery of it during the course of his investigations on electricity. Despite its long and rich history, the mechanism of electric taste has yet to be elucitdated. There are, however, two main hypotheses. The first maintains that electrical taste is the result of a direct action of the current on either the nerve fibers and/or the taste cells. The second, the electrolytic-chemical hypothesis, describes electrical taste as the result of a chemical stimulation of taste receptors by ions (which may include electrolytic products of saliva) iontophoresed to the taste cells.

In the present set of experiments, anodal current (10 - 50 μamps) was delivered through various subthreshold salt solutions (0.001 M) and neural responses recorded from the chorda tympani nerve of the rat. Current passed through any solution may be divided into two fractions, that carried by the cation and that carried by the anion. Salts with a larger less mobile anion will transfer more current through the cationic current than salts with a smaller more mobile anion. A larger cationic current should result in a greater accumulation of cations at the tongue surface and thus elicit a larger neural response. Such responses were measured for sodium and potassium salts with the following series obtained:

Butyrate > Propionate > Acetate > Formate > Chloride.

Moreover, sodium responses were much larger than potassium responses. These observations were interpreted in support of the electrolytic-chemical hypothesis since those salts with a greater cation accumulation elicited larger neural responses and the same transducing ability of cations is reflected in both chemical and electrical stimulation.

217.16 MODELING THE CONVERGENCE OF GUSTATORY NEURONS. Stephen L. Bieber* and David V. Smith. Depts. of Statistics and Psychology, Univ. of Wyoming, Laramie, WY 82071.

Taste-responsive fibers in the hamster chorda tympani (CT) nerve are relatively narrowly tuned, especially those that respond best to sucrose. The breadth of responsiveness of hamster taste neurons to anterior tongue stimulation increases systematically at the levels of the nucleus tractus solitarius (NTS) and parabrachial nuclei (PbN). Response profiles within a best-stimulus class of neurons are relatively homogeneous at each of these levels. Thus, it should be possible to model the nature of the convergence process leading to the increase in breadth of responsiveness at each level.

Responses (impulses/5 sec) to each of the four basic taste stimuli (0.1 M sucrose, 0.03 M NaCl, 0.003 M HCl and 0.001 M quinine hydrochloride) in 78 CT, 57 NTS and 53 PbN neurons were used to classify these cells into S-, N-, H- and Q-best categories, depending upon which of the four stimuli elicited the maximum response in each neuron. The values of the average response profiles of each best-stimulus class of fibers in the CT were used as the independent variables in a multiple regression analysis to predict the values of the average response profiles of each best-stimulus category of cells in the NTS. Similarly, the values of the NTS profiles were used to predict the profiles of cells in each best-stimulus class in the PbN. This approach estimates the convergence from one synaptic level to the next (i.e., it calculates the proportional contribution of each best-stimulus class). For example, reproduction of the average response profile of S-best neurons in the NTS requires a convergence ratio of approximately three S-best CT fibers to each H-best fiber, with the contribution of N-best CT fibers being essentially zero. In this regression, the predicted average response profile of NTS S-best neurons is: 86.98 impulses/5 sec to sucrose, 31.87 to NaCl, 28.79 to HCl and 10.31 to quinine hydrochloride. The actual response profile of this class of neurons in the NTS was: 87.50 impulses/5 sec to sucrose, 29.20 to NaCl, 30.15 to HCl and 10.35 to quinine.

The response profiles of all best-stimulus classes of cells at both the NTS and PbN could be predicted quite well from various combinations of converging input from the preceding level. This analysis suggests a way in which the increased breadth of responsiveness of brainstem taste neurons could occur from converging input from fungiform papillae on the anterior portion of the tongue.

Supported in part by NINCDS Grant NS10211 and Research Career Development Award NS00168 to D.V.S. Chorda tympani (CT) responses were provided by Dr. Marion Frank.

217.17 CONVERGING TASTE RECEPTOR INPUTS TO THE HAMSTER SOLITARY NUCLEUS. Robert D. Sweazey, J. David Dickman* and David V. Smith. Dept. Psychology, Univ. Wyoming, Laramie, WY 82071.

Taste receptors are distributed over various parts of the tongue and intraoral cavity. Only about 15% of the rat's taste buds are located in fungiform papillae on the anterior portion of the tongue (Miller, 1977). Most electrophysiological studies of taste, however, have restricted stimulation to the anterior tongue. Processing of taste information through the hamster brainstem is understood only with respect to this anterior receptor area. In the rat, responses can be elicited in the nucleus tractus solitarius (NTS) by both anterior tongue and posterior oral stimulation, with considerable overlap of multi-unit responsiveness to both fields (Halpern & Nelson, 1965). In the rat pons, many of the same neurons can be driven by both anterior and posterior stimulation (Norgren & Pfaffmann, 1975).

The present investigation examines the overlap in sensitivities to anterior tongue and posterior oral gustatory stimulation in the hamster NTS. Stimuli (0.1 M sucrose, 0.03 M NaCl, 0.003 M HCl and 0.001 M QHCl) were delivered to the anterior area through a glass chamber and to the posterior area through an inserted tube, both at a rate of 3.6 ml/sec.

Multi-unit responses could be recorded from the anterior lateral NTS over a span of about 400-500 μm in the rostral-caudal and about 200-250 μm in the medial-lateral dimension. Although there was some tendency for the most rostral penetrations to respond primarily to stimulation of the anterior portion of the tongue, the majority of sites responded to both anterior tongue and posterior oral stimulation. This overlap suggests, but does not demonstrate, the convergence of single peripheral fibers onto individual medullary neurons. However, of the several neurons isolated thus far, most were driven by both anterior and posterior stimulation. Often the pattern of responsiveness of a neuron was different to stimulation of these two receptor fields. For example, one neuron responded well to sucrose delivered to either the anterior or posterior fields, but to NaCl following only posterior stimulation. Another responded exclusively to HCl on the anterior tongue and to quinine delivered to the posterior oral cavity. Additional cells possessed similar sensitivities to both anterior and posterior stimulation.

Since the response profile of an NTS neuron may differ, depending upon the receptors stimulated, characterization of the sensitivities of these cells may be more complex than when considering anterior tongue input alone. Complete understanding of taste information processing by brainstem neurons depends upon input from divergent receptor areas.

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217.18 ELECTROPHYSIOLOGICAL EVIDENCE FOR THE TOPOGRAPHICAL ARRANGEMENT OF TASTE AND TACTILE NEURONS IN THE FACIAL LOBE OF THE CHANNEL CATFISH. T. Marui* and J. Caprio. Department of Zoology and Physiology, Louisiana State University, Baton Rouge, LA 70803.

In some teleosts, Cyprinidae and Ictaluridae, large numbers of taste buds are distributed across the whole body surface. These taste buds are innervated by the facial nerve (VII) which projects to the hindbrain. Behavioral ablation studies have confirmed the importance of the medullary taste centers in the catfish (Atema, 1971). In the present study, responses in the facial lobe of the channel catfish, Ictalurus punctatus, to mechanical and chemical stimulation of the external skin were investigated. Responses were recorded with glass microelectrodes filled with 3M KCl solution (tip diameter, 2-3 μm; impedence, 2-7MΩ). Finger (1976) reported that facial nerve fibers in bullhead catfish (I. nebulosus and I. natalis)which innervate the trunk, nasal barbel and maxillo-mandibular barbets terminate in the lateral, intermediate and medial lobules of the facial lobe, respectively. In the present study, an electrophysiological analysis of neurons in each of the lobules of the channel catfish was performed. Most neurons in the channel catfish facial lobe responded only to mechanical stimulation. Neurons responsive to amino acids which are known to be highly stimulatory to catfish from peripheral recordings (Caprio, 1975, 1978) were located in the tactile sensitive area. Taste neurons were located generally in the superficial layer (between 0.2-1.0 mm below the surface) of the lobe. In the deeper layer (1.0-2.5 mm in depth) of the anteromedial part of the lobe, only tactile responses were obtained. These deeper neurons have large receptive fields (100mm^2-the whole body surface) and some of them showed lateral inhibition. Secondary taste neurons responded best to L-alanine or L-arginine HCl which is similar to the recordings obtained from peripheral neurons in the same species.

The antero-posterior axis of the face including the barbels is represented in the medial and intermediate lobules of the facial lobe in a postero-anterior axis; in addition, regions associated with maxillary and mandibular barbels are greatly enlarged. The antero-posterior axis of the trunk is represented in the lateral lobule of the facial lobe in an antero-posterior axis descending from dorsal to ventral position. This somatotopic arrangement in the facial lobe of the Ictaluridae is markedly different from that found in the Cyprinidae (Peterson, 1972; Marui, 1977). This discrepancy is probably due to a real difference between these two families of fishes.

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217.19 LAMINAR AND COLUMNAR ORGANIZATION OF THE VAGAL LOBE IN GOLDFISH: POSSIBLE NEURAL SUBSTRATE FOR SORTING FOOD FROM GRAVEL. T.E. FINGER. Dept. of Anatomy, Univ. Colo. Med. Sch., Denver, CO 80262

Goldfish possess a highly muscular palatal organ located in the roof of their mouth. Scanning electron microscopy of this organ reveals numerous taste buds as well as microscopic "teeth". Sensory and motor innervation to the palatal organ is via palatine branches of the vagus nerve. The central processes of this nerve enter the vagal lobe, a large, laminated structure protruding from the dorsolateral surface of the mid-medulla. The sensory fibers terminate in a distinct laminar pattern within the superficial layers of the lobe. Motor fibers are believed to originate from α-motor neurons lying in the deep, motor layers of the lobe (Herrick, 1905; McGlone, 1977). McGlone's physiological studies suggest that some degree of somatotopy (palatotopy) exists in the organization of the sensory input to the vagal lobe.

In order to test this, 1-5 μl of 30-50% horseradish peroxidase solution (HRP; Sigma Type VI) was injected just below the surface of different areas of the palatal organ in 15 goldfish. Subsequent histological analysis of the palatal organ demonstrated that the enzyme was restricted to relatively small areas of the organ. Retrograde and transganglionic anterograde transport of the HRP labeled only small areas within the vagal lobe. These studies indicate both a motor and sensory mapping exists between the vagal lobe and palatal organ such that the anterior end of the palatal organ is represented in the anterior end of the vagal lobe and vice versa.

In 8 other goldfish, small amounts of HRP were placed in either the sensory (superficial) or motor (deep) layers of the vagal lobe. Sensory layer injections revealed anterogradely labeled fibers extending radially inward from the point of injection to end in the motor layers. Similarly, the motor layer injections of HRP retrogradely labels a column of cells in the sensory layer extending radially outward from the point of injection. Thus the intrinsic connections of the vagal lobe consist of a topographically organized projection from cells in the sensory layer to the underlying motor layers.

The palatotopically arranged connection from taste receptors in the palatal organ, to sensory part of the vagal lobe, to motor layers of the vagal lobe, to intrinsic musculature of the palatal organ is suggestive of an oral gustatory reflex mechanism. The goldfish may use this hypothesized gustatory mechanism to sort food particles from a mouthful of gravel or other bottom substrate by local contraction of muscles in that part of the palatal organ in contact with a "tasty" morsel.

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217.20 PREFERENCE BEHAVIOR OF DOGS FOR SUGARS IN A COMPLEX MIXTURE Dawn Brown* (SPON: D.M.Easton) Department of Biological Science, Florida State University, Tallahassee, Florida 32306.

Previously reported research has shown that beagles prefer sugar solutions to plain water (Brown and Ewen, AChemS, 1981). In the present experiment preference behavior of the same dogs was assessed for sugars added to a soft-moist dog food. Dogs were adults (3-5 years) who had a great deal of experience with a variety of sweetners.

The dogs were housed in groups of three to five in indoor-outdoor runs. They had access to water ad lib and were maintained on dry food. At the beginning of each experimental session they had been food deprived for 23 hours. Brief, 2-pan tests were conducted in a rotating pan apparatus.

Tests were conducted using a commercially prepared soft-moist dog food which contained no sugar or other preservative. To this product 3% by weight corn syrup was added. In preference tests this mixture was paired with the same mixture to which a sugar had been added. Sugar was added on a percent by weight basis and concentration curves plotted for each of the four sugars: Maltose, xylose, glucose, and fructose.

At the lowest concentrations, maltose and xylose flavored food was not prefered to the plain food. All othertests, however, a strong preference was shown for the food containing sugar.

In order to compare these data with that obtained in the previous workconcentrations were converted using the formula:

$$\frac{\text{Grams sugar per kg mixture}}{\text{molecular wt. of sugar}}$$

When these data are plotted with the concentration curves obtained for solutions there is no consistent relationship. It is, therefore, not possible to generalize between preferences for a simple sugar solution and a more complex mixture such as dog food.

217.21 EFFECTS OF TEMPERATURE ON THE TASTES OF NACl, CITRIC ACID, QUININE, SUCROSE AND SACCHARIN. L.M.Bartoshuk and J.E.Hooper*. J.B.Pierce Fndn. Lab. and Yale Univ., New Haven, CT 06519.

Early studies of the effects of temperature on the sense of taste have two limitations: taste function was assessed by threshold measurements, and the tongue was often adapted to the temperature of the stimulus to be tasted to remove "troublesome" thermal sensations. In the real world, individuals typically taste substances at concentrations far above threshold and the tongue is at body temperature so that taste substances that are warmer or cooler than the tongue produce taste and thermal sensations simultaneously.

In the present study, we used the psychophysical procedure of magnitude estimation to assess taste function over the whole dynamic range from threshold to very strong taste sensations. In order to maintain a body temperature baseline, we required subjects to rinse with water at approximately body temperature (36°C) before tasting each stimulus. Concentration series of sucrose, NaCl, citric acid, quinine hydrochloride (QHCl), and saccharin were tested at 4, 12, 20, 28, 36, and 44°C. A single session consisted of: several concentrations of one of the taste substances, each concentration presented at each of the 6 temperatures. An additional stimulus, .32 M NaCl at 36°C, was used to normalize data across sessions. An experiment on one taste substance consisted of two sessions run with each of ten subjects. For saccharin the number of subjects was doubled to include 10 tasters and 10 nontasters of phenylthiocarbamide (PTC).

The most dramatic effects of temperature occurred for sucrose (reported at the last meeting of the Society for Neuroscience). The functions showing sweetness of sucrose for different temperatures converged at about .5 M sucrose. Below that concentration, lower temperatures produced less sweetness. High concentrations of NaCl, citric acid, and QHCl, like those of sucrose, were essentially unaffected by temperature. Although there were some effects of temperature at low concentrations (e.g., 44°C QHCl tended to taste less bitter and 4°C citric acid tended to taste less sour), the effects were small compared to those observed with sucrose.

The sweetness of saccharin did not vary with temperature as that of sucrose did. This is important since the sweet theory of Shallenberger maintains that increases in temperature produce conformational changes in sucrose molecules that increase the amount of sweet stimulus present. Saccharin is not affected in this way by temperature.

217.22 PERCEPTION OF TASTE QUALITY AND INTENSITY IN SUBJECTS WITH ANOREXIA NERVOSA. P. Jirik-Babb and Jack L. Katz*. Dept. of Psychiatry, Albert Einstein Col. of Med. at Montefiore Hosp., Bronx, N.Y. 10467.

Previous reports have indicated that poor gustatory sensitivity is associated with loss of appetite and a decrease in food intake (Hambridge et al, 1972 and Henkin et al, 1971). In order to determine whether or not gustatory impairment is associated with abnormal eating behavior characteristic of persons who have anorexia nervosa, 10 subjects diagnosed as having primary anorexia nervosa (ANs), ages 19 to 28 yrs, and 7 normal control women (NCs), ages 16 to 21 yrs, were tested for perception of taste quality and intensity using the method of magnitude estimation. The 4 taste stimuli used were sucrose, hydrochloric acid (HCl), sodium chloride (NaCl) and quinine hydrochloride (QHCl). Four different log molar concentrations of sucrose (-1.5, -1.0, -0.5, 0.0), HCl (-3.0,-2.5, -2.0, -1.5), NaCl (-3.0, -2.0, -1.0, 0.0) and 3 of QHCl (-5.0, -4.0, -3.0) were employed. All solutions were made with distilled water and reagent grade chemicals except for sucrose solutions which were made with commercial grade sucrose. At the time of testing, solutions were at room temperature (~21°C). Each of the concentrations of the 4 qualitatively different solutions was presented 3 times in random order, along with about 10 blanks consisting of distilled water, thereby making a total of about 55 stimuli presentations. Each trial consisted of swishing 15ml of solution over the tongue. (Before each trial, the mouth was thoroughly rinsed with distilled water.) Magnitude standards of the following log molar concentrations were given at the beginning of the test and arbitrarily assigned a magnitude of 10: sucrose,-0.5; HCl, -2.0; NaCl, -1.0; and QHCl, -4.0. Subjects were instructed to mark both the quality and magnitude of each stimulus on a response sheet. The results indicated no significant differences between the ANs and the NCs in the ability to identify taste quality; however, there were significant differences ($p<.05$) in magnitude estimates of the 2 higher concentrations of sucrose, HCl and NaCl solutions and the QHCl solution of the highest concentration. The magnitude estimates of the ANs were consistently lower than those of the NCs. It is possible that poor gustatory sensitivity is an antecedent, concomitant, or consequence of the low food intake characteristic of anorexia nervosa.

218.1 ANATOMICAL AND OPTICAL CHARACTERISTICS OF THE SPECIALIZED PHOTORECEPTORS IN THE DORSAL RIM AREA OF THE BEE'S EYE. Ernst W. Sommer* (SPON: Karl H. Pfenninger). Department of Zoology, University of Zurich, CH-8057 Zurich (Switzerland).

It is a characteristic of the regular type of ommatidia that the rhabdomers are twisted. This structural twist causes a reduction in polarizational sensitivity (PS<2.0) in spite of the assumed high dichroic ratios of the microvilli. On the other hand, a group of ommatidia can be found at the dorsal rim of the eye in which the rhabdomers are not twisted but straight. Here a high polarizational sensitivity can be expected (Wehner et al., J. Comp. Physiol. 104:225, 1975). We have found that the dorsal rim area (DRA) consists, on the average, of 141 specialized ommatidia (or less than 3% of the about 5500 ommatidia in the compound eye). By morphological comparison with the 3 known UV-receptors (UV-R) in the regular ommatidia, we determined by serial section electronmicroscopy which photoreceptors in the specialized ommatidia are the UV-R. By comparing the diameter of the microvilli, the level of the nuclei, and other characteristics, it is postulated that there are also 3 UV-R in the specialized ommatidium: two UV-R (R1, R5) have their microvilli arranged parallel to one another but, unlike that in the regular ommatidium, the third UV-R (R9) has its microvilli oriented perpendicular to those of R1 and R5. As a further specialization in the DRA the microvillar orientation of R1 and R5 is not constant in adjacent ommatidia but changes up to a maximum of 180° in a fan-like way. The antidromically illuminated corneal pseudopupil technique was used to define the directions of view of single ommatidia and the border lines of the binocular visual field and the DRA. The greatest extension of the DRA is through $90^{\circ} \pm 2.2^{\circ}$ (n=7) within the paramedial plane of the head, lying symmetrical about the zenith, and in the plane perpendicular to this, through $22^{\circ} \pm 1.35^{\circ}$ (n=7) in the contralateral portion of the binocular visual field. Microvillar directions of R1, R5, and R9 of DRA are mapped onto the positions in the sky, defined by polar coordinates, at which the receptors are aimed. This allows us to determine the systematic variations in the retinal excitation pattern evoked by rotation of the bee around its vertical body axis during flight. Intracellular electrophysiological recordings confirm that the UV-R in the DRA exhibit high polarizational sensitivities (average PS=6.6); indeed, two populations of UV-R have been found, their directions of maximal sensitivity to the e-vector of linearly polarized light (Φmax values) differing by 90° (Labhart, J. Comp. Physiol. 141:19, 1981). Behavioral experiments show the important role of the DRA for skylight navigation (Wehner, in prep.). Supported by Swiss National Science Found. (grants #3.529-0.75 & 3.313-0.78).

218.2 THE EXACT NUMBERS OF FLY PHOTORECEPTOR SYNAPSE AND SOME FACTORS BY WHICH THESE ARE REGULATED. D. Nicol & I.A. Meinertzhagen, Life Sciences Centre, Dalhousie University, Halifax, N.S., Canada.

When nerve cells establish correct synaptic interconnections, one may ask how many synapses are deployed between any two identified neurons, whether their number is regulated and if so by what determinants. A solution to this problem has been sought in the first optic neuropile (lamina) of the fly's visual system, where a systematic perturbation in the distribution of photoreceptor axons occurs at the equator. This perturbation results in the convergence of 8 and 7 receptor axon terminals to the unit synaptic columns, or cartridges (making 8R and 7R cartridges respectively) instead of the normal 6 elsewhere (making 6R cartridges). This predictable variation has been exploited to examine the effects of altering the number of presynaptic cells innervating a fixed complement of postsynaptic cells in each of the modular cartridges situated adjacent to the equator.

We examined the chief afferent class of synapse, a population of tetrads at which every receptor axon is repeatedly presynaptic to a fixed constellation of four postsynaptic elements (Burkhardt & Braitenberg: Cell. Tiss. Res. 173, 287). Where these four could be clearly identified one each is invariably contributed from two second-order monopolar interneurons L1 and L2, the other two are usually (55-60%) both α elements of amacrine cells or sometimes (17-20%) both glial elements. The number of these synapses was calculated and compared in 6R, 7R and 8R cartridges from counts of their profiles in electron micrographs of transversely sectioned cartridges from a 1 hr. old female fly (Musca domestica). Counts from many cartridges in single sections were confirmed by counts from single cartridges sectioned serially and reveal that each receptor in 6R cartridges has, on average, a remarkably constant number of synapses (168±17). Small variations in synapse number between 6R, 7R and 8R cartridges correlate with variations in presynaptic membrane area, each synapse occupying on average 1.91μm^2 membrane. Thus, a major determinant of the total cartridge synapse complement appears to be presynaptic.

The number of synapses which are serviced by the same postsynaptic cells therefore increases by 25% in 8R cartridges over 6R cartridges. The membrane area of L1 and L2 dendrites, which accommodate about 87% of all receptor synapses, was measured from serial electronmicroscopy of 6R and 8R cartridges. The increase in synapse number 6R:8R was reflected by an equivalent increase in dendritic membrane area, so regulating the average postsynaptic membrane area per synapse. Overall, the results suggest the regulation of membrane area per synapse and the importance of the presynaptic influence in determining total synapse number. Supported by NSERC of Canada

218.3 THE EYES OF THE GIANT CLAM TRIDACNA: PHOTORECEPTOR ELECTROPHYSIOLOGY. Lon A. Wilkens. Department of Neurobiology, Research School of Biological Sciences, Australian National University, Canberra, A.C.T. 2601, AUSTRALIA.

The mantle surfaces in the giant clam Tridacna maxima contain numerous eyes, ca. 400 in a 50 cm-long clam. The eyes were previously referred to as hyaline organs whose sole function, it was hypothesized, was to focus light on the surrounding, densely packed symbiotic zooxanthellae (Yonge, 1936). More recently, Stasek (1966) has described structural features of the eyes consistent with a visual function, including retinal cells and an optic nerve. I now report the electrophysiological response of photoreceptor cells to light.

Tridacna photoreceptors, as recorded from intracellularly, hyperpolarize to a light stimulus. A decrease in illumination produces a rapid membrane depolarization. Although all photoreceptors display this response polarity, two categories of cells have been observed. Type A cells, when fully dark adapted, give large hyperpolarizing light responses (57.2 mV ±11.5 S.D.) which undershoot the light adapted potential level by an average of 25 mV. In Type B cells, hyperpolarizing responses do not undershoot and are equal in amplitude to depolarizing responses (7.0 mV ±4.2 S.D.). Furthermore, the amplitude of the hyperpolarizing response depends on the extent of dark adaptation in Type A cells whereas, in Type B cells, dark adaptation seemingly has no effect. In nine cells held for a period of time sufficient to record both dark- and light-adapted responses, Type A cells (n = 6) had membrane potentials of 13 mV and 38 mV, respectively, in the dark and light; correspondingly, Type B cells (n = 3) were 35 mV and 45 mV. Light adaptation had little effect on the amplitude of the depolarizing response in either cell type.

In half of the cells a short burst of spikes, 3-4 mV in amplitude, accompanied the depolarizing light-off response. Following light adaptation at relatively high intensities (0.04 W/cm^2), spike frequencies were 60-80 Hz, although they followed the onset of membrane depolarization by 60-70 ms. Spikes were never spontaneous and burst durations were typically 100-400 ms for 1 s shadows. For shorter intervals, the onset of light rapidly terminated spiking. Spike output was also markedly decreased by successive shadow stimuli, i.e. by dark adaptation, even though the depolarizing receptor potential was not. The eyes in Tridacna, therefore, contain primary off receptors similar to those in the distal retina of scallops. (Supported in part by a grant from the Whitehall Foundation).

Stasek, C.R. Occ. Papers Calif. Acad. Sci. 58:1-9, 1966.
Yonge, C.M. Sci. Reports, Gt. Bar. Reef Exped. 1928-29. 1:283-321, 1936.

218.4 MECHANISM OF d-TUBOCURARINE BLOCKADE OF MECHANORECEPTOR TRANSDUCTION IN THE PROTOZOAN, STENTOR. D.C. Wood. Psychobiology Program, University of Pittsburgh, Pittsburgh, PA 15260.

The funnel-shaped ciliate protozoan, Stentor coeruleus, contracts in an all-or-none fashion when stimulated by a suprathreshold mechanical, photic or electrical stimulus. Mechanical stimuli elicit receptor potentials of up to 25mV amplitude. Large receptor potentials trigger all-or-none action potentials which are correlated with the onset of the contraction. These mechanoreceptor potentials are produced by a large ($\cong$180 fold) increase in Ca^{++} permeability accompanied by a smaller ($\cong$20 fold) increase in Cl^{-} permeability.

d-Tubocurarine (d-TC) and other curariform drugs depress the probability of eliciting a mechanically stimulated contraction (ED_{50} = 14 uM for d-TC) but do not alter response probabilities for photic or electrical stimuli. d-TC also produces a dose dependent blockade of mechanoreceptor current (ED_{50} = 7.5 uM) while resting potentials, action potentials, electrical thresholds, membrane resistance and mechanoreceptor current reversal potentials are not significantly affected. The behavioral and electrophysiological effects of d-TC are well correlated with the binding of d-TC (^{14}C) to the cell's surface (K = 22 uM). Thus d-TC appears to bind rather specifically to the mechanical stimulus transduction mechanism and block it.

Mechanoreceptor currents are increased by depolarizing the membrane from the -50mV resting potential to -25mV though this procedure reduces the driving force. Conversely hyperpolarizing the membrane reduces receptor currents while increasing the driving force. These changes in mechanoreceptor current occur exponentially with a rate constant which is a function of the direction and magnitude of the voltage step. These data indicate that the unstimulated mechanoreceptor transduction mechanism is voltage dependent and can exist in at least 2 forms; one of which increases its conductance in response to a mechanical stimulus (responsive form) while the other does not increase its conductance upon stimulation (unresponsive form).

d-TC appears to block mechanoreceptor currents by binding to the unresponsive form of the mechanoreceptor. This conclusion is supported by the inhibition of d-TC (^{14}C) binding observed in depolarizing K^{+} solutions, in which the proportion of unresponsive mechanoreceptors should be decreased. Secondly large depolarizing voltage steps (to +20mV or more) relieve the previously induced d-TC blockade of receptor current seen at resting potential. This effect is observable both during and immediately after the voltage step. The relaxation kinetics of this relief suggests that d-TC is dissociated from its binding site when the depolarizing voltage step converts it to the responsive form.

218.5 BEHAVIORAL AND ELECTRICAL RESPONSES TO AMINO ACIDS IN PARAMECIUM. Robert M. Rivera. Dept. of Biology, UCLA, Los Angeles, CA 90024.

Sensory-motor behavior in Paramecium has been shown to be controlled through ionic mechanisms present in the surface membrane. Electrophysiological methods were used to study the bioelectric basis of behavioral responses of P. caudatum to the amino acids glutamate and serine. Observations were made of direction and velocity of locomotion in response to addition of a small volume of amino acid solution (10^{-6}M) to a slide containing a suspension of swimming cells (bath solution: 3.5mM KCl, 0.5mM KOH, 1.0mM $CaCl_2$, 1.0mM HEPES, pH 7.1). Addition of serine produced a slight increase in forward-swimming velocity within the region of the added amino acid. No increase in the occurrence of reversed swimming was observed in reponse to serine. Addition of glutamate, however, resulted in reversed swimming, causing movement away from the stimulus solution.

Membrane potential was measured with intracellular recording methods while each of these amino acids (10^{-2}M) was pressure pulsed from a micropipette (~5μm tip) into the bath solution near the cell surface. A dye, fast green, added to the stimulus solution, served as a visual indicator of delivery of the solution. Fast green alone had no electrophysiological effect. Application of serine with a 1s pressure pulse caused a hyperpolarization of 3-5mV lasting more than 10s. Glutamate, delivered with a 0.5s pressure pulse, caused a 15s depolarization of 10-20mV.

Depolarization of the cell membrane of Paramecium by a variety of stimuli has been found to elicit reversed swimming, while hyperpolarization induces accelerated swimming in the forward direction. The magnitude of the change in membrane potential from rest determines the frequency of ciliary beating in either direction (Machemer, H., J. Exp. Biol., 65:427-448, 1976; Brehm, P., & Eckert, R., J. Physiol., 283:557-568, 1978). The electrical and behavioral responses to amino acids reported here show these same relations. Locomotor responses to chemical agents in the medium are likely to be important to the foraging ciliate in locating food sources, or in avoiding undesirable conditions. Supported by NSF BNS 80 12346.

219.1 DEVELOPMENT OF MULTITERMINAL INNERVATION IN A LOBSTER MUSCLE. C. K. Govind and Joanne Pearce*. (SPON. N. W. Milgram). Scarborough College, University of Toronto, West Hill, Ontario, Canada, M1C 1A4.

The development of multiterminal innervation from a single identifiable excitatory motoneuron to the lobster distal accessory flexor muscle (DAFM) was examined by serial section electron microscopy. Cross-sections of the entire DAFM were obtained in newly hatched, 24 hr old, 1st stage lobsters, in 2 week old 4th stage lobsters and in a year old 12th stage animal. At each of these developmental stages, the number, size, and location of neuromuscular synapses within the peripheral branching pattern of the axon was determined. The mean size of synapses was similar in all three stages though the number of synapses per unit length of fiber increased more than 20 fold from the 1st to the 4th stage and more than five fold from the 4th to the 12th stage. A similar increase was found for the synaptic surface area per fiber length, thus demonstrating a proliferation of multiterminal innervation. Simultaneously the innervation is delineated from supplying the entire muscle in the 1st stage, to groups of fibers in the 4th stage and to individual fibers in the 12th stage.

The amount of innervation provided by the main axon, the primary branches and other branches differed in the three stages. In the 1st stage, the main axon and its branches which make up the entire branching pattern provide almost equal amounts of innervation. In the 4th stage, the main axon provides 20-25%, the primary branches 45% and the other finer branches 30-35% of the total innervation. In the 12th stage, the innervation is provided completely by the fine branches and none from the main axon and its primary offshoots. This shift in innervation to the ever more distal sites of the axonal branching pattern provides synapses to the enlarging target muscle in an uninterrupted fashion and may occur via nerve terminal sprouting. Research supported by NSERC and MDA of Canada.

219.2 NOREPINEPHRINE AFFECTS SYNAPTOGENESIS IN THE VISUAL CORTEX OF THE RAT. M.E. Blue* and J.G. Parnavelas (SPON: E.D. Ross). Dept. of Cell Biology, The Univ. of Texas Health Science Center, Dallas, TX 75235.

The influence of the noradrenergic (NE) system on cortical synaptogenesis was examined in the visual cortex of rats whose NE afferents were selectively eliminated with the neurotoxin 6-hydroxydopamine (6-OHDA). Experimental animals received up to four subcutaneous injections of 6-OHDA (100 µg/g body weight) during the first four days of postnatal life. Control littermates were injected with equal volumes of vehicle containing 1 mg/ ml ascorbic acid in saline. Depletion of the NE projection to the visual cortex was confirmed in adult animals with glyoxylic acid histofluorescence. Using the electron microscope, photographic montages of 50 µm-wide strips of cortex were prepared from experimental and control littermates at 2,4,6,8,14 and 90 days of age. Synapse counts revealed a significantly higher synaptic density in the cortex of 6-OHDA treated rats during the first week of postnatal life (paired-sample t-test; $p<0.025$ at days 2 and 6 and $p<0.05$ at day 4). The difference between experimental and control rats was less apparent during the second postnatal week, and at day 90 synaptic densities were similar for the two groups of animals. The increased synaptic density was primarily the result of an increased number of Gray's type I synapses. The effect was confined to the lower cortical plate at day 2 but became more widespread in the cortex at subsequent stages of development. These results suggest that in the visual cortex, the NE system exerts an inhibitory influence on synapse formation in early postnatal life.

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219.3 POSTNATAL DEVELOPMENT OF RAT SUPERIOR CERVICAL GANGLION AS MONITORED BY BINDING OF MONOCLONAL ANTIBODIES K.F. Greif, W.D. Matthew* and L.F. Reichardt Dept. Physiol., Sch. Med., Univ. of Calif., San Francisco, CA 94143

Monoclonal antibodies raised against rat forebrain synaptic junctions were used to monitor the appearance of developmental antigens in the rat superior cervical ganglion (SCG). Two antibodies have binding specificity for synaptic vesicles; the other three bind surface proteoglycans. The localization of determinants defined by these antibodies was examined in sections of adult SCG and in ganglia from rats aged one to thirty days postnatal, using PAP and HRP-IgG immunocytochemical staining. Of five antibodies staining adult tissue, only one stained neonatal ganglia. The remaining antibodies stained developing ganglia beginning in the first month after birth. Quantitation of antigen levels by inhibition radioimmune assays indicates that these antigens are present in very low levels at birth and increase during early postnatal life. These antigens are present in very low levels in adult sensory ganglia, suggesting possible association with developing synapses in the SCG. Immunoelectron microscopy will reveal the precise location of these antigens in the SCG.

This research was supported by a postdoctoral fellowship from the Muscular Dystrophy Association, NIH Training Grant, and the NSF.

219.4 SYNAPTOGENESIS OF PYRAMIDAL NEURONS IN RAT VISUAL CORTEX USING THE COMBINED GOLGI-ELECTRON MICROSCOPE METHOD. M. Miller and A. Peters. Dept. of Anat., Boston Univ. Sch. Med., Boston, MA 02118.

The synaptology of maturing pyramidal neurons has been insufficiently studied because of the difficulty of identifying from which type of cell a specific process originates. Hence, the maturation of pyramidal cells in layer V of rat visual cortex has been examined during the first three postnatal weeks by both light and electron microscopy using the correlative gold toning method of Fairén, Peters and Saldanha (1977; J. Neurocytol., 6: 311-337).

Somata of pyramidal cells form only symmetric synapses presumably with multipolar stellate neurons. These synapses are not evident until day 6. Presynaptic processes in immature axosomatic synapses have smaller diameters and contain fewer pleomorphic vesicles than mature ones. On day 12, about four axosomatic synapses are evident in a thin section through the center of the soma, and by day 21 a mean of eight such synapses can be counted. Dendrites, however, form symmetric and asymmetric synapses which are present by day 3 and by day 9, respectively. Each dendritic spine forms a single synapse. In its immature form a spine is a low, broad protrusion of the dendrite which forms a symmetric junction with a small caliber axonal process containing a few clear vesicles. With time, the spine protrudes further and appears as a taller stump. Eventually, a mature spine with a distinct head and neck is produced, and the synapse with the now enlarged axonal bouton becomes asymmetric. Other dendritic appendages, i.e. growth cones and filopodia, form symmetric junctions. These processes are transient pioneer structures and are important in extension and branching of dendrites. Thus, the junctions they form are probably precursors of synapses seen on mature dendrites and spines. Like axosomatic synapses, axoaxonic ones involving the axon hillock and initial segment of pyramidal cell axons synapse with dendritic spines and shafts of other pyramidal cells. Before becoming asymmetric, these synapses pass through a stage during which the junctional densities are symmetric.

The sequence of development of all synapses on pyramidal cells is similar in that a narrow presynaptic process containing a few vesicles matures into an axonal varicosity. During the development of asymmetric synapses, the junctional densities go through a stage when they appear symmetric. The next phase of this study will be to examine the maturation of nonpyramidal neurons to gain a fuller understanding of the synaptogenesis of the rat cortex. Supported by NS 07016 and EY 07054.

219.5 CHOLINESTERASE LOCALIZATION AT SITES OF NERVE CONTACT ON EMBRYONIC XENOPUS MUSCLE CELLS IN CULTURE. F. Moody-Corbett*, P.R. Weldon*, D.F. Davey*, and M.W. Cohen. Dept. Physiology, McGill University, Montreal, Quebec.

Acetylcholine receptors (AChRs) become localized at sites of nerve-muscle contact in cultures of embryonic Xenopus spinal cord and muscle cells. In the present study we have found that cholinesterase (ChE) also becomes localized at these sites.

Cultures were prepared from spinal cord and myotomal muscle cells of 1-day-old embryos. After two or more days, they were stained for AChRs with fluorescent α-bungarotoxin, and then were fixed and stained for ChE so that the staining patterns of both molecules could be examined on the same cells. More than 70% of the nerve-contacted muscle cells had ChE reaction product localized along the path of nerve-muscle contact. In some instances the ChE stain extended along the whole length of the contact but in most cases it was restricted to shorter segments. In a high proportion (more than 80%) of the examples where ChE stain was located along the path of nerve-muscle contact there were also regions of overlapping AChR stain. The localization of ChE (and AChRs) at sites of nerve-muscle contact developed within 2 days in culture and was also observed 1) when muscle cells were derived from younger embryos, prior to any nerve-muscle contact in vivo; 2) when cultures were maintained in 15 μM-curare in order to prevent spontaneous twitching of nerve-contacted muscle cells; and 3) when cultures were maintained in 1.2 μM-tetrodotoxin alone or together with 2 mM-manganese or 2 mM-lanthanum in order to prevent action potentials.

Electron microscopy showed the ChE reaction product at nerve-muscle contacts to be located in the intercellular cleft and often on the sarcolemma beyond the area of contact. At these contacts the neurites frequently contained accumulations of small clear vesicles, the cleft was about 80 nm wide, and in instances where the reaction product was not too dense, basal lamina-like material was apparent in the cleft. By contrast, at contacts which did not exhibit ChE activity the axolemma and sarcolemma were usually closely apposed (less than 10 nm), the cleft did not contain basal lamina-like material, and vesicle accumulations were rarely observed in the neurites.

It is concluded that ChE develops at sites of neuromuscular synaptic specialization in these cultures even in the absence of muscle contraction and nerve and muscle action potentials.

(Supported by the Medical Research Council of Canada).

219.6

WITHDRAWN

219.7 NERVE-INDUCED DEPOSITION OF BASAL LAMINA DURING THE DEVELOPMENT OF AN AMPHIBIAN NEUROMUSCULAR JUNCTION IN CELL CULTURE. M.J. Anderson* and D.M. Fambrough, Carnegie Institution of Washington, Dept. of Embryology, Baltimore, MD. 21210

Monoclonal antibodies have been generated to components of basal lamina from the amphibian Xenopus laevis. In adult skeletal muscle the antigens are distributed within capillaries and over the entire surface of each muscle fiber, but appear concentrated both at tendons and at the neuromuscular junction. Immunocytochemical experiments using antibody and α-bungarotoxin labelled with contrasting fluorochromes reveal that this junctional concentration of basal lamina antigen extends into the junctional folds and throughout the synaptic cleft.

Similar experiments with cultured embryonic myotomal muscle cells reveal a complex ordered array of basal lamina antigen. This consists of a diffuse background over the entire muscle cell surface, along with a scattering of discrete plaques and fibrils. In such cells, developing in the absence of innervation, dense aggregates of acetylcholine receptors invariably appear associated with correspoinding specializations of the adjacent basal lamina. At these sites the striking homology in the organization of receptor and basal lamina suggests that their distributions are co-ordinately regulated even in the absence of innervation.

When embryonic muscle cells become innervated by cholinergic neurons in vitro there is a progressive nerve-induced accumulation of acetylcholine receptors in the developing post-synaptic membrane. This accumulation of receptors is closely paralleled by a corresponding deposition of new basal lamina associated with the newly-formed acetylcholine receptor clusters. It is concluded that the junctional specialization of basement membrane is induced by the developing motor nerve, and appears virtually concomitantly with the receptor aggregates in the post-synaptic membrane.

219.8 GUANOSINE 3':5'-MONOPHOSPHATE (cGMP) ACCUMULATION BY SPINAL CORD CELLS DURING NEUROMUSCULAR JUNCTION FORMATION IN VITRO AND IN VIVO. C.L.Weill, Department of Neurology, Louisiana State Univ.Med.Ctr. New Orleans, La., 70112

The molecular signals that subserve neuromuscular junction formation have eluded detection. That communication must take place between these cells is evidenced by the anatomical elaborations that result in the functional alignment of pre- and post-synaptic structures of the mature junction.

cAMP and cGMP were measured by radioimmunoassay in cultured and embryonic chick tissue during synapse formation. Dissociated 6 day spinal cord cultures and post fusion muscle cultures contain constant levels of cAMP, 0.130pm/mg and 0.326pm/mg respectively and cGMP, 0.020pm/mg and 0.025pm/mg respectively over 9 days. Spontaneously twitching cocultures display a 5.7±2.7X increase in cGMP over 4 days while cAMP remained constant. cGMP in cocultures continues to rise at variable rates from day 4-10. The increase in cGMP is specific for cocultured spinal cord and muscle. Spinal cord cocultured with muscle derived fibroblasts and muscle co-cultured with 10 day cerebellar cells showed no increase in cGMP over 8 days.

Increases in cGMP in stimulated muscle have been observed (Beam, et al. Nature 267:534, 1977). Cocultures of nerve and muscle treated with curare (50uM) and tetrodotoxin (10^{-7}g/ml) to block action potential and synaptic potential activity showed increases of 4.4 and 5.5X respectively in cGMP over 4 days. Landmesser (J.Physiol.284:391, 1978) has shown that motoraxons invade their target muscle and begin to form functional contacts at St27. cGMP was measured in lumbar spinal cord and dorsal thigh muscle dissected from 5-8 day embryos. cGMP remained nearly constant at 6.93±0.75pm/mg in muscle while in spinal cord there was a 5.4X increase to 35.7pm/mg on day 8. In a correlate culture experiment with 6 day spinal cord explants, cGMP remained nearly constant in muscle over 10 days at 0.075±0.032pm/mg while in cord explants cGMP rose 2.3X to 0.598pm/mg. Mediation of this effect by a secreted factor or cell contact was tested by growing cells in medium conditioned by the counter cell. cGMP in muscle grown in SC/CM showed no change over 4 days and then rose to 0.964pm/mg on day 5. Spinal cord cells grown in M/CM showed a 24X increase in cGMP to 5.94pm/mg on day 5. A 23X increase in the rate of $^{45}Ca^{++}$ influx by spinal cord cells was also induced by M/CM over 4 days in culture.

It is concluded that a specific, synaptic activity independent increase in cGMP is induced in spinal cord cells upon contact with muscle or a muscle derived factor and a muscle factor induced increase in calcium influx in spinal cord cells may be causal.

(Supported by NIH, BRSG funds.)

219.9 QUANTITATIVE STAINING OF SYNAPSES ON FROG CARDIAC GANGLION CELLS USING ZINC-IODIDE AND OSMIUM. Peter B. Sargent and C. Douglas Evans*, Department of Structural Biology, Sherman Fairchild Building, Stanford University School of Medicine, Stanford, CA 94305.

The zinc-iodide osmium (ZIO) staining procedure has been adapted for use in the Xenopus cardiac ganglion to stain synapses on parasympathetic neurons lying within the interatrial septum. The most critical parameters affecting the staining were found to be osmotic strength, pH, osmium concentration, and the duration of staining. We have been able to label synapses consistently from cell to cell and from preparation to preparation by staining for one hour with a hyperosmotic solution that is buffered to pH 4.5 with potassium acetate and that contains 0.28% osmium tetroxide (w/v). Under these conditions all neurons in adult Xenopus cardiac ganglia have stained synaptic boutons and preterminal axons on their surface. Electron microscopy of stained synapses reveals electron-dense deposits within all small "agranular" synaptic vesicles and in the extravesicular cytosol. Such heavy staining was observed in each of 150 synapses examined on 266 cells in 6 cardiac ganglia. Thus the staining procedure consistently and quantitatively labels synapses in this preparation. Light microscopic analyses of ZIO-stained adult Xenopus hearts indicate that ganglion cell bodies receive an average of 11 synaptic boutons, which emanate usually from one or two axons (S.D.=6 boutons, n=200 neurons taken from 4 animals with body lengths of 5.5-6.0 cm). The number of boutons is strongly correlated with cell body surface area ($r=0.78$, $p<0.001$).

The ZIO-staining conditions suited for adult Xenopus did not label boutons in hearts taken from larval or recently metamorphosed individuals. By modifying the staining conditions, we were able to consistently and quantitatively stain boutons in post-metamorphic frogs; the critical modifications entailed raising the pH to 5.1 and lengthening the staining time to 2.5 h. Under these conditions, 99% of 280 vesicle-containing nerve contacts on ganglion cells were heavily stained, with electron-dense deposits within all "agranular" synaptic vesicles and in the extravesicular cytosol (6 hearts examined from young frogs having body lengths of 1.6-1.9 cm; the remaining 1% of the contacts were "lightly" stained, with reaction product within vesicles only). The modified staining conditions label boutons on ganglion cells at all larval stages beginning with Nieuwkoop and Faber stage 46, when ganglion cells generally can first be recognized in the heart. Ganglion cells in larvae and young frogs have fewer synaptic boutons than those in adults; we are presently quantifying the change in bouton number that accompanies development.

220.1 NEUROGENESIS IN CAT RETINA. A STUDY USING ^{3}H-THYMIDINE AUTORADIOGRAPHY. E. H. Polley, C. Walsh* and T. L. Hickey. Depts. of Anatomy, Ophthalmology and Neurosurgery, U. of Illinois School of Medicine, Chicago, IL 60680, Comm. on Neurobiology, U. of Chicago, Chicago, IL 60637, and School of Optometry/The Medical Center, U. of Alabama, Birmingham, AL 35294.

The pattern of neurogenesis of retinal cells has been determined by making single injections of 500μCi of ^{3}H-thymidine in a series of fetal cats 21-30 days of gestation (E21-E30), which were sacrificed 60-250 days after birth. Some of these animals have been used in a different study by Hickey and Cox (Neurosci. Abstr., 5:788, 1979). The retinae were flattened, embedded in methacrylate resin, and sectioned at 2μm either radially or en face. Autoradiographs were prepared using standard techniques.

An injection at E21 led to no detectable retinal label. An E22 injection labelled a few cells near the optic disc. The retinal area containing labelled cells was greatly increased after injections made slightly later. An E23 injection led to labelled ganglion cells up to 3-5 mm from the optic disc, and after an injection at E24 labelled ganglion cells extended more than half the distance from the disc to the ora serrata. In this material (E24) labelled ganglion cells were very common at the area centralis, and all labelled ganglion cells were small- or medium-sized. Label was also seen in the outer nuclear layer adjacent to the pigment epithelium. In the inner nuclear layer, label was limited to a cytologically distinct cell type adjacent to the outer plexiform layer and tentatively identified as horizontal cells.

Injections on E26 led to label which extended further toward the ora serrata, but there were still many labelled ganglion cells at the area centralis. Again, no large ganglion cells were labelled. Later injections (E29, E30) resulted in labelled ganglion cells which extended from disc to ora serrata and which were primarily large or small, with few labelled medium-sized cells. Other cell types in the inner nuclear layer were also labelled, but label in the outer nuclear layer, now quite heavy, was still seen only adjacent to the pigment epithelium.

These preliminary results suggest that cell production may start roughly synchronously in all three retinal layers for at least some retinal elements, but continue later in the receptor and inner nuclear layers. The material shows that at a given gestational age ganglion cells are produced over a very wide area of retina, so that the retina does not seem to demonstrate a simple radial pattern of development. Lastly, we have found that there is no simple linear relation between date of birth and ganglion cell size.

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220.2 THE VOLUME OF THE LATERAL GENICULATE NUCLEUS AND STRIATE CORTEX AS A FUNCTION OF POSTNATAL AGE IN MONKEYS. Michael D. Gottlieb, Pedro Pasik and Tauba Pasik, Dept. Neurol., Mount Sinai Sch. Med., CUNY, New York, N.Y. 10029.

The volumes of the lateral geniculate nucleus, pars dorsalis (LGNd) and the striate cortex (SCx) were measured in monkeys (M. mulatta) at birth and at 1, 2, 4, 8, 16 and approximately 100 weeks of age. The brains were fixed by perfusion and marked before processing with two needles inserted 5 mm apart perpendicularly to the future plane of sectioning, thus allowing the calculation of a shrinkage factor per individual specimen. Volume estimates for the left LGNd were derived from camera lucida tracings (41.3X) of the cell laminae (the interlaminae were not included) appearing in 7-9 equally spaced Nissl-stained coronal 40 μm sections through the nucleus. Estimates for the left SCx were obtained from microprojection drawings (13.8X) of 11-14 similar sections through the rostrocaudal extent of the structure. The area measurements of the outlines were used to calculate the volumes by Simpson's rule for irregular solids and the resulting values further corrected by the shrinkage factors.

The results show that the total volume of the LGNd cell laminae increases almost twofold from birth (35 mm^3) to the oldest age sampled (68 mm^3). This growth seems to take place in two stages, namely between birth and 2 weeks, and between 8 and 16 weeks, most of the expansion occurring in the latter period. The changes in total laminar volume parallel those shown by the parvocellular laminae whereas those of the magnocellular laminae exhibit a delayed and slow increase that continues beyond 16 weeks. Similarly to the LGNd, the volume of the SCx increases nearly twofold from birth (1856 mm^3) to adolescence (3262 mm^3). These data also indicate two periods of growth, one between birth and 4 weeks, and the other between 8 and 16 weeks. As in the LGNd, the major development occurs between 8 and 16 weeks. The dynamics of the SCx expansion differs, however, from that of the LGNd in that the first growth period peaks two weeks earlier in the latter structure. In addition, the volume of the SCx decreases beyond 16 weeks, whereas that of the LGNd remains constant. A high correlation is found between the volume of the LGNd cell laminae and that of the SCx across all ages sampled ($r = 0.97$, $p < 0.001$). The slope of the line of best fit for this function indicates an approximately constant 1:50 ratio of LGNd:SCx volumes, thus allowing the prediction of one on the basis of the other.

The findings are consistent with previous data indicating a differential development of the parvocellular and magnocellular LGNd laminae, and with the maturation of the LGNd preceding that of the SCx. Aided by USPHS Grants #MH-02261, EY-01926, EY-01867, and F32NS06251.

220.3 ORGANIZATION OF VISUAL PROJECTIONS IN NEONATAL KITTEN. Z. Henderson* (SPON: R.J.W. Mansfield). University Lab. of Physiol., Parks Road, Oxford OX1 3PT, U.K.

The methods of labelled axonal transport, which have proved to be very sensitive for tracing projections in newborn animals (e.g. Innocenti, G.M., et al., Neurosci. Letts. 4: 237-242, 1977), were used to make a detailed study of connections in the visual system of newborn kitten. The main problem is that standard doses of horseradish peroxidase (HRP) and ^{3}H-proline diffuse rather extensively after injection into neonatal brain. Improved results were obtained by using HRP conjugated to wheatgerm agglutinin (HRP-WGA) and by injecting the markers in very small quantities. Unilateral injections of 1-2 μCi ^{3}H-proline and 2-4 μg HRP-WGA in 0.05 μl H_2O were made, through a stereotaxically-placed 32 gauge needle, into lateral geniculate nucleus (LGN) or superior colliculus (SC) of kittens anaesthetized with halothane. Bilateral or unilateral injections into areas 17 or 18 were made by implantation of 1mm^2 pieces of Millipore filter paper containing the same amounts of tracers in dried form. After 24h the brains were fixed by perfusion with glutaraldehyde and paraformaldehyde. Frozen sections were cut and processed either for autoradiography or for HRP activity with cobalt intensification of the diaminobenzidine-H_2O_2 reaction.

The results indicate that all the major trajectories of the visual system are present in newborn kitten and that most of them have the same gross organization as that in adult. There are ipsilateral and contralateral projections from retinal ganglion cells to SC and LGN. The LGN projections to areas 17 and 18 arise from cells in all the major LGN laminae, are topographically arranged and entirely ipsilateral; small injections of HRP-WGA into area 17 label cells in narrow projection columns in the LGN. The LGN terminal axons end mainly in cortical layers I, III and IV. There is a projection, strictly ipsilateral, arising from layer VI cells of visual cortex and terminating in all the major laminae of the LGN; the topography of this projection closely follows that of the geniculo-cortical pathway. Cells in layer V of the visual cortex form an ipsilateral and topographically organized projection to the superficial layers of the SC above the stratum opticum. In conclusion, a careful application of the labelled axonal transport methods shows that in the cat there is a high degree of precision in the organization of the visual pathways, at birth, before the retina becomes fully functional and when synapses are still relatively sparse (Cragg, B.G., J. Comp. Neurol., 160: 147-166, 1975).

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220.4 PLASTICITY IN THE RETINOGENICULATE PROJECTION OF THE CHICK: TEMPORAL PARAMETERS. R. H. Granda* and W. J. Crossland, Department of Anatomy, Wayne State University, School of Medicine, Detroit, Michigan 48201.

Recently we have reported a region of increased innervation density in the chick ventral geniculate nucleus (GLv) which is in topographic correspondence with a lesion placed in the ipsilateral optic tectum on the day of hatching. The increase in retinogeniculate projection could be observed 21 to 70 days after the lesion had been made. We have extended these observations by examining two temporal parameters of the plasticity phenomena: 1) the time course of appearance of the augmented GLv projection following lesions at hatching, 2) the period in which the GLv is subject to plasticity.

The time course of appearance of augmented GLv projection was investigated by lesioning chicks on the day of hatching, followed at three-day intervals by labeling the retinofugal projections with an intravitreal injection of 200 μCi of ^{3}H-proline in the eye contralateral to the lesion. Six to eighteen hours later the chicks were perfused with formalin, the brains were embedded in paraffin and sections were prepared for autoradiography. Using brightfield and darkfield light microscopy, we observed a patch of heightened grain density in the GLv corresponding topographically to the ipsilateral tectal lesion by the sixth day after hatching. No qualitative differences were noticed in the appearance of the zone of plasticity between six and twenty-one day post-operative survival periods.

To determine the period of susceptibility to plasticity tectal lesions were made three to ninety-six days after hatching. Six weeks later the animals were injected and sacrificed as described above. We have found that the plasticity phenomena is demonstratable through the 96th day after hatching. The labeling pattern at 96 days appears qualitatively similar to that observed in chicks lesioned at hatching. Whether the chick continues to express the ability to alter the retinogeniculate projection in still older animals is under investigation.

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220.5 CATECHOLAMINE DEPLETION, OCULAR DOMINANCE SHIFT AND DIRECTION SELECTIVITY IN KITTEN VISUAL CORTEX. J. D. Daniels, Mary Kay Ellis*, S. A. Bianco*, Mary Garrett*, S. B. Nelson* and Marjory Schwartz*.

If a kitten is dark-reared until about 5-7 weeks of age, one day of normal visual experience through one eye only can cause ocular dominance shift of area 17 neurons to the opened eye, and cause those neurons to develop direction and orientation selectivities. We have used this paradigm in four kittens in which one hemisphere was injected with the selective catecholamine neurotoxin 6-hydroxydopamine (6-OHDA), and the other hemisphere was sham-operated.

The injections (10 micro ℓ. of 2mg/ml fresh 6-OHDA in vehicle of Ringer's solution with 0.4% ascorbic acid) were given three days before the visual experience. We allowed twelve hours for overnight consolidation after the visual experience before beginning single unit recording (nitrous oxide anesthesia). Recording sites were spaced every 75-100 microns in the medial bank of the postlateral gyrus (area 17).

In the control hemispheres 76% (50/66) of the visual units were driven by the opened eye (ocular dom. groups 1-2 or 6-7) and 52% of the units were selective for the stimulus' direction of movement. In the 6-OHDA-treated hemispheres 28% (18/64) of the visual units were dominated by the opened eye and only one unit of the 64 was genuinely direction selective. The two groups didn't differ significantly in percentage of non-visual sites--about 20% of the total in each case.

We recorded from two dark-reared kittens and found the results similar to the drugged hemisphere recordings. Both had similar percentages of binocular (gp 3-5) units, about 55%. The dark-reared actually had more selective units (17%). Twenty-eight percent of the dark-reared recording sites were non-visual.

We confirmed catecholamine depletion by subjecting tissue near each injection site to a high performance liquid chromatography (HPLC) assay. The drugged hemispheres averaged 35% the norepinephrine (NE) context of the control hemispheres. Control values were about 80 ng/gm. One kitten injected with 6-OHDA did not show any depletion and did show ocular dominance shift and selectivity.

We have verified and extended the results of Kasamatsu and Pettigrew (J. Comp. Neurol. 1979); we think both ocular dominance and selectivity can be altered only when proper levels of catecholamines are present in cortex during development. We are continuing to test this idea by studying kittens with NE delivered to cortex via osmotic minipumps.

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220.6 EFFECT OF 6-HYDROXYDOPAMINE ON PLASTICITY OF DIRECTION SELECTIVE CELLS IN VISUAL CORTEX. N.W. Daw, R.K. Rader* and T.W. Robertson.* Physiology Dept., Washington Univ. Med. School, St. Louis, MO, 63110.

Plasticity of direction sensitivity in the visual cortex is known to have a different critical period from the plasticity of ocular dominance. Moreover, the synapses responsible for direction sensitivity (inhibitory GABA synapses) are almost certainly different from the synapses that determine ocular dominance. It is therefore important to know whether 6-hydroxydopamine (6-OHDA) has the same effect on direction sensitivity as it does on ocular dominance, in order to test the generality of the effect of 6-OHDA on plasticity in the visual cortex.

Six kittens were reared in the dark. At the age of 3 1/2-4 weeks a minipump containing 1 mg/ml 6-OHDA in 0.4% ascorbate in saline was inserted under the skin, leading to a needle in the left cortex. During the next 1-2 weeks the animals were exposed to an environment continually moving in one direction (left in three cases, right in three) for approximately 50 hours. Shortly after that recordings were made from the cortex and a sample of approximately 40 cells was accumulated from each kitten. The number of cells preferring the direction of movement of the drum was compared to the number of cells preferring the opposite direction. As one control, the opposite cortex was recorded in two animals; as a second control group, four animals were reared similarly with a minipump containing ascorbate but no 6-OHDA; as a third control group, four animals were reared similarly, but no minipump was inserted.

The ratio of cells preferring movement with the drum to cells preferring movement against the drum was 67:33, 70:30 and 65:35 in the three control groups and 41:59 in the experimental group. The ratios from the control groups are not significantly different from each other, but the ratio from the experimental group is significantly different from the control groups ($p < .001$) and is close to the ratio in normal animals (50:50). Therefore 6-OHDA does prevent the plasticity of direction sensitivity in the visual cortex. It appears that the effect of 6-OHDA on plasticity in the visual cortex is a general phenomenon, affecting the connections for direction sensitivity as well as the connections for ocular dominance.

220.7 CHANGES IN THE DORSAL LATERAL GENICULATE INDUCED BY MONOCULAR DEPRIVATION IN HOODED RATS. D. McClearn* and E. Fifková (Spon.: S.K. Sharpless). Dept. of Psychology, Univ. of Colorado, Boulder, CO 80309.

The dorsal lateral geniculate (GLD) was studied (volume and cell density) in 26 hooded rats in which the right eye was suture shut prior to the physiological eye opening at the 14th postnatal day. Twenty-six controls with both eyes open were kept together with their lid-sutured littermates for varying periods of time prior to the sacrifice. Controls and lid-sutured rats were matched as to their sex, weight and survival time. Volume of the rostral and caudal half of the GLD were calculated separately. While no consistent difference in the volume was found in the rostral part, the caudal part was significantly smaller by 9.8% ($p<0.05$) in deprived animals which survived for 8-14 weeks. In those animals which survived for longer periods of time (15-23 weeks), no significant volume reduction could be observed in either part of the GLD. The cell density was calculated in the caudal half of the GLD in two separate regions. One forms the dorsolateral aspect of the nucleus and receives mostly crossed optic fibers. The other region is located ventromedially and was shown to receive a crossed as well as an uncrossed visual input (Cunningham and Lund, Brain Res., 34:394). In the dorsolateral region, the cell density was significantly decreased by 13.7% ($p<0.01$) in deprived rats which survived for 8-10 weeks, while no systematic variation of the cell density was observed in the ventromedial region. In rats which survived for more than 10 weeks, the cell density in the dorsolateral region of the deprived GLD reached control levels. Monocular deprivation thus seems to retard the development of GLD neurons in hooded rats, however, without halting it. The severity of the postdeprivation change appears to be determined by the length of deprivation period, since the decreased volume and cell density of the deprived GLD were observed only in shorter intervals after the lid-suture. This result agrees with the time curve of postdeprivation changes observed in the rabbit visual system (Chow and Spear, Exp. Neurol., 42:129; Grobstein, et al., Neurosci. Soc. Abstr., IV, 239). (Supported by NIH Grant EY 01500-07.)

220.8 POSTNATAL CHANGES IN THE NUMERICAL DENSITY AND THE TOTAL NUMBER OF NEURONS AND SYNAPSES IN THE DIFFERENT LAMINAE OF THE STRIATE CORTEX OF MACAQUE: A STEREOLOGICAL ANALYSIS. J. O'Kusky* and M. Colonnier, Lab. de Neurobiologie, 1401, 18e Rue, Québec, Qué. G1J 1Z4.

The surface of area 17 and the thickness of its laminae were measured in a series of new-born, 3-month, 6-month and adult macaques. The numerical density (N_v) of neurons was determined by the method of size frequency distribution (Weibel '69, Int. Rev. Biol: 26, 260) for each of the lamina. The N_v of synapses was similarly estimated in the new-born, 6-month and adult specimens. The total number of neurons and synapses were derived from these measures. Except where noted all the described changes are statistically significant.

The thickness of the cortex increases from birth to 6 months and diminishes back to near new-born values in the adult. The 6-month cortex is 19% thicker than that of the adult, and this overshoot is greatest in layers II-III which are 43% thicker at 6 months. The average surface of area 17 in each age group shows a similar trend, being 23% greater at 6 months, but the differences are not statistically significant because of large within group variability.

The N_v of neurons decreases from birth to 6 months and increases back to near new-born values in the adult. The 6-month N_v is 30% smaller than that of adults and again the greatest difference is found in layers II-III where it is 38% smaller. When the total number of neurons in a hemisphere is calculated, however, the undershoot in N_v is compensated by the overshoot in thickness and surface. The pattern of change with age becomes that of a gradual decrease in the number of neurons from new-born to adult (16%) but this overall decrease is not statistically significant. There is a significant decrease, however, in layers I, IVC , V and VI. In layer I the number of neurons in adult is decreased by 73%, while in layer VI the number at 3 months is decreased by 34%.

The N_v of synapses on the other hand undergoes a pattern of change similar to that of cortical thickness, increasing from birth to 6 months and decreasing back to near new-born values in adult. The 6 month overshoot is 35% for the total cortex, and is greatest in layers I-III, at 41%. The total number of synapses in the striate cortex of one hemisphere is 90% greater at 6 months than in the adult. For layers I to III the synapses are 120-155% more numerous (i.e., more than twice as numerous) at 6 months.

These data demonstrate an increase in neuronal connectivity in the striate cortex from new-born to 6-months, especially in layers I, II and III, and a subsequent decrease in connectivity in the adult. It is as if at the time of cortical plasticity an overabundant neuropil develops, capable of adapting to several potential environments, and is later resorbed as the most appropriate circuits are selected.

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220.9 PRESERVATION OF STEREOPSIS IN CATS FOLLOWING NEONATAL SECTION OF THE OPTIC CHIASM. Brian Timney and Gordon Lansdown*. Psychology Department, University of Western Ontario, London, CANADA, N6A 5C2.

Section of the optic chiasm (OX) eliminates the descussating pathways from the nasal retinae to the contralateral visual cortex, thus preventing the direct convergence of optic fibres onto binocular cells in the visual cortex. The relatively high proportion of binocular neurones found in the cortices of OX sectioned cats results from an indirect pathway through the corpus callosum (Cynader et al., Neurosci. Abs. 1979, 5, 781). Blakemore (J. Physiol. 1969, 205, 471) has suggested that such a pathway might subserve midline stereopsis, when disparate images of a target lying directly behind or in front of a fixation point fall on opposite hemiretinae and therefore should project to different cortical hemispheres.

We assessed the functional integrity of the indirect pathway by measuring stereoacuity in 2 cats which had undergone OX section at the age of 3 weeks. Control measurements of visual fields and acuity also were taken. Perimetric examination, using the Sherman procedure, revealed the expected losses in the temporal hemifield of each eye. Visual acuity of both cats was low compared to normals, with a maximum of approximately 2 cycles/degree.

Binocular and monocular depth thresholds were measured using the jumping stand technique. Thresholds for the OX cats were somewhat worse than normals, but their respective binocular thresholds were still very much better than when they were tested monocularly, suggesting that these animals were still able to use binocular cues to make depth judgements. These results suggest that the indirect callosal projections, which maintain binocularity in OX section cats, are sufficient also to mediate binocular depth discrimination.

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220.10 EFFECTS OF SELECTIVE EXPOSURE TO LINES ON DIFFERENT CELL TYPES IN CAT VISUAL CORTEX. M. McCall*, A.G. Leventhal, D.G. Tieman* and H.V.B. Hirsch. SUNY Albany, Albany, N.Y. 12222.

We have suggested previously that several receptive field properties of a cortical cell help to predict the extent to which development or maintenance of its orientation preferences requires exposure to appropriate patterned stimuli (Leventhal and Hirsch, 1980). To test this further we have now raised cats in controlled visual environments. Three groups of cats were raised wearing masks within which each eye could view lines of a single orientation. Animals in one group viewed the same orientation with both eyes (0 deg or 90 deg); animals in the second group viewed 0 deg lines with one eye and 90 deg lines with the other eye; animals in the third group viewed 45 deg lines with one eye and 135 deg lines with the other eye. When animals were not wearing masks they were housed in a dark room. Once animals received about 150 h of exposure to the patterns, we recorded extracellularly from single units in Area 17.

Exposure to one or two orientations produced a matching bias in the distribution of orientation preferences of cortical cells; the distribution had one peak in cats exposed to a single orientation and two peaks in cats exposed to two orientations. The proportion of non-selective cells was inversely proportional to the number of lines the cats had seen, suggesting there are constraints on the preferred orientation a cell may develop. The bias in the distribution of orientation preferences of cortical cells was greater for cells dominated by the ipsilateral eye than for cells dominated by the contralateral eye. Furthermore, the bias was greater for cells with high cutoff velocity (suggestive of an excitatory input from Y-type cells in the LGNd; Dreher et al., 1980) than for cells with a low cutoff velocity and narrow receptive field (suggestive of an excitatory input from X-type cells in the LGNd; Dreher et al., 1980). The results provide further evidence that there is a class of cortical cells whose members do not require visual stimulation for either development or maintenance of their orientation preferences, while there are other cells which are dependent upon visual stimulation for either the development or maintenance of an orientation preference. (Support provided by EY01268 to H.V.B.H.).

220.11 EVIDENCE FOR A "CONSOLIDATION" EFFECT DURING CHANGES IN THE OCULAR DOMINANCE OF CORTICAL NEURONS IN KITTENS

V. S. Ramachandran and M. Ary, Division of Biology 216-76, California Institute of Technology, Pasadena CA 91125.

In a previous study (Soc. Neurosci. Abstr., 1980), we had compared the effects of massed and distributed sessions of brief visual experience in changing the ocular dominance of cortical neurons and had suggested that a "consolidation"-like effect might be taking place. We now present further evidence for such a process.

Eight kittens, 5-6 weeks of age were used to study the effects of brief monocular visual experience on the ocular dominance of neurons in area 17. They were monocularly deprived for 2½ days and in all of them ocular dominance was found to have shifted almost completely towards the experienced eye. Two of them were recovered from anesthesia and paralysis and the deprived eye was then stimulated for 4 hours with high-contrast rotating gratings. They were then anesthetized and paralysed again to obtain 'Post-Stimulation' ocular dominance histograms - and only a slight change in ocular dominance was observed. In two of the kittens the deprived eye was stimulated for 4 hours as in the previous experiment, but we put the kittens in total darkness for 3-4 days before obtaining a 'Post-Stimulation' histogram. In these 2 animals the two eyes became almost equally effective in driving cortical neurons suggesting that the effects of brief visual experience become more obvious if some time is allowed to elapse after stimulation. As a control we monocularly deprived two kittens for 2½ days and placed them in the dark for 3-4 days without any prior visual stimulation. Only a slight recovery was observed in these animals.

Lastly, two of the monocularly deprived animals had their deprived eye stimulated in 4 one-hour sessions distributed over 4 days instead of a single 4 hour session. (They were kept in total darkness whenever they were not being stimulated.) The observed recovery was even more striking in this experiment suggesting that 'distributed' practice may be more effective than 'massed' practice.

We conclude that a) For reversing the effects of monocular deprivation one needs only 4 hours of visual experience i.e. one doesn't need 3-4 days of on-going stimulation. b) The effects of this brief experience are more obvious after some time has elapsed in the dark. This implies that even after active synaptic stimulation has ceased, some time-dependent process akin to 'consolidation' must continue to occur in the dark.

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220.12 BINOCULAR COMPETITION DETERMINES THE SIZE AND SHAPE OF OCULAR DOMINANCE COLUMNS IN CATS. Nina Tumosa* and Suzannah Bliss Tieman. Neurobiology Res. Ctr., SUNY Albany, Albany, NY 12222.

To determine the effect of binocular competition on the development of ocular dominance columns (ODC) in cats, we used the C14-2-deoxyglucose technique to map the ODC in cats reared with different degrees of competitive advantage to one eye. Cats were reared normally, with monocular deprivation (MD), or with unequal alternating exposure (unequal AME). In unequal AME, each eye receives normal patterned vision but on alternate days and for unequal periods (8 h/day vs. 1 h/day). It was previously shown that this imbalance in the duration of stimulation gives a competitive advantage to the 8 h eye (Tumosa et al., Science, 208: 421, 1980). One eye was removed from each of 4 MD cats, 4 AME 8/1 cats, and 2 normal cats under halothane anesthesia when they were 12-16 wks old. One week later, the cat was reanesthetized with halothane, a flexible catheter was inserted into the femoral vein, and the cat was allowed to recover from the anesthetic. A neck ruff prevented the animal from removing the catheter. The next day, 175 µCi C14-2-deoxyglucose was injected intravenously, and the animal was allowed to explore the lighted laboratory for 45 min before it was deeply anesthetized and perfused with buffered saline followed by 3 l of buffered formalin and then by 500 ml of 30% sucrose. The visual cortex was removed, rapidly frozen, and sectioned in the horizontal plane at 30 µm with a cryostat. The sections were picked up onto warm coverslips, dried at 60°C, and exposed with Kodak SB-5 film for 1-3 wks. The size of the resulting ODC varied with the rearing of the cat and with which eye was stimulated: the eyes with the greatest competitive advantage activated the largest ODC. In order of increasing percentage of Area 17 activated by the stimulated eye, they are: deprived eye (DE) of MD cats, 1 h eye of AME 8/1 cats, either eye of normal cats, 8 h eye of AME 8/1 cats, and experienced eye of MD cats. The ODC of the DE of MD cats were widest in layer IV, where they were about the same width as those of the 1 h eye of the AME 8/1 cats; in other layers they were narrower, sometimes disappearing altogether. In contrast, the ODC of the 1 h eye were the same width in all layers. Thus the degree of competitive advantage determines both the size and shape of ODC in the cat. These results suggest that when one eye is placed at a severe disadvantage, as in MD, both geniculocortical connections and intracortical connections may be disrupted, but when the disadvantage is less, as in unequal AME, only the geniculocortical connections are disrupted. (Supported by PHS grants EY02609 to SBT and EY01268 to HVBH).

220.13 MONOCULAR AND BINOCULAR VISUAL FIELD LOSSES IN KITTENS WITH OPTICALLY INDUCED STRABISMUS. A.J. Elberger, E.L. Smith, III* and J.M. White*. Dept. of Neurobiology and Anatomy, The Univ. of Texas Med. Sch. at Houston, Houston, TX 77030, and College of Optometry, Univ. of Houston, Houston, TX 77004.

Optically induced strabismus was produced in 4 kittens by the following procedure: the kittens were reared in the dark, and in addition from 28 days through 14 weeks old, were given 2-3 hours daily light exposure through visual goggles. The right eye covering was a 15 Diopter base in prism and the left eye covering was a zero power lens. A control cat was reared identically except that zero power lenses were placed over both eyes in the goggle exposure; a second control cat was given normal laboratory rearing. All 4 prism-reared cats demonstrated a range of esotropia as a result of the rearing conditions. The direction and magnitude of the deviation was determined using the corneal reflex technique. The resulting fixation pattern was determined from qualitative results of cover testing. The extent of monocular and binocular visual fields was determined using perimetry testing.

The normal and goggle control cats demonstrated normal interocular alignment and normal extents of monocular and binocular visual fields (Elberger, Exp. Brain Res. 36:71-85, 1979). In comparison, all 4 experimental cats demonstrated significant contralateral field shrinkage in monocular tests. In addition, in binocular tests 2 of the 4 cats, those which exhibited constant unilateral esotropia (8^{o} and 16^{o} compared to normals) in cover testing and corneal reflex photography demonstrated a significant shrinkage in the peripheral field (monocular segment) ipsilateral to the deviating eye. However, the other 2 cats, exhibiting an alternating fixation pattern in cover testing, and 4^{o} and 17^{o} esotropia, demonstrated no consistent reduction in the peripheral binocular visual field.

It is interesting to note that both unilateral and alternating fixation patterns were exhibited by the identically reared experimental kittens; these two patterns also occur in human esotropes. Nevertheless, the contralateral field loss under monocular testing of both unilateral and alternating fixators is qualitatively similar to the results of other investigators using surgically induced esotropes. This indicates the field loss is due to anomalous visual experience rather than abnormal eye movement patterns associated with a surgically induced noncomitant squint.

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220.14 THE CRITICAL PERIOD FOR FUNCTIONAL COMPENSATION IN LATERAL SUPRASYLVIAN VISUAL AREA FOLLOWING NEONATAL VISUAL CORTEX REMOVAL IN CATS. L. Tong, P.D. Spear, and R.E. Kalil., Depts. of Psychology & Ophthalmology, Univ. of Wisconsin, Madison, WI 53706

Following a unilateral lesion of visual cortex (areas 17 & 18) in adult cats, lateral suprasylvian visual cortex (LS) exhibits a decrease in direction selective cells, an increase in cells responsive to stationary stimuli, and a decrease in percent of cells responsive to the ipsilateral eye. In LS of cats with a lesion on day of birth, however, cells develop normal response properties. Thus, in cats with day 1 lesions, there is functional compensation in LS not found following adult lesions.

In this study we investigated the critical period for the functional compensation. Cats received visual cortex (VC) lesions at 1 day, 2,4,8,12,18, and 26 wks of age or as adults. After a survival of at least 6 months, single cells were recorded in LS. Results for different response properties are as follows.

Direction selectivity: Following a day 1 lesion, the percent of responsive cells that are direction selective appears normal (80%) whereas following an adult lesion 18% are direction selective. The ability to develop direction selectivity following a lesion decreases between 12 and 18 wks of age, and by the age of 26 wks a lesion results in only 22% direction selective, comparable to an adult lesion. The loss in direction selectivity is accompanied by an increase in the percent of cells responsive to stationary stimuli.

Ocular dominance: A day 1 lesion results in a normal ocular dominance distribution while an adult lesion reduces the percent of cells driven by the ipsilateral eye (OD 2-7) from 70% to 40%. Visual cortex lesions at ages up to 18 wks result in normal ocular dominance, but by 26 wks of age a lesion decreases the percent OD 2-7 to that found after an adult lesion.

Orientation selectivity: Orientation selective cells are not found in LS in normal cats. Following a day 1 or an adult VC lesion, less than 4% of LS cells are orientation selective. By contrast, 35% of the responsive cells are orientation selective in cats with a lesion at 2 wks. Increased orientation selectivity also is found following lesions at 4, 8 and 12 wks, but is less than 4% after a lesion at 18 or 26 wks.

In summary, the critical period for functional compensation in LS following visual cortex lesions extends at least to 18 wks of age. In addition the anomalous property of orientation selectivity can develop after VC lesions at some ages. The critical period for this property differs from the critical period for development of direction selectivity and normal ocular dominance.

220.15 REGROWTH OF CENTRAL CATECHOLAMINERGIC FIBERS IN CAT VISUAL CORTEX FOLLOWING LOCALIZED LESION WITH 6-HYDROXYDOPAMINE

K. Nakai*, G. Jonsson* and T. Kasamatsu, Division of Biology, California Institute of Technology, Pasadena, CA 91125, U.S.A. and Department of Histology, Karolinska Institute, Stockholm, Sweden.

We have been studying roles played by the central catecholamine (CA)-containing system in regulating synaptic plasticity in cat visual cortex. In the kitten visual cortex perfused with 6-hydroxydopamine (6-OHDA) to place chemical lesions specific to CA-containing nerve endings, we physiologically demonstrated the prolonged suppression, with a waning trend, of recovery of cortical cells from the effects of monocular lid suture which was followed by reopening the previously closed eye (Kasamatsu et al., 1981a). The CA system is known for its remarkable capability of regrowth of terminal fields or sprouting after partial lesions, provided that the proximal axons and the somatas of CA cells are saved from initial lesions. Thus, we may explain the observed incomplete suppression of recovery in cortical physiology by the quick regrowth of intracortical CA-fibers after localized chemical lesions with 6-OHDA, in conjunction with some biochemical changes such as dennervation supersensitivity.

The visual cortex of 5-week-old kittens, which had priorly been subjected to the bilateral resection of superior cervical ganglia, were locally perfused with 4mM 6-OHDA for a week. With varying the survival time (0, 2 and 4 weeks) after stopping the 6-OHDA perfusion, such cortex was then studied by either glyoxylic acid fluolescence histochemistry or a biochemical assay for endogenous CAs using liquid chromatography. Immediately after the end of the 6-OHDA perfusion no CA terminals were seen within a cortical area whose radius was about 5mm away from the perfusion site (Kasamatsu et al., 1981b). In the vicinity of this primary lesion area, especially in superficial layers, CA terminals and fibers were seen more densely than the normal. Two weeks after stopping the 6-OHDA perfusion, the CA-terminal-depleted area seemed to be shrunken (a radius, 2-3mm). Some of regrowing CA fibers were observed even at the edge of the gliosis caused by the placement of cannulae. By four weeks after stopping the 6-OHDA perfusion, CA fibers with terminals were virtually seen everywhere in the visual cortex including the previous site of cannulation. At the perfusion center, however, the intensity of CA fluolescence did not yet return to the normal level. In an area 3-4mm away from the perfusion site, complexity and intensity of fluolescent fibers were already indistinguishable from those in the normal. Reappearance of moderately bright fluolescent fibers, parallel to the cortical surface, were especially notable in the superficial layers. In corollary with the above results in histofluorescence biochemical assays also revealed the quick recovery of CA contents in a cortical area which had been once depleted of its CA terminals with 6-OHDA. (USpHS EY 03409-01; Swedish MRC 04X-2295)

220.16 POSTNATAL MATURATION OF LATERAL GENICULATE NEURONS IN RELATION TO DEVELOPING RETINAL AFFERENTS IN THE KITTEN. C.A. Mason. Dept. of Pharmacology, New York Univ. Med. Centr., New York 10016.

Dendritic maturation of cells in the lateral geniculate nucleus (LGN) was studied in kittens of 1-8 postnatal weeks in tissue impregnated by the Golgi-Kopsch method. During the first few postnatal days, all impregnated cells display many stubby perikaryal extensions. By 1 week, recognizable dendrites have emerged. Dendrites bear growth cones at their tips and are covered with irregular protrusions. At primary and secondary branch points, sprays of finger-like extensions occur. Cell class distinctions that can be made in the adult (Guillery, J. Comp. Neur. 128: 21, 1966) cannot yet be applied. By 2 weeks, large cells bear much longer dendrites that are shaggy with spines, hairs, and growth cone-like protrusions. Smaller cells have more finely divided dendrites that also bear sprays of finger-like structures at branch points. Assignment to cell classes can more easily be made. At 3-4 weeks, dendrites are longer and characteristic 'grape-like' appendages can be recognized on larger cells. By 5-6 weeks, dendrites of all cell types have thinned out, but they bear many more spines than in the adult. Obvious class I cells have more finely sculpted appendages, and smaller cells bear some immature appendages. By 8 weeks all cell types look virtually mature, although spines are still seen on some peripheral dendrites.

Golgi material was taken from littermates of kittens used to study postnatal development of terminal arbors of retino-geniculate axons. Axon arbors were diffusely labeled with horseraddish peroxidase and analyzed in the light and electron microscopes (Mason, Soc. Neurosci. Abstr. 6:660, 1980). At 1 week postnatal, axon arbors look mature in their extent but terminal swellings are highly immature. During the major phase of dendritic outgrowth at 1-2 weeks, however, axon terminal arbors undergo little change. New ultrastructural findings reveal that broad expansions on axons make proto-glomerular contacts, but the more numerous filopodial structures make frequent simple contacts with dendrites and perikaryal blebs. These latter synaptic patterns are not seen in the adult. From 3-6 weeks, refinement of shape both of axonal terminals and of specialized dendritic appendages at proximal branch points where most retinal input occurs, proceeds in tandem. Since retinal axons have reached the LGN one month prenatal (Shatz and DiBerardino, Soc. Neurosci. Abstr. 6:485, 1980), these results suggest that waiting afferent retinal axons may influence primary dendritic outgrowth and subsequent modeling of appendages of geniculate neurons. (Supported by NIH Grants NS-14283 to R.W. Guillery and NS-16951).

220.17 IRREVERSIBLE DISRUPTION OF CORTICAL BINOCULARITY FOLLOWING EARLY EXPERIENCE WITH 32° OF ROTATIONAL DISPARITY BETWEEN THE LEFT AND RIGHT VISUAL FIELDS. Michael R. Isley*, Diane C. Rogers*, William C. Owen*, and Paul G. Shinkman. Univ. North Carolina, Chapel Hill, NC 27514.

Previously, we reported that when kittens' early visual experience (between the ages of 4-12 wks.) consists of left- and right-eye visual fields optically rotated in opposite directions about the visual axes (16° of disparity between the two eyes), the distribution of interocular differences (IOD) in visual cortical cells' preferred stimulus orientations is found subsequently to be centered about the rotation experienced during early development. In other respects, the organization of visual cortex resembles that found in normally reared kittens.

At last year's Neuroscience meeting, we described experiments designed to assess the parametric limits of this developmental plasticity. Kittens were reared viewing a normal environment through goggles fitted with prisms that introduced 32° of rotational disparity between the two eyes' visual fields. The results differed in three principal ways from the earlier (16°) experiments: (1) the ocular dominance distribution was U-shaped rather than normal; (2) the distribution of preferred interocular orientation disparities was somewhat rectangular, with significantly greater variance than that of normally reared or control kittens; and (3) there was a substantial overrepresentation of cells with preferred stimulus orientations in the dominant eye near horizontal or vertical (± 22.5°).

The present experiment was designed to assess the permanence of this effect, and its susceptibility to modification by subsequent normal visual experience during adulthood. Kittens that had worn 32° prism goggles during early development, with results as described above, were subsequently returned to the main colony without goggles where they experienced a normal visual environment for 4-8 mos. They were then retested to assess the effects of this exposure upon the physiological organization of visual cortex. No changes were found; the pattern of results was the same as in the earlier measurements. We conclude that the disruption of binocularity and also of interocular matching of preferred stimulus orientations produced by early experience with a rotational disparity of 32° between the left and right visual fields is permanent and not subject to modification by subsequent prolonged visual experience without the goggles during adulthood.

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220.18 THE EFFECT OF IMPULSE BLOCKAGE ON CYTOCHROME OXIDASE ACTIVITY IN THE CAT VISUAL SYSTEM. M. Wong-Riley and D.A. Riley. Dept. of Anatomy, Univ. of Calif., San Francisco, CA 94143

The use of cytochrome oxidase histochemistry as an indicator of oxidative metabolism in neurons has proven effective in revealing functional changes following sensory deprivation in developing and mature nervous systems (Wong-Riley and Welt, '80). Since sensory deprivation can involve several parameters, such as natural stimuli, action potentials and axoplasmic transport, we wished to know if the cessation of impulse transmission alone could bring about an adjustment of oxidative metabolism in the postsynaptic neurons. We used tetrodotoxin (TTX) in a dosage that blocked action potentials without blocking axoplasmic transport (Ochs and Hollingsworth, '71; Stryker, personal communication). TTX was injected intravitreally into one eye of 8 adult cats every 3 days for total periods of 1,2,4,5½ or 6 weeks respectively. The effect of the drug could be monitored by the disappearance of the pupillary reflex and its return in about 3 days. At the end of the experimental periods, the animals were perfused and their brains were processed for cytochrome oxidase histochemistry. The results indicated that: (1) A decreased level of C.O. activity was observed in the LGN laminae innervated by the TTX-treated eye as well as in area 17 of all animals. (2) Changes were discernable after one week, but became progressively more prominent up to 6 weeks. (3) Within the LGN, the ipsilateral lamina A1 consistently appeared more severely affected than the contralateral lamina A. This pattern was similar to that seen previously in unilaterally lid-sutured cats (Wong-Riley,'79). (4) The monocular segment of the affected lamina A exhibited a high level of enzyme activity, indicating that its oxidative metabolism might be sustained by other factor(s) or synaptic activity. (5) Within the striate cortex, banding pattern of high and low C.O. activity was seen in lamina IV. This was consistent with the pattern observed in monocularly sutured cats. Cortical changes after one week survival appeared milder than that in the LGN, suggesting that alterations in the LGN preceded that of the cortex. Experiments were also initiated to determine whether the histochemical changes were reversible following the termination of drug treatment. Preliminary results in cats TTX-treated for 4 weeks with 6 weeks recovery showed that the enzymatic levels in the affected LGN and cortical laminae were comparable to that of the normal. This indicates that the dosage used apparently did not damage the neural pathways. Thus, impulse blockage alone can cause a decrease in the level of cytochrome oxidase activity in the affected postsynaptic neurons, and the maintenance of functional and enzymatic integrity of postsynaptic neurons in the adult is dependent upon viable presynaptic impulse conduction.

220.19 PATTERN PROCESSING AND SLOW WAVES IN VISUAL CORTEX OF PATTERN DEPRIVED CAT. J. D. Glass and R. W. Hall*. Dept. Pharmacol., Univ. of Pgh., Sch. of Med. and Elec. Eng., Univ. of Pgh., Pgh., PA 15261.

We recently showed that pattern processing mechanisms of cat visual cortex can be studied with the slow-wave response (Glass and Hall, 1981). In the context of the relationship between pattern processing and the slow wave, we have now examined the effects of monocular lid closure upon the slow-wave response.

Kittens received lid closure over one eye prior to the time of the normal opening of the lids. After at least 13 months of closure, bipolar electrodes were implanted under anesthesia into the visual cortex. Recordings were taken with the cats awake and restrained. A diffuse and a checkerboard pattern stimulus were used. In each test condition 15 responses were digitized and peak amplitudes were compared with T tests.

Comparisons between the responses evoked from the non-deprived (ND) eye by the diffuse and patterned stimuli showed significant peak amplitude differences primarily for those components occurring between 50-230 msec. Components before and after this critical interval rarely showed significant differences. However, for the deprived (D) eye, when the responses were recorded immediately after opening the lids, the percentage of significant comparisons in the critical interval was substantially reduced. The response evoked from the D eye was of decreased amplitude and altered waveform compared with the response evoked from the ND eye.

Single-unit studies in cats have shown a visual cortex that is virtually unresponsive to edge stimuli as a result of the deprivation. Our findings with the slow-wave response show that pattern processing abnormalities are present in the visual cortex confirming the single-unit evidence. However, the existence of a reliable and complex slow-wave response within the visual cortex demonstrates that visual stimulation of the deprived eye is indeed activating the visual cortex. The maintained responsiveness of visual cortex following visual deprivation has also been demonstrated with the slow-wave response recorded from humans. The role of this retained cortical activity in visual function is not known. The use of the slow-wave response in the visually deprived cat may provide a useful animal model for studying the retained visual function in humans.

220.20 DARK REARING EFFECTS ON CORTICAL CELLS OF CATS FOLLOWING POSTNATAL MONOCULAR DEPRIVATION. U. Yinon and S. Goshen (SPON: S. YEHUDA). Physiol. Lab., Maurice and Gabriela Goldschleger Eye Inst., Tel Aviv Univ. Sch. of Med., Sheba Med. Ctr., Tel Hashomer 52621, Israel.

The effect of prolonged periods of dark rearing on early visual experience was studied in five age groups of kittens (N=13) subjected postnatally to 0-85 days of normal binocular vision. Following this they were all monocularly deprived for one month and dark reared for 9.5-20.5 months. Unit recording was performed from visual cortex area 17 and compared to kittens (N=2) postnatally monocularly deprived (MD)for 10 months and to normal control adult cats (N=13).

The ocular dominance distribution shows already a minor bias toward the formerly exposed eye in the age group of kittens which has received the earliest monocular visual experience (8-38 days). The highest bias in the ocular dominance was found in the second age group of kittens in which the monocular visual experience has covered the most critical part of the susceptibility (Hubel and Wiesel, J. Physiol. 206: 419, 1970) period (23-53 days postnatally). Accordingly the maximal diminution in the proportion of binocularly driven cells was found in this group age. The smallest bias was obtained in the last age group of kittens which has received the period of monocular deprivation at the latest age (93-124 days postnatally). Of the visually unresponsive and direction and orientation nonselective cells the highest proportion was found in the first group age of kittens while in the other group ages it was similar to that of the MD or the control groups.

It was concluded that the age factor is more important than the duration at which a certain visual experience within the susceptibility period was given. In addition, it was proved that brief periods (several days) of monocular deprivation are ineffective if followed by a complete absence of visual experience; a continuous visual exposure is needed for the effect of the binocular competition to persist.

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221.1 HISTOCHEMICAL EVIDENCE THAT HEART CONDITIONED MEDIUM PROMOTES CENTRAL CHOLINERGIC REGENERATION IN VIVO. Amy Rothman Schonfeld and Robert Katzman. Dept. of Neurology, Albert Einstein College of Medicine, Bronx, N.Y. 10461.

The ability of central cholinergic neurons to undergo axonal regeneration after injury has been demonstrated both histochemically (Svendgaard et. al., Brain Res. 102:1, 1976) and biochemically (Emson et. al., Brain Res. 135:87, 1977) in adult rats. In these studies, iris tissue implanted into the anterior hippocampus, served as targets for newly regenerating cholinergic septo-hippocampal fibers which had been transected during the implantation procedure. Recently, the activities of several "cholinergic growth factors" have been studied in vitro. One of these, heart conditioned medium (HCM), has been shown to promote both the survival and neuritic outgrowth of chick ciliary ganglion cells in culture (Helfand et. al., Devel. Blo. 50:541, 1976). In a recent report from this laboratory (Schonfeld et. al., submitted for publication, 1981), we observed that daily application of HCM near cholinergic soma in the septum significantly stimulated axonal regeneration, as measured by choline acetyltransferase activity, into iris implants in the anterior hippocampus. Using the same experimental paradigm, the aim of the present study was to examine the effect of HCM on cholinergic regeneration histochemically.

For this, heterologous iris implants were inserted in the anterior hippocampus in female rats previously subjected to ipsilateral superior cervical ganglionectomies. At the same time, a cannula was stereotaxically placed in the medial septum. Animals were administered either HCM (prepared by Dr. R. Johnston of Stanford University according to Helfand et. al., 1976) or saline as control vehicle. Rats received either daily injections for 0 or 8 days post-op or alternate day injections for 16 days. Animals were sacrificed by decapitation. The brains were rapidly removed, frozen, sectioned on a cryostat, stained for acetylcholinesterase by the direct-coloring thiocholine technique (Karnovsky and Roots, J. Histochem. Cytochem. 12:219, 1964) with modifications (Hardy et. al. Neurosci Lett. 3:1, 1976) and analyzed.

Differences between treatments were most evident 16 d post-op. In the saline treated implants, areas of reinnervation were generally limited to those peripheral areas most proximal to the hippocampal fimbria. In contrast, HCM appeared to stimulate both the number of regenerating fibers as well as extend the distribution of these fibers to include central portions of implant and sections in dorsal hippocampus relatively distal to the fimbria. These observations support our earlier neurochemical findings and indicate that HCM stimulates cholinergic regeneration in vivo.

221.2 IN THE SNAIL MELAMPUS EXCISED CEREBRAL GANGLIA INTRODUCED INTO THE HEMOCOEL BECOME INTEGRATED INTO CNS CIRCUITRY. Stacia Moffett, Dept. of Zoology, Washington State University, Pullman, WA 99164.

When a cerebral ganglion is implanted in the hemocoel of a normal adult snail, the ganglion often forms afferent and efferent connections. Extracellular recordings from the implant surface and nerves reveal reflex activity that mimics the host snail's responses to stimulation. Projections from the implanted ganglion to the host snail's CNS are more rare, but occasionally do form. In these cases, stimulation applied to the implanted ganglion is capable of eliciting reflex responses from the host's nervous system. In addition, many of the implanted ganglia induce supernumerary eyes and tentacles (Moffett and Austin, Amer. Zool 20, p. 891, 1980; J. Exp. Zool. 216(2), 1981).

Removal of one cerebral ganglion results in formation of a ganglion bud and some restoration of normal behavior on the operated side (Moffett, Soc. Neurosci. Abs. 5 p. 754, 1979). If, however, the cerebral ganglion has all its nerves and central connections severed and is then replaced in approximately its former position, it is reincorporated into the CNS circuitry. In six of seven animals examined 6-8 mos. postoperative, the restored ganglion had assumed its normal position relative to the other cerebral ganglion. The ganglion bud development was rudimentary and the bud connected directly to the restored ganglion. In the exceptional case, the restored ganglion was displaced and was connected to the contralateral cerebral ganglion via the ganglion bud. Nickel chloride backfilling has confirmed the reestablishment of central tracts and electrophysiological recordings show that the restored ganglion motoneurons effect motor responses on the operated side.

When, in addition to reintroduction of the snail's own excised ganglion, another cerebral ganglion is also inserted, the ganglia fuse or are closely associated and joined by a commissure. In four of the five preparations examined six weeks postoperative, the two united ganglia are incorporated as one into the brain pathways, with varying degrees of redundancy in peripheral nerve connections. For instance, the tentacle nerve in one preparation bifurcated and entered both cerebral ganglia. In two preparations, cerebrobuccal connectives extended from both reintroduced ganglia. Communication from the ring ganglia via the ganglion bud extended to both of the united ganglia and one or two cerebral commissures were formed with the contralateral ganglion. Despite the possibilities for maladaptive responses inherent in these operations, the snails appear quite normal, and preliminary physiological recordings indicate that reflex control is fairly normal.

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221.3 LONGITUDINAL EVALUATION OF THE EFFECTS OF NEONATAL HIPPOCAMPAL X-IRRADIATION IN THE RAT. Dr. R. B. Wallace, Mary-Beth McLaughlin and John Gustafson (Spon: D. Oliver). Developmental Psychobiology Laboratories, University of Hartford, West Hartford, Ct. 06117. Studies employing fine-resolution thymidine H^3 autoradiography have established the existence of a pool of mitotically active cells in the subependymal layers of the ventricles in the brains of mammals, particularly altricial species. The technique of focal x-irradiation of the rat hippocampus provides a method to produce selective lesions of the granular cells of the dentate gyrus; one can then study the behavioral consequences of this manipulation. In a series of papers dealing with the effects of focal cerebellar irradiation (Wallace and Altman 1970) a number of qualitatively assessed motor deficits were noted in young adult rats and these were correlated with a massive reduction (80%) in the granular cells of the IGL. We then noted and reported in a later paper (Wallace, Daniels and Altman 1972) that some of these animals with 2 year survival times showed behavioral recovery. Histological analysis revealed in these animals a recovery of the IGL to within normal values. In an attempt to determine the recovery potential of the proliferative matrix of the hippocampus, the following experiment was conducted; 42 male Long-Evans rats from our animal colony were used. All animals received focal x-irradiation of the hippocampus according to the following schedule: irradiation treatment began on postnatal day 2. On the 2nd and 3rd days after birth, animals received 200 rads. Thereafter, irradiation received was 150 rads on alternating days (5th through 15th postnatally). Non-irradiated control subjects remained with their mothers throughout the period. To determine the long term effects of neonatal degranulation 3 experimental and 3 control animals were sacrificed and examined at ages 1 month, 2 months, 4 months, 6 months, 12 months, 18 months, and 24 months. Animals were sacrificed with an overdose of sodium pentobarbital and perfused with 10% buffered formalin transcardially. The brains were removed, embedded in paraplast and sectioned at 6μ in the coronal plane. Every 10th section was saved and stained with H&E for histological analysis. The number of granule cells in matched sections of the dorsal hippocampus was counted at 400X magnification. Results indicated approximately a 70% reduction in the granule cells of the dentate gyrus in the irradiated animals up to 6 months of age. Following this, however, there was a recovery to approximately a 45% reduction against the control level in the 24 month animals. Thus, the proliferative matrix of the hippocampus would appear to possess some reconstitutive capacity although not to the extent earlier shown in the IGL of the cerebellar cortex. The implications of this will be further examined.

221.4 FUSION OF SEVERED AXONAL STUMPS WITH POLYETHYLENE GLYCOL: AN ARTIFICIAL MECHANISM FOR AXONAL REGENERATION. George D. Bittner, Dept. of Zoology, Univ. of Texas, Austin, Tx. 78712.

Since neurons have long cytoplasmic processes (axons), some traumatic lesions to nervous tissue can result from axonal severance rather than cell body ablation. Repair (if any) of such lesions in all vertebrate and some invertebrate axons studied to date occurs via rapid (12-36 hour) degeneration of severed distal stumps together with slow (1-2mm/day) outgrowth of processes from proximal stumps to reform synases on denervated cells. In contrast, many invertebrate neurons initially repair severed axons by the slow outgrowth of processes from proximal stumps which functionally activate surviving distal stumps by gap junctions or ephaptic current spread. (Severed distal stumps may survive and be activated for days, months, or years). In such cases, the final repair mechanism appears to be the reformation of synapses on target tissues. We and others have now reported a great variety of distal stump survival times, distal stump activation mechanisms, and synaptic reformation times. However, in no case have we or others confirmed that any organism regenerates axons by morphological fusion of proximal stumps with surviving distal stumps. (See data or references given in Bittner, Comp. Biochem. Physiol., 68A: 299-306, 1981; Birse and Bittner, J. Neurophysiol., 45: 724-742, 1981; Bouton and Bittner, Cell Tiss. Res., in press, 1981.)

We (Bittner and Ballinger, manuscript submitted for publication) now report an ability to reconnect severed CNS axons in an invertebrate (crayfish medial giant axon) in vitro and axons from a vertebrate hybrid cell line (NG 108-15 mouse neuroblastoma/rat glioma: Nelson et. al., PNAS USA 73, 123-27, 1976) in tissue culture. In both preparations, severed axons were fused with 40-50% polyethylene glycol (PEG) solution focally applied to the lesion site for 30-60 seconds (PEG, $HOH_2C(CH_2OCH_2)_nCHOH$, has been used for some years to fuse various cell lines grown in tissue culture). Our results suggest that in ~ 5% of all trials PEG can produce axoplasmic continuity between two severed medial giant stumps, although axolemmal continuity is not complete. Our data also show that ~ 8% of severed NG 108-15 processes reconnected to the cell body using PEG survive at least 24 hours. Our initial in vivo trials using mammalian myelinated axons have not yet been successful, perhaps because PEG dissolves both the myelin and axolemmal membranes so that they flow together. However, the possibility exists that PEG or similar membrane-active compounds may be used in vivo to repair axonal severance much more rapidly than ever occurs via various mechanisms evolved by different organisms. Supported by NSF grants BNS 77-27678 and 80-22248 and NIH grant NS-17275.

221.5 MOLECULAR CHANGES IN REGENERATING RETINOTECTAL NERVE TERMINALS. L. I. Benowitz and K. Padda*. Dept. of Psychiatry, Harvard Medical School, McLean Hospital, Belmont, MA 02178, and Harvard College.

During the regeneration of the optic nerve in goldfish, the labeling spectrum of proteins in the rapid phase of axonal transport shifts radically from that seen in the intact state (Benowitz, Shashoua & Yoon, 1981, J. Neurosci. 1:300-307). The rapid phase of transport includes material which becomes incorporated into the developing nerve terminals, and might be expected to include proteins involved in axonal guidance, target recognition and synaptogenesis. In our previous studies, double-isotope labeling and 1-dimensional gel separation methods were used to contrast the protein labeling patterns of intact and regenerating optic nerves. More recently, we have used a modification of O'Farrell's two-dimensional procedure to be able to examine the metabolic shifts for individual molecular species. Differentially-labeled proteins from intact and regenerating optic nerves were co-separated first by isoelectric focusing, then by molecular weight on SDS-polyacrylamide slab gels. Labeled spots on the gels, identified by fluorography, were cut out and counted for [^{3}H] and [^{14}C] to measure the synthetic activity of proteins in the intact and regenerating sides, respectively. The labeling of a 44,000 dalton species with a pI of 4.3 was found to increase 50-fold during regeneration, while 5- to 10-fold increases were seen for proteins of 210,000 daltons (pI 5.3) and 24,000 (pI 5.7). Major decreases in labeling were also observed, particularly for several high molecular weight proteins. Using subcellular fractionation methods to separate various components of the optic nerve terminals in the tectum, the greatest overall labeling increases during regeneration appeared at the 0.6/0.8 and 0.8/1.0 M sucrose interfaces when a lysed P_2 pellet (17,000 x g ppt.) from tectal homogenates was separated on a discontinuous sucrose density gradient (53,000 x g, 2 h). Analyses of these fractions on gels showed that the same protein changes which had been found in the optic nerve also appear in the nerve terminal membranes. Thus, these proteins may be considered as potential candidates for mediating cell-cell interactions during development. Nearly identical results have been reported in the regenerating frog optic pathway and in the developing rabbit visual system (Skene & Willard, 1981, J. Cell Biol. 89:86-95, 96-103; J. Neurosci. 1:419-426). Current studies using lectin-affinity chromatography indicate that overall, glycoproteins containing α-D methyl mannoside, glc-NAc, sialic acid or galactose are not being turned on in regeneration over and above the overall protein synthetic changes. Supported by NINCDS grants NS 14674, NS 16943, and by a Fellowship from the Alfred P. Sloan Foundation.

221.6 EMBRYONIC CNS TISSUE IMPLANTED INTO ADULT SPINAL CORDS H. Nornes, A. Björklund* and U. Stenevi*. Dept. of Anatomy, Colorado State University, Ft. Collins, CO 80523, and Dept. of Histology, University of Lund, Sweden.

Embryonic CNS tissue was implanted into adult spinal cords of rats to replace missing supraspinal systems, or to make a tissue bridge for the regeneration of host spinal cord axons across a site of injury or transection.

In the first type of experiment, the supraspinal noradrenaline (NA) system was lesioned at level C_2 with bilateral injections of 6 hydroxydopamine (3μg in 2μl/injection). Embryonic NA tissue from the brain stem (locus coeruleus) was then implanted into the NA denervated cords. The donor tissue was dissected from rat fetuses with crown-rump lengths (CRL) of 14-24 mm (embryonic days 15-17) and implanted into subpial cavities in the dorsolateral aspect of level T_{11} in adult female rats (180-200 g.). Following a 3-4 month survival time, the implantation sites were analyzed by Falck-Hillarp histofluorescent methods. Tissue union formed between the embryonic implants and host tissues with little or no cavitation. Noradrenaline axons from the implants crossed the tissue junctions and grew up to 10-12 mm in the grey matter of the hosts.

In the second type of experiment, complete subpial transections were made in the lower thoracic regions of adult female rats (180-200 g.). Pieces of embryonic spinal cord (CRL 10-16 mm), brain stem (CRL 7-8 mm), or hippocampus (CRL 16-20 mm) were implanted into the 1-2 mm subpial gaps in the spinal cords. Following a 3-4 month survival, a fluorescent dye (propidium iodide or true blue, 0.5μL, 5% in saline) was injected into the distal stumps of the spinal cords 2-15 mm from the site of the implant. The host animals were finally processed by Falck-Hillarp histofluorescence methods 5-7 days post injection. Tissue union formed between the hosts and transplants, and in some cases, fluorescent dye had been transported retrogradely from the distal stump into cell bodies in the transplant. In these same animals, NA axons had regenerated from the rostral stump of the cord into the transplant.

These experiments show that embryonic CNS tissue survives when placed into subpial cavities or complete transections of adult spinal cords of rats; further, axons from the transplants grow into the gray matter of the spinal cord and axons from the host regenerate into the transplant to reconstruct a potential relay. (Supported by Paralyzed Veterans of America Fellowship NBR-101, and Swedish [illegible] grant 04X-3874).

221.7 EFFECTS OF NERVE GROWTH FACTOR AND ITS ANTISERUM ON NEURITE OUTGROWTH FROM GOLDFISH RETINAL EXPLANTS. James E. Turner, Martin Schwab and Hans Thoenen. Dept. of Neurochemistry, Max Planck Institute for Psychiatry, Martinsreid, West Germany.

Our previous in vivo studies have strongly suggested that the goldfish visual system is dependent in part on nerve growth factor (NGF) for a successful regenerative response to axotomy (Turner, J.E. et al., Brain Res., 197:319, 1980). This presentation compliments our earlier in vivo work and reports that NGF stimulates neurite outgrowth from goldfish retinal explants and that the antiserum inhibits this process.

Goldfish retinal explants, whose ganglion cells were primed in vivo by an optic nerve crush of varying periods prior to culture (ie, post crush interval) were found to respond to low nanogram levels of NGF (1-5 ng/ml). This in vitro response was dose dependent, specific for NGF and could be reduced by 80% with NGF antiserum treatment. The magnitude and sensitivity of the NGF response was dependent on the post crush interval. Without a prior crush there was no NGF response unless explants remained in culture 1-2 weeks before treatment. NGF elicited the greatest increase in neurite outgrowth when administered to explants with a 7 day post crush interval (ie, 7 DPA). With increasing post crush intervals (ie, 14 DPA) NGF demonstrated a decreased capacity to enhance neurite outgrowth until by 35 DPA no response could be elicited.

Dr. Turner is the recipient of an NIH Research Career Development Award. Dr. Turner's present address is the Dept. of Anatomy, Bowman Gray School of Medicine, Wake Forest University, Winston-Salem, N. C. 27103.

221.8 CORTICOSPINAL TRACT (CST) PLASTICITY IN THE EARLY POSTNATAL RAT. D.R. Bernstein and D.J. Stelzner, Dept. of Anatomy, SUNY Upstate Medical Center, Syracuse, New York 13210.

Animals received midthoracic spinal cord (T_8-T_{10}) "over-hemisection," including right hemicord and left dorsal funiculus (DF), at birth (N=13) or 21 dpn (N=14). In a second experiment, the right sensorimotor cortex (SMC) was ablated at the same time the midthoracic lesion was made at birth (N=6), 6 (N=3) or 12 dpn (N=7). All operates survived 3-12 mo. prior to stereotaxic unilateral ^{3}H-proline injection of either, or the intact, SMC. Similarly injected adult rats (N=6) served as controls. After 48 hrs., rats were sacrificed and the brain and spinal cord examined using standard autoradiographic techniques. In control rats, injections labeled the contralateral CST primarily in the DF, and sparsely in the dorsal lateral funiculus (dLF) and upper central gray.

Since the CST develops postnatally, spinal lesion at birth and 6 dpn severs the pathway through which most CST axons will traverse at a later stage, but at 12 and 21 dpn, CST axons are also cut. Following injection of the appropriate SMC, the right or left CST was studied. In neonatal operates, the left CST bypassed its severed pathway by growing through the remaining cord. The right CST also grew through the remaining tissue, but only in the double lesion neonatal and 6 dpn operates where growing left CST axons were absent. The misrouted axons were found only in the dLF and upper central gray, normal CST positions, and terminated bilaterally but to correct CST sites (Neurosci. Abst. 6: 683).

In 12 and 21 dpn operates, CST axons severed by the spinal lesion did not regenerate. Within 3-4 mm rostral to the lesion site, fibers sprouted to include nonspecific areas, especially near the scar border. Tightly-packed label concentrations, av. 8.5 μm diam., similar in size and location to reactive ends of cut axons (Anat. Rec. 193: 483), were common rostral to the lesion only in 21 dpn operates. Spared CST axons in the left dLF were more heavily labeled than normal and there was evidence of both collateral and terminal sprouting in the zone caudal to the lesion site. Evidence of labeled CST axons was usually not detectable in lumbar spinal cord.

Our data show that the availability of an intact matrix allows neogenic CST axons to grow around a lesion which destroyed their primary pathway. Growth is enhanced by CST axons being "channeled" into secondary CST routes and is limited by an interaction with opposite side CST axons. On the other hand, by 12 dpn there is axonal sprouting of the spared CST axons, but only regenerative sprouting of cut CST axons near the lesion site. (NS Grant 14096)

221.9 ANALYSIS OF 4S RNA IN REGENERATING OPTIC NERVES OF GOLDFISH. N.A. Ingoglia, Dept. of Physiology and Neurosciences, CMDNJ-New Jersey Medical School, Newark, NJ 07103

Previous experiments have demonstrated that 4S RNA is transported axonally during the reconnection period of optic nerve regeneration in goldfish (Ingoglia, Science, 206:73, 1979). The present experiments were performed to analyze further the characteristics of the RNA undergoing axonal transport.

In the first experiments, ^{3}H-uridine was injected into both eyes of fish 10 days after the right optic nerves were crushed. Fish were sacrificed 2, 3 or 5 days later and radioactivity in the normal and regenerating nerve tract complexes were analyzed for TCA soluble and insoluble radioactivity. Results indicated a several-fold increase in radioactivity in both fractions in regenerating nerves. In other experiments, the nature of the RNA present 2 days p.i. was determined by extracting nerves in cold phenol and fractionating the RNA by polyacrylamide gel electrophoresis (PAGE). Approximately 40% of the RNA in regenerating nerves was present as 4S RNA, whereas only 20% was present as 4S RNA in normal nerves, indicating that 4S RNA is transported axonally prior to reconnection of regenerating optic axons. In other experiments ^{3}H-uridine was injected into both eyes of fish 18 days (near the time of reconnection) after bilateral optic nerve crush and fish were sacrificed at various times up to 180 days p.i. ^{3}H-RNA was extracted from 24 pooled tecta and fractionated by PAGE. 70% of the ^{3}H-RNA is present as 4S RNA 12 days p.i., and 60 days p.i. this value is still approximately 50%. When nerves were cut 36 days p.i. (54 days post crush) and allowed to degenerate for 6 days before determining tectal ^{3}H-RNA, the majority of the ^{3}H-4S RNA was lost from the tectum indicating significant amounts of axonally transported 4S RNA are not transferred out of the axon to surrounding cells. In other experiments the time of sacrifice after injection was held constant and the time of injection after nerve crush was varied. Analysis of tectal ^{3}H-RNA showed that the major period of axonal transport of ^{3}H-4S RNA was in the early stages of regeneration. Further analysis of the data suggests a long 1/2 life for intra-axonal 4S RNA.

In conclusion, these experiments show that 4S RNA: 1) is present in growing axons prior to reconnection; 2) is transported maximally during early stages of regeneration; 3) remains intra-axonal following transport; and 4) turns over at a slow rate following transport.

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221.10 FAILURE OF AXON FASCICULATION: A CLUE TO CNS REGENERATIVE FAILURE? S. Goldberg and B. Frank*. Department of Anatomy, University of Miami School of Medicine, Miami, Florida 33101.

We produced lesions in retinas of regenerating (newt, goldfish) and non-regenerating (mouse, rat, chick, rabbit) species and noted, via silver-stained flat mounts, the qualities of axonal sprouting from severed optic axons in the ganglion cell fiber layer of the retina.

In unoperated controls, all retinas had a bundled (fascicular) pattern of optic axons converging upon the optic nerve. Lesions in the regenerating species resulted in sprouts that formed fascicles as they grew. A fascicular pattern of growth has recently also been noted (Bohn and Reier, '80) following lesions in the optic nerve of Xenopus, another animal which regenerates.

Lesions in non-regenerating animals resulted in sprouts that had no tendency to adhere to one another as fascicles. Lack of fasciculation was also noted among axons that succeeded in bypassing the lesion site. On the other hand, adult mouse sciatic nerve axons (which do regenerate) formed fascicles when lesioned and diverted within the scarred region.

The absence of fasciculation among non-regenerating axons could reflect a lack of extracellular growing space in the CNS of these animals (see Krayanek and Goldberg, these meetings) or a deficiency in the mechanism of adhesive specificity between neuron and environment.

221.11 REGENERATIVE AXONAL ELONGATION IN THE ADULT MAMMALIAN CNS. S. David* and A.J. Aguayo. (SPON: M. Rasminsky). The Neurosciences Unit, Montreal General Hospital and McGill University, Montreal, Canada.

Using a new experimental technique that permits axonal growth to be directed into or from specific regions of the CNS, we have examined whether spinal and medullary neurons in the adult mammalian CNS are capable of extended axonal elongation. In a series of adult Sprague-Dawley rats and C57BL/6J mice, autologous segments of sciatic nerve (3.5 cms in rats and 2 cms in mice) were used as bridges to connect the medulla oblongata to the lower cervical or upper thoracic spinal cord. These bridging nerve grafts were placed extraspinally with one end inserted into the dorsolateral spinal cord and the other end inserted into the dorsolateral medulla. The spinal cord between these two levels was left intact. The animals survived without apparent neurologic deficits.

Grafts examined by light (LM) and electron microscopy (EM) 1-7½ months after surgery were well innervated by axons ensheathed by Schwann cells. The origin of these axons was determined by transecting the regenerated grafts and applying horseradish peroxidase (HRP) to the cut ends. LM of tetramethylbenzidine and H_2O_2 reacted sections showed retrogradely labelled neurons in the medulla and spinal cord. These neurons were located near the sites of insertion of the graft and their axons had elongated a maximum of 3 cms in rats and 1.5 cms in mice. The terminal course of axons in the graft was examined by anterogradely labelling the fibers with HRP. Using LM-HRP, anterogradely labelled fibers penetrated the CNS for approximately 2 mm. With EM-HRP (tissue reacted with diaminobenzidine and H_2O_2), these fibers were found to be ensheathed by CNS glia along their terminal course.

These results indicate that PNS bridges become innervated by axons from spinal and medullary neurons and that in this altered glial environment these CNS neurons are capable of marked axonal elongation. In addition, axons from the graft penetrate the CNS for short distances, where they are ensheathed by CNS glia.

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221.12 REGENERATION OF OPTIC AND PERIPHERAL AXONS IN THE FROG OPTIC TECTUM. Elliot I. Kaplan and Carmine D. Clemente. Brain Research Institute, UCLA Sch. Med., Los Angeles, CA. 90024.

Previous reports from this laboratory (Neurosci. Abstr. 5:679, 1979; Anat. Rec. 196:92A, 1980) have described the successful regeneration of peripheral axons into the optic tectum of Rana pipiens. Aberrant axons derived from surface grafts of the mandibular nerve were found to invade the entire rostro-caudal extent of the dorsal tectum, primarily within superficial layers 8 and 9. Since these layers also play host to the retinotectal projection, it was of interest to ask: (1)does optic denervation have an effect upon the aberrant fiber pattern? (2)how does the regeneration pattern of aberrant axons compare with that of optic fibers? (3)how might optic and peripheral axons simultaneously regenerate into the same target tectum?

To answer these questions, 23 nerve-grafted and 6 sham operated frogs received a contralateral optic nerve crush or retinal enucleation. These lesions were made either before or after (-2 to +7 weeks) the graft/sham surgery. Following a graft survival of 6-67 weeks, regenerating peripheral or optic nerves were labelled with horseradish peroxidase. The present results are based on light microscopic analyses of 40 μ tectal sections treated with tetramethyl benzidine or Hanker-Yates reagent.

Removal of the optic input to the host tectum produced no significant effect upon the rostro-caudal or medio-lateral extent of aberrant growth, but it did influence the laminar distribution of peripheral axons. If the retinotectal projection underwent degeneration before or during the active phase of aberrant ingrowth, peripheral fibers were able to assume a wider field in layer 9 than if the optic fibers were left intact. However, peripheral axons which had already entered and established themselves in a fully innervated host were unaffected by subsequent optic lesions. These findings suggest that the intact optic fiber layers may serve as mechanical constraints for growing peripheral axons. The denervation effect, then, would reflect a breakdown of such "barriers".

Comparisons between optic and peripheral regeneration patterns revealed a number of disparities. While optic fibers formed many fascicles and reestablished discernible layers in the tectum, aberrant axons failed to form organized bundles or layers. Most peripheral fibers grew singly and in a tortuous manner, occasionally in close association with blood vessels and myelinated axons of the host. Even when both fiber projections regenerated concurrently, they each displayed their respective growth patterns. Thus, both types of axon are able to grow and survive in the same CNS environment, but they do so differently and independently of one another.

221.13 EVIDENCE THAT TRANSFER RNA IS TRANSPORTED AXONALLY IN REGENERATING OPTIC AXONS OF GOLDFISH. M. Zanakis*, G. Chakraborty* and N. Ingoglia (SPON: F.P.J. Diecke), Depts. of Physiol. and Neurosci., CMDNJ-New Jersey Medical School, Newark, NJ 07103

Experiments were designed to determine if 4S RNA, which is transported axonally in regenerating optic axons of goldfish, is transfer RNA.

Eighteen days following bilateral optic nerve crushes, ^{3}H-uridine was injected into both eyes of 12 fish (labe]ing axonal and perikaryal RNA in the tectum). Four days later, ^{14}C-uridine was injected intracranially (labeling only perikaryal RNA in the tectum). Two days later, fish were sacrificed, left and right tecta were removed and pooled, and RNA was extracted with phenol and precipitated by adding 2.5 volumes of ethanol. 4S RNA was obtained by DEAE cellulose chromatography. The individual transfer RNA species were then fractionated either by methylated albumin kieselguhr (MAK) or BD cellulose chromatography. Optical density profiles of labeled 4S RNA were similar to commercially obtained E. coli tRNA and to profiles already described in the literature. Radioactivity profiles for both ^{3}H and ^{14}C followed O.D. profiles, and comparisons of ^{3}H to ^{14}C showed no significant differences between the two, within the experimental error of this study. Since at least half of the ^{3}H-4S RNA is intra-axonal (Ingoglia, 1981, Neurosci. Abstr.) and all of the ^{3}H-4S RNA has the same chromatographic mobility as the perikaryal 4S RNA as well as the E. coli tRNA, we conclude that intra-axonal 4S RNA is likely to be tRNA. Furthermore, since there was no difference in the level of labeling of various tRNA species in regenerating axons compared with tRNAs in surrounding cells, we suggest that the tRNA in regenerating axons is composed of a variety of tRNA species and not a unique species of tRNA.

Since ribosomes are not present in axons, it is unlikely that axonal tRNA is participating in classical protein synthesis. However, it might be participating in non-ribosomal, post-translational protein modification as described by Soffer (Adv. Enzymol., 1974, 40:91-97). If this is the case, then the enzyme responsible for this reaction, peptidyl transferase, should be present in these axons. Preliminary data indicate the presence of this enzyme in rat and goldfish brain, in rat sciatic nerves and in regenerating optic nerves and tracts of goldfish. Further experiments are planned to determine if this enzyme is axonally transported.

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221.14 NERVE GROWTH FACTOR PROMOTES GOLDFISH OPTIC NERVE REGENERATION. Henry K. Yip* and Bernice Grafstein. Dept. of Physiology, Cornell Univ. Med. Col., New York, NY 10021.

Previous studies have shown that NGF enhances the effects of axotomy on retinal ganglion cells in both the newt and in the goldfish (Turner, et al., Brain Res., 171:197, 1979; Brain Res., 197:319, 1980). These studies have demonstrated that following NGF treatment, there is a marked increase in perikaryal size and an acceleration of morphological changes. Cultured retinal ganglion cells pretreated with NGF also showed enhanced neurite outgrowth.

In the present study, we have further examined the role of NGF in promoting regeneration of goldfish optic nerve. NGF was administered by intraocular injection or by local application to the lesion site with silastic cuffs. The effects of NGF on the retinal ganglion cells were evaluated behaviorally and morphologically. The behavioral measure was the mean recovery time for the startle reaction to a sudden increase in illumination. Measurements of silver-stained regenerating axons were made by light microscopy. To determine whether the enhanced outgrowth was accompanied by changes in the cell body, we also examined the effects of NGF on nucleolar incidence and cell size.

Both β-NGF and 7S-NGF were effective in enhancing axonal outgrowth following optic nerve crush. The time for recovery of visual function was decreased by 20-40% as a result of NGF treatment. The number of regenerating axons and the mean axonal length were also significantly increased. The size of the retinal ganglion cell bodies and nucleolar incidence, however, did not change up to 14 days after the lesion. These results suggest that the outgrowth-promoting effect of NGF does not require a concomitant increase in RNA and protein synthesis above that which normally occurs during regeneration.

The effects produced by a single intraocular injection given at the time of the lesion were not improved by giving repeated injections throughout the experiment. The effect of NGF appeared to be dose-dependent, since doses smaller than 900 BU were not effective.

In a similar experiment, we have studied the effects of bovine brain gangliosides on regenerating goldfish retinal ganglion cells. Preliminary results indicate that gangliosides also have a promoting effect on axonal outgrowth.

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221.15 GLIAL CELL FACTOR(S) AFFECT REGENERATING GOLDFISH RETINA, IN VITRO. M. Schwartz, Y. Mizrachi*, A. Shahar*†and Y. Kimhi*. Dept. of Neurobiology, The Weizmann Inst. of Sci., Rehovot, Israel. Dept. of Virology, Israel Inst. for Biol.Res., Ness-Ziona, Israel.†

The regenerative capacity of the goldfish visual system is exhibited, in vitro, by the extensive outgrowth from retinal explants in the presence of 10% fetal calf serum (FCS), provided that the optic nerve had been crushed 10-14 days prior.

We have shown that neurotrophic factor in the goldfish brain can induce and maintain neuritic outgrowth from the explanted regenerating retina (Schwartz et al., submitted). The source of the neurotrophic factor(s) in the brain is not clear as yet.

As indicated in the present work, cloned rat glioma cells, in culture, release neurotrophic molecules that replace the ultimate need for FCS in the induction and maintenance of neurites from retinal explants. Light and scanning electron microscopy studies indicate that the morphology and the growth pattern of the neurites growing out of the goldfish regenerating retina in presence of glial factor(s) is different from that observed in FCS-containing media: The neurites in the former case are longer, have a lower tendency to fasciculate and show high density of varicosities. This observation may indicate that the glial conditioned medium does not provide all of the factor(s) participating either in the optic nerve regeneration in vivo or in the induction of outgrowth in vitro.

Preliminary results suggest that the active component is a protein of about 5.5S, that can be partially purified on a DEAE column. Addition of antibodies specific to mouse nerve growth factor (NGF) does not affect the growth induced by the glial factor.

The present work further supports the hypothesis that non-neuronal cells can supply their own neurons with trophic stimuli during development and during regeneration.

221.16 AXONAL CHANGES IN THE RETINAL STUMP OF THE TRANSECTED OPTIC NERVE. P. M. Richardson, V.M.K. Issa*, S. Shemie*, Dept. of Neurosurgery, McGill University & Neurosciences Unit, Montreal General Hospital.

The retinal stumps of rat optic nerves were examined by light and electron microscopy from one to 32 weeks after the nerves were cut intracranially. By one week, a zone of necrosis, perhaps due to infarction, had developed, extending from the cut end of the nerve approximately one half way to the globe but sparing the peripheral region of the nerve. During subsequent progressive degeneration, changes became evident sequentially in the intracranial portion of the nerve, the orbital segment, and finally the retina. At four weeks, the population of myelinated axons in the optic nerve was reduced to 10% normal; by 32 weeks, less than 1% remained. Ganglion cells in the periphery of the retina (identified by retrograde labelling with horseradish peroxidase) appeared to degenerate more slowly than those close to the optic disc. Although meagre compared to other fibre systems, some evidence of axonal outgrowth was seen. Single unmyelinated axons were scattered through the optic nerve stumps at all times after transection. Newly formed structures resembling synapses were found near the pial surface in some nerves.

Peripheral nerve grafts and cross-unions did not appear to enhance either the survival or regrowth of retinofugal fibres. Despite adverse local tissue conditions, some grafted cells survived and some optic nerve axons could be traced with radioautography to the tips of cut nerves. However, none of them have been seen yet to extend into a graft or become ensheathed by Schwann cells. The lack of response of optic nerve axons to peripheral nerve grafts may be due to intrinsic neuronal properties, poor local tissue perfusion or both.

221.17 EFFECTS OF LESIONS OF THE NEONATAL STRIATUM ON THE ORGANIZATION OF MIDBRAIN DOPAMINERGIC SYSTEMS. C.B. Jaeger, T.H Joh, and D.J. Reis. Lab of Neurobiology. Cornell Univ. Med. Coll. New York, NY 10021.

Unilateral lesions were placed in the corpus striatum (CS) in newborn rats to determine whether (a) the topographic organization of the nigrostriatal dopaminergic (DA) projection characteristic of adult rats (Moore & Bloom, Ann. Rev. of Neurosci. 2:113,1979) is established at a time when the striatum is still undergoing morphological and functional maturation; (b) neonatal injury would result in any reorganization of the pathway. The CS was ablated unilaterally by gentle aspiration in newborn rats, 6-24h of age, anesthetized with ether. At various times thereafter the distribution and connectivity of the mesencephalic DA neurons was studied by immunoctyochemical localization of tyrosine hydroxylase (TH), which catalyses the biosynthesis of DA, histochemical localization of acetylcholinesterase (ACHE) and retrograde transport of horseradish peroxidase (HRP). Lesions of the CS were varied. Complete ablation of the CS and ventral forebrain resulted in a unilateral loss of the majority of DA neurons in A9, A10 and A8. However, even after extensive unilateral lesions of the forebrain all of midbrain DA neurons did not disappear. Removal of the dorsolateral CS affected mostly A9 neurons. Lesions of the tail had no appreciable effect on DA neurons in the mesencephalon. ACHE, normally present in the neuropil of the zona compacta of the substantia nigra and ventral tegmentum of the midbrain, was strikingly absent in A9 and A10 regions coincident with loss of DA neurons. This observation indicates a close correspondence between neurons synthesizing ACHE and TH. HRP was injected into the unlesioned striatum of neonatally injured rats when they were adults. There was a small projection to the contralateral substantia nigra and mesencephalic tegmentum. Some of the retrogradely labelled neurons occupied similar positions as surviving DA neurons. However, HRP injected into unlesioned controls demonstrated a comparable cross nigrostriatal pathway of similar density. We conclude: (a) The topographic organization of the nigrostriatal projection is established prior to maturation of the target. (b) Interruption of this pathway in neonatal animals does not lead to evident reorganization of the remaining nigrostriatal system nor in hypertrophy, of a crossed collateral nigrostriatal pathway. (Supported by NIH grants HL 07379 and N.S.O. 3346).

221.18 AUTORADIOGRAPHIC TRACING OF TRITIUM-LABELLED CULTURED PERIPHERAL NON-NEURONAL CELLS IMPLANTED IN THE TRANSECTED CAT SPINAL CORD. J.R. Wrathall*, M.R. Braford and C.C. Kao*. Departments of Anatomy and Neurosurgery Georgetown University and Veterans Administration Medical Center, Washington, D.C. 20007.

Peripheral non-neuronal cells appear to provide support for regenerating PNS axons. Results from implantation of peripheral nerve segments into the spinal cord indicate that PNS non-neuronal cells may also facilitate regeneration of axons in the CNS. In order to further study the behavior of peripheral non-neuronal cells in the CNS, we prepared autologous cultures of such cells and labelled them in vitro with ^{3}H-thymidine so that they could be traced by autoradiography after implantation into the CNS. Each adult female cat was anesthetized and a segment of sciatic nerve removed, dissociated, and used to initiate cultures of autologous non-neuronal cells enriched in Schwann-like cells (J. Cell Biol. 83:97a, 1979). When sufficient cells had been generated, the cultures were exposed to ^{3}H-thymidine (0.33μCi/ml medium) for 72 hours, harvested, and a cell pellet ($\sim$2 x 10^{7}v.c.) taken to surgery. The cat was re anesthetized, a laminectomy performed, the dura opened and a "slit" transection performed through a small longitudinal incision in the pia-arachnoid at T10. In this microneurosurgical procedure, the spinal cord is completely transected but only about a 1mm gap is produced between the two spinal cord stumps. The labelled cells were then implanted to completely fill the gap and the wound closed in layers. In one group of cats, the exposed spinal cord was bathed in hypothermic saline ($<4^{\circ}$C) for 2 hours prior to and 1 hour post-transection, before the cells were implanted. Three or 7 days post-operatively the cats were perfused with saline and fixative and the cord processed for autoradiography. By 3 days after implantation the labelled PNS non-neuronal cells were observed to have penetrated 2-3 mm into the spinal cord stumps and were observed in areas of CNS axon terminals. Pre-implantation hypothermia had no observable effect on this rapid penetration by the peripheral cells into the cord but did reduce the later degeneration and cavitation within the spinal cord stumps. Thus, the cultured PNS non-neuronal cells appeared to rapidly move into the spinal cord and reach positions where interaction with spinal cord axons would be possible. (Supported by The Veterans Admin. & NINCDS NS 14413)

221.19 BEHAVIORAL ANALYSIS of SPINAL CORD REGENERATION in the SEA LAMPREY. Joseph Ayers, Gail A. Carpenter, Scott Currie* and James C. Kinch*. Marine Science Institute, Dept. of Biology and Dept. of Mathematics, Northeastern University, Boston, MA 02115

Recovery from complete transection of the spinal cord has been examined in ammocoete larvae, transforming larvae and fully transformed adults of the sea lamprey, Petromyzon marinus. The present investigation addressed two issues: (1). Do different behaviors recover with different time courses? and (2). How complete is the recovery of behavioral function?

The time course of behavioral recovery was ascertained by scoring animals for the reappearance of four behaviors in addition to forward swimming, including turning, backward crawling, burrowing and aversive withdrawal. Our findings indicate that some behaviors such as burrowing can appear prior to the reappearance of coordinated swimming, while others such as aversive withdrawal recover with even longer time courses. Thus, the processes which underly these different recoveries appear independent.

In order to assess the normalcy of recovered behaviors, computer algorithms which analyze digitized images from motion pictures (Abstr. Soc. Neurosci., 6: 466) were extended to calculate new movement parameters as well as measures of swimming performance. When compared to normal specimens, recovering transectees typically exhibit significant deficits in several of these parameters. Thus, while the regenerates appear normal in the quality of the behavior there are essential differences in quantitative aspects of the recovered motor output. Supported by an Alfred Sloan Foundation Fellowship and NSF Grant MCS 80-04021.

221.20 EFFECT OF OPTIC NERVE CRUSH ON RETINAL GANGLION CELLS IN HOODED RATS. L. Misantone, K. Barron, M. Gershenbaum*, V. Cipolla*, M. Zanakis* and M. Murray. Departments of Anatomy and Neurology, Phila. College of Osteopathic Med., Philadelphia; Albany Medial College, Albany, N.Y.: The Medical College of Pennsylvania, Philadelphia, PA. 19129

The failure of mammalian central neurons to regenerate after axotomy is a poorly understood phenomenon. One approach to defining the causes is to compare a mammalian central neuron with a successfully regenerating nonmammalian neuron. We compared the reaction to axotomy of non-regenerating retinal neurons (rat) with a regenerating retinal neuron (goldfish). Rat ganglion cells were axotomized by unilaterally crushing the optic nerve intracranially, and the rats were allowed to survive 1-28 days. The intact side served as a control. RNA content of retinal ganglion cells was measured cytophotometrically. Optic nerves were examined proximal and distal to the crush with LM and EM. Axonal transport was assessed in animals in which 25 uCi ^{3}H proline-leucine had been injected intraocularly 3-18 hr before sacrifice.

Cytophotometric examination of ganglion cells showed evidence consistent with a depletion of RNA content and cellular atrophy by 7d p.o. These changes became more pronounced at later times. Concurrently, the axons in the proximal nerve stump showed clear morphological signs of degeneration by 7d p.o. which becomes well defined by 14d. By 14d there was a significant loss of axons, a marked increase in glial cytoplasm and a noticable reduction in optic nerve diameter. By 28d few recognizably normal axons are observed. Distal to the crush degenerative changes are apparent within 7d p.o. and occur in the large diameter axons first. Reactive gliosis is well advanced by 14d. Transport studies over 1-28d p.o. demonstrate that the intraocularly labeled protein accumulates at the crush site, suggesting that axonal transport continues in proximal axons up to 28d p.o. In contrast to the rat, goldfish retinal ganglion cells show cellular hypertrophy and increased RNA metabolism after axotomy. There is also an increase in amount of axonally transported material. These changes are early and dramatic components of the regenerative response to axotomy. These events do not appear to occur in the nonregenerating rat optic neurons, although some morphologically normal axons persist for several weeks, and there is an indication that some degree of axonal transport continues.

(Supported by EY 03360; NS 16101; NS 16556; Veterans Administration).

221.21

WITHDRAWN

221.22 AXON REACTION IN TRIGEMINAL NEURONS FOLLOWING MANDIBULAR NERVE GRAFTS TO THE FROG OPTIC TECTUM. Carmine D. Clemente and Elliot L. Kaplan. Brain Research Institute, UCLA Sch. of Med., Los Angeles, CA. 90024.

How does the cell body respond to aberrant regeneration of its axon? In an attempt to answer this question, the following experimental paradigm was used: the proximal stump of a transected mandibular nerve was grafted onto the surface of the optic tectum in adult Rana pipiens. Details of the procedure and of the non-specific peripheral fiber growth within the host tectum have been reported previously (Neurosci. Abstr. 5:679, 1979; Anat. Rec. 196:92A, 1980; this meeting). In the present study, parent neurons in the trigeminal motor (Mot V) and mesencephalic (Mes V) nuclei were analyzed morphometrically and in terms of cell numbers.

In response to nerve graft surgery, there was a dramatic axon reaction in cell bodies of the ipsilateral Mot V and Mes V. This was characterized by a transient increase in nucleolar, nuclear, and somal cross sectional areas, beginning within 1 week post surgery (WPS), peaking by 6 WPS, and gradually returning toward normal levels thereafter (followed for as long as 67 WPS). Although the recovery rates for the two nuclei were similar, Mot V neurons tended to reach their maximal response levels more rapidly than Mes V cells (2 WPS as opposed to 6 WPS). The most severely affected cell component was the nucleolus, which swelled by as much as three times in Mot V and two times in Mes V. At no time was chromatolysis observed. As a matter of fact, during the first 2-3 WPS, cytoplasmic basophilia increased somewhat over control levels.

These changes in neuronal morphology reflect a strong anabolic reaction, one which has a temporal coincidence with the regeneration of grafted axons. Also, the distinctions between Mot V and Mes V suggest that the motoneurons may be better equipped to respond effectively to nerve transection and translocation. However, the more vigorous Mot V response might instead be due to the fact that these neurons are situated closer to the nerve end than are the Mes V cells.

To test whether the nerve graft might provoke a loss of trigeminal neurons, cell counts were carried out from 1 day to 24 WPS. Whereas Mot V cells were maintained at all survival times studied, there was a delayed drop-out of 25-30% of Mes V cells between 12 and 24 WPS. This, taken together with a qualitative estimate of sensory cell loss in the trigeminal ganglion, suggests a greater viability of regenerating motoneurons. Whether the Mot V neurons are permanently stable and therefore correlated with the long term survival of peripheral axons in the host tectum is still under investigation.

221.23 DIMETHYL SULFOXIDE, TRYPSIN AND HYPERBARIC OXYGEN TREATMENTS FOLLOWING SPINAL CORD TRANSECTION IN RATS, J. B. Gelderd, D. E. Bowers, Jr.*, S. F. Deschner*, W. P. Fife*, and D. W. Welch*. Dept. of Anatomy, Coll. of Med. and Dept. of Biology, Coll. of Sci., Texas A&M University, College Station, TX 77843.

A previous study in our laboratory indicated a synergistic therapeutic effect between dimethyl sulfoxide (DMSO) and hyperbaric oxygen (HBO) following spinal cord transection in rats (Gelderd et al., Undersea Biomed. Res., 7:305, 1980). The present study was designed to assess the efficacy of combining trypsin, DMSO and HBO treatments in promoting regeneration and return of function following spinal cord transection.

Sixty, adult, female rats served as experimental animals. Following spinal cord transection at the T-8 vertebral level, animals were separated into groups of 15 each for treatment as follows: Group I received only normal postoperative care required for paraplegic animals; Group II received a 0.5cc subcutaneous injection of 20% DMSO in phosphate buffer (pH 8.0) over the lesion site every 12 hrs for 15 days; Group III received the identical treatments given animals in Group II, with the addition of 0.4mg trypsin included in each injection medium; Group IV received the identical treatments given animals in Group III, in addition to daily HBO treatments of 90 min. duration at 2.82 atmos. absolute. All treatments were initiated within 15 min. following spinal cord transection. Systematic behavioral testing was conducted at 30-day intervals to assess hindlimb function. All animals were sacrificed 180-200 days postlesion by intracardiac perfusion with fixatives. The spinal cords at the lesion sites were removed and processed for either transmission electron microscopy, scanning electron microscopy or light microscopy (Bodian silver stain, Gomori's trichrome).

No return of hindlimb function was observed during the postoperative period in any of the animals. Histological data revealed scar formation at the lesion site with cavitation formation rostral and caudal to the lesion. The rostrocaudal extent of these cavitations varied within and between treatment groups (3mm-11mm), with Group I revealing the most extensive cavitations. The walls of the cavitations were lined by occasional ovoid cells with branching processes. Nerve fibers were seen within the lesion in all animals at the light and electron microscopic levels with ultrastructural data revealing both myelinated and unmyelinated axon profiles. At this time, the origin and termination of these nerve fibers has not been determined. It was concluded that the combined trypsin, DMSO and HBO therapy used in this study did not produce a demonstrable, synergistic, therapeutic effect. Supported by a grant from Paralysis Cure Research.

222.1 TENSION OVERLAP IN SPLIT VENTRAL ROOTS OF SKELETAL MUSCLES OF THE CHRONIC SPINAL CAT. V. R. Edgerton, E. Eldred, L. A. Smith*. Dept. Kinesiology, Anat. and Brain Res. Inst., UCLA, CA. 90024.

In the developing soleus (SOL) of a kitten, the muscle fibers revert to slower contracting fibers of slow-twitch staining profile; during a comparable period the polyinnervation characteristic of the very young kitten gives way to a single anonal innervation of each fiber. Cutting the spinal cord prevents to some degree the conversion of fiber types, whether it also interfers with loss of polyinnervation is not known.

Tests with the tension occlusion method were made on SOL and medial gastrocnemius (MG) muscles of 8 cats cordotomized (C) at 2 or 12 wk and sacrificed at 14 to 18 wk of age. As controls 9 normal (N) kittens of 2 or 12 wk were tested. Other hindlimb muscles were denervated, the SOL and MG were freed of fascia and the tendons connected to a stiff myograph. Ventral roots L_7 and S_1 or portions thereof were placed on two bipolar electrodes so as to yield approximately equal tension responses at L_0 muscle length.

For the SOL no significant overlap of either twitch or tetanic tensions was found in either group of (C) cats, nor in the 12 wk intact kittens. In the MG, overlap was consistently noted in the 2 and 12 wk transected cats, as well as the intact 2 and 12 wk cats. These findings suggest that after cordotomy polyinnervation in the SOL disappears as in normal animals. The detection of MG tension overlap under near isometric conditions, even in the adult animal may be a result of the more, complex pennate arrangement of fibers in this muscle. In any case, in neither muscle did the cord transection appear to affect the picture of tension overlap presented.

	$\bar{X}$ % Overlap SOL				$\bar{X}$ % Overlap MG			
Group	Tw	(n)	Tet	(n)	Tw	(n)	Tet	(n)
2 wk N	/		/		+13	4	+30	4
2wk C	-2	4	+6	4	+16	4	+22	4
12 wk C	-2	3	/		/		/	
Adult N	+1	4	+2	3	+20	5	/	

Supported by NIH grant NS 16333

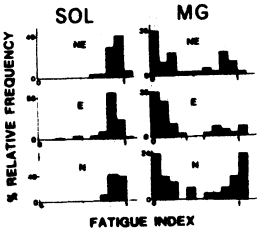
222.2 FATIGUE PROPERTIES OF SINGLE MOTOR UNITS IN EXERCISED AND NON-EXERCISED CHRONIC SPINAL CATS. L.A. Smith*, V.R. Edgerton, E. Eldred. Depts. Kinesiology, Anat and Brain Res. Inst., UCLA Los Angeles, CA 90024

Spinalization in the cat results in a reduction in the contraction time (CT) of the soleus (SOL) as well as a conversion to a fast twitch staining profile of its fibers (Edgerton, Neurosci. Abst., 1979). It has also been reported that the alteration in CT was not related to the amount of neuromuscular activity the cat received.

The present study was made to determine the effects of chronic spinalization and exercise on fatigue of single motor units in the SOL and medial gastrocnemius (MG) muscles. Twelve cats were cordotomized at the T_{12} level at either 2 or 12 weeks (wk) of age. Six were exercised on a motor driven treadmill 15 to 20 min/day for 12 to 14 wk post-surgically. Normal animals were also studied as age group controls. Motor units (MU) were identified by the teasing of ventral root filaments.

The measn MU CT for the SOL for the non-exercised (NE), exercised (E) and normal (N) groups was 49 (± 1.901), 50 (± 1.307), and 62 (± 1.815) respectively. The mean values for the MG for the same groups was 37 (± 1.426), 32 (± 0.943) and 36 (± 1.078). Mean fatigue index (FI) values for the MU of the SOL were .89 (± 0.014), 0.84 (± 0.016), and 0.88 (± 0.009) for NE (n=28), E (n=60), and N (n=30). MG mean FI values were 0.39 (± 0.054), 0.30 (± 0.054), and 0.52 (± 0.073).

The figure below contrasts the FI for MU of MG and SOL. The FI of MU in both muscles were not appreciably affected by exercise. The persistence of a high index in the SOL muscle which shows a shortened CT and altered histochemical profile, supports the conclusion that the conversion of fibers is from SO to FOG. The properties responsible for changing the CT apparently are not the same as those that regulate the fatigue properties.



Supported by NIH grant NS 16333

222.3 THE MYOELECTRIC SIGNAL VERSUS FORCE RELATIONSHIP IN DIFFERENT HUMAN MUSCLES. J. H. Lawrence*, C. J. De Luca, A. P. Xenakis* and R. S. LeFever* (SPON: G. Bilotto). Neuro Muscular Research Lab., Children's Hospital Medical Center, Harvard Medical School, Boston, MA 02115.

The present study has been initiated to investigate if the normalized surface myoelectric (ME) signal vs. normalized force relationship varies in different human muscles and if it is dependent on exercise. The data were obtained from a carefully controlled set of experiments which involved the biceps, deltoid and first dorsal interosseous muscles of three pianists, four long-distance swimmers, three powerlifters and six normals. This study presupposed that if type of training influences the ME signal - force relationship or if this relationship is muscle-dependent then this would be revealed through the ten "specialists". These specialists, chosen from among the world's best, exhibit varying degrees of fine motor control, endurance training and power training in different muscles. A total of 400 isometric linearly force-varying contractions peaking at either 40% or 80% of the maximal voluntary contraction (MVC) level were processed, grouped and averaged. The rms amplitude of the ME signal was smoothed with a Hamming window filter. The resulting data were normalized and plotted as a function of normalized MVC.

The results indicate that for the group-averaged data the shape of the ME signal - force curve is primarily determined by the muscle under investigation and is generally independent of the subject group and the force rate. Whereas this relationship is linear for the first dorsal interosseous ($r>0.98$), it is nonlinear for the biceps and deltoid. Paired t-tests revealed a significant ($p<.0001$) difference between these three curves at 20%, 40% and 60% of MVC. A large intersubject variation was noticed for the same muscle. The ME signal - force relationship may be associated with the different anatomical architecture, function and motor unit control properties of each muscle [R.S. LeFever and C.J. De Luca, Abstracts of the Soc. for Neuroscience, 1979].

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222.4 POSSIBLE FIBER TYPING BY ANALYSIS OF SURFACE EMG SIGNALS. R.G. Rosenthal,* M.A. Sabbahi, R. Merletti* and C.J. De Luca. NeuroMuscular Research Laboratory, Children's Hospital Medical Center, Boston, MA., Liberty Mutual Research Center, Hopkinton, MA., and Politecnico di Torino, Italy.

Fast twitch muscle fibers have long been known to have higher conduction velocities than slow twitch muscle fibers. Furthermore, intramuscular conduction velocity has previously been reported to correlate with the median frequency of the EMG signal. This is possibly due to the association between the spectral compression of the EMG signal and the change in the muscle pH during a contraction. The median frequency of the EMG signal and the pH of the muscle have been observed to decline during a sustained contraction [F.B. Stulen, Ph.D. Thesis, M.I.T., 1980]. Yet, there have been no reports of the correlation between the surface EMG signal and the fiber composition of a muscle.

In this study, surface electrodes were placed on various muscles of 12 normal male subjects. They performed isometric contractions equivalent to 20%, 40%, 80% and 100% of their maximal voluntary contraction level. EMG signals were monitored from contractions of the soleus, medial gastrocnemius, vastus medialis, vastus lateralis and first dorsal interosseous muscles. The median frequency of the signals obtained was calculated on-line and in real-time using a newly developed analog Muscle Fatigue Monitor (MFM). The percentage changes of the initial median frequency of the EMG signal measured at the beginning of the muscle contraction was obtained at the various force levels.

Our preliminary results indicate that at higher levels of contraction, the percentage change of the initial median frequency was minimal in the soleus muscle, whereas in the other muscles, it gradually increased in direct correlation with the increased percentage of fast twitch fibers within these muscles.

Such a non-invasive technique for the detection of muscle fiber predominance in a given muscle has potential values in both physiological and clinical settings. (Supported in part by Liberty Mutual Insurance Co. and NIHR, grant #23-P-55854.)

222.5 ISOMETRIC TORQUE PRODUCED BY THE CAT HAMSTRINGS MUSCLE ABOUT THE ANKLE AS A FUNCTION OF HINDLIMB POSITION. Roger W. Wicke* and Felix E. Zajac (SPON: C.C. Boylls). Rehab. Eng. Res. Dev. Ctr.(153), VA Med. Ctr., Palo Alto, CA 94304.

Cuff electrodes were implanted around semitendinosus (ST) and posterior biceps (PB) muscle nerves in two cats anesthetized with pentobarbital. Each cat was placed in a frame which rigidly restrained the pelvis. To measure torque about the ankle, a pin through the tibia was rigidly fixed to the frame, and a force transducer was placed around the foot. The moment arm was measured from the force transducer to the ankle joint. Isometric torque was recorded during maximal simultaneous stimulation of the electrodes for a range of combinations of hip, knee, and ankle angles.

Torque is highly dependent on all joint angles. Maximum torques are produced at the physiological extremes of knee extension and ankle dorsiflexion, and torque approaches zero at opposite extremes. Active torque of up to 20 kg-cm about the ankle can be produced. This indicates that the hamstrings must be considered as a three joint muscle group in the cat. After the torque measurements had been obtained from the intact hindlimb, active torque was once again measured at *various stages of dissection of skin and fascia.* Almost all of the torque was produced by transmission of PB and ST force through their tendinous insertion on the calcaneus via the interconnecting fascial sheath. After additional dissection to isolate soleus muscle and nerve, we measured ankle torque generated by maximal stimulation of soleus at all ankle joint angles. Hamstrings can produce up to three times greater torque than soleus for certain hindlimb positions.

In the range of hindlimb positions occurring during the E2 phase of locomotion in the cat, the hamstrings are capable of producing 5 to 8 kg-cm of torque at the ankle in comparison with 6 to 7.5 kg-cm produced by soleus. The fact that ST and PB EMG activity occurs in E1 (Rasmussen et al, J. Morph. 155:253, 1978) suggests that ankle torque contributed by the hamstrings may be an important component during E2.

Supported in part by NIH grant NS 11971.

222.6 MECHANICAL INTERACTIONS OF FAST-TWITCH AND SLOW-TWITCH MUSCLES DURING PLANTAR FLEXION OF VARYING SPEEDS. R. S. Hutton and R. M. Enoka. Dept. of Kinesiology, Univ. of Washington, Seattle, WA 98195

It has been proposed that recurrent inhibition, which is presumed to act more powerfully on tonic than on phasic alpha motoneurons (Cullheim & Kellerth, J. Physiol., 281:301, 1978; Eccles et al., J. Physiol., 159:479, 1961), and selective recruitment order of synergistic motoneurons (Kanda et al., Exp. Brain Res., 29:57, 1977; Smith et al., J. Neurophysiol., 43:612, 1980) may function to prevent slowly contracting muscles from impeding rapid contraction. The possibility that such mechanical interactions do occur was investigated kinematically by analysis of plantar flexion in rat hind limb (Sprague-Dawley, 300-350g) under loaded and unloaded conditions. The left limb was denervated to isolate the LG-Sol nerve and muscles. Distal attachments of MG, PL, BF, ST and Q were cut. The knee was clamped at 98° (σ±9). Plantar flexion was induced before and after Sol denervation by posterior tibial nerve stimulation (100Hz) at 3x twitch threshold with the ankle in a 102° (σ±11) resting position (unloaded), 52° (σ±9) dorsi-flexed position (held by a stimulus-synced retractable bar) or with ankle position set by a 50-500g load applied in series to the intact Achilles tendon. Foot movements were filmed at 500 f/s, digitized, smoothed by digital filter, and differentiated to determine velocity and acceleration. Kinematic data before/after denervation are shown in the following table:

Load (g)	n	Peak Vel. rad/s (σ)	t-Peak Vel. ms (σ)	Peak Accel. rad/s/s	t-Peak Accel. ms (σ)
0	4	18±5/18±4	14±3/13±5	475/423	9±2/9±2
50	3	24±3/23±2	25±1/25±2	983/1176	15±6/12±2
100	4	21±3/18±4	36±4/32±2	851/782	20±3/17±3
200	4	17±3/15±4	44±6/41±5	591/552	18±4/17±4

With the ankle near maximal dorsi-flexion at stimulus onset, peak velocities associated with passive elastic muscle properties approached values achieved by active contractile mechanisms. Under unloaded and loaded conditions no evidence was found in support of mechanical interference by Sol of maximal contractile LG responses. Contributions of Sol tension to foot velocity and acceleration began to emerge under loaded conditions between 100-300g but did not become prominent until loads >300g were applied. Findings were in agreement with previous reports (Walmsley et al., J. Neurophysiol., 41:1203, 1978; Spector et al. J. Neurophysiol., 44:951, 1980) that the slow twitch Sol muscle, though fully activated, cannot contribute effectively to plantar flexion movements falling within a critical dynamic range.

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222.7 ELECTRICAL AND MECHANICAL FATIGUE PROPERTIES IN CAT MOTOR UNITS OF IDENTIFIED TYPE. H.P. Clamann and A.J. Robinson* Dept. of Physiology, Medical College of Virginia, Richmond, Va. 23298.

Although it has been suggested that muscle or motor unit fatigue may occur by failure in the electrical or the mechanical mechanism, only mechanical fatigue of the different motor unit types has been studied in detail. The present experiments were designed to compare the susceptibility to electrical fatigue of the three motor unit types and to relate electrical to mechanical fatigue in these same units. In cats single motor axons of medial gastrocnemius or soleus motor units were isolated in finely divided ventral root filaments. Force was recorded from the distal tendon of the appropriate muscle, and the electrical activity of motor units was led off from bare pins inserted into the same muscle. The electromyogram (EMG) was full-wave rectified and electronically integrated to permit recording the integral (IEMG) of individual motor unit potentials during stimulation of the motor axon. Motor units were classified as type FF, FR, or S according to criteria of Burke and others. Motor unit fatigue was induced by tetanic stimulation at 80 pps. This stimulus rate was greater than the tetanic fusion frequency of nearly all units including type FF, and was slow enough to prevent overlap of successive EMG waveforms. All motor units were stimulated at the same rate to allow direct comparison of fatigue properties.

Type FF motor units lost the ability to produce force after 10-15 seconds of stimulation; a smooth decline almost to 0 of the EMG waveform followed loss of force. Such a pattern of force fatigue followed by EMG failure is seen with other stimulus patterns also. Partial recovery of twitch force and full restoration of EMG amplitude occurred within seconds. FR units generally showed a smooth simultaneous decline of electrical and mechanical activity; force declined by over 75% in 45-60 seconds. S units showed marked changes in the EMG before any loss of force was measured. As with other unit types, the EMG waveform increased in duration and then began to decline smoothly in amplitude. After 30-45 seconds abrupt fluctuations in EMG amplitude (jitter) appeared and persisted. IEMG declined by over 50% before any force decrement occurred.

It is concluded that during fatiguing contractions, readily fatiguable motor units lose the ability to contract while the EMG is still virtually normal; EMG fatigue precedes mechanical failure in fatigue-resistant units. Electrically as well as mechanically, FF units are best suited to phasic use while S units respond best to tonic drive. Supported by Grant # NS-11677 from NIH, and a United Nuclear predoctoral grant to AJR.

222.8 EFFECTS OF STEROID TREATMENT ON PROPERTIES OF MOTOR UNITS IN RED AND PALE CAT HINDLIMB MUSCLES. A.J. Robinson*and H.P.Clamann (SPON: A.J. Szumski). Dept. of Physiology, Medical College of Virginia, Richmond, Virginia 23298

The administration of glucocorticoids has been shown to induce atrophy and weakness in whole skeletal muscle. These effects are more pronounced in pale muscle than in red muscle. The present study was designed to examine the effects of steroid treatment on the mechanical,electrical, and fatigue properties of single motor units in the pale, medial gastrocnemius(MG) and red soleus muscle of the cat. The properties of MG and soleus motor units from normal cats were examined and compared to those of MG and soleus units in steroid-treated(3-4mg triamcinolone acetonide per kg per day, 10-16 days) animals. Steroid treatment produced alterations in the strength- and speed-related properties of motor units that were more pronounced in fast-twitch than in slow-twitch units. The mean maximum tetanic tension($\overline{P_{max}}$) for types FF(fast-twitch, fatiguable) and FR(fast-twitch,fatigue-resistant) units were reduced by 63% and 71% respectively. The $\overline{P_{max}}$ for type S (slow-twitch,fatigue-resistant) units in MG was reduced by 25% but was unchanged for type S units in soleus. The mean rate of rise of tetanic tension($\overline{dP/dt}$) for FF, FR, and S units in MG were reduced by 79%, 54%,and 25% respectively following steroid treatment. The $\overline{dP/dt}$ for type S units in soleus in steroid-treated animals was increased by 73% over controls. Steroid treatment lengthened the mean twitch contraction time($\overline{CT}$) of MG type FF units and shortened the $\overline{CT}$ of type S units in both MG and soleus. The mean integrated EMG signals elicited from FF and FR units in MG were unchanged following steroid treatment but were significantly increased for type S units in both pale and red muscles. The susceptibility to fatigue of the three classes of motor units in MG and of soleus motor units was unchanged as a result of steroid administration. These findings suggest that those units most frequently activated in muscular contractions(type S units) are the least susceptible to steroid-induced changes in their contractile properties. In contrast, those units activated only occasionally in muscular contractions (FF and FR units) appear to undergo preferential changes in their strength- and speed-related contractile properties. Since the overall frequency of activation of motor units correlates well with the size of their motoneuron cell bodies, these results imply that the Size Principle can be extended as a general rule to not only account for the recruitment order and overall degree of use of motor units in muscular contractions but also to account for the patterns of motor unit involvement seen in steroid-induced myopathy. Work supported by grant #NS11677 from NIH to HPC and United Nuclear predoctoral grant to AJR.

222.9

WITHDRAWN

222.10 A COMBINED EXPERIMENTAL AND COMPUTATIONAL METHOD FOR THE REMOVAL OF CROSS TALK FROM AN ELECTROPHYSIOLOGICAL SIGNAL. P.L. Weiss,* I.W. Hunter and R.E. Kearney. (SPON: L. Wolfe). Biomedical Engineering Unit, McGill University, Montreal, Canada, H3G 1Y6

Surface electrodes are commonly used to record electrophysiological signals since they can be rapidly applied and can monitor potentials emanating from a broad section of tissue. However, these electrodes also pickup other adjacent signals, which tend to contaminate the waveform of interest. EEG is often marred by noise from eye movement potentials and the ECG often impinges on respiratory muscle EMG. Since the spectra of the signals being studied (EEG and EMG in the examples above) and the unwanted, contaminating signals (EOG and ECG, respectively) overlap considerably, the use of selective filters is impractical.

One traditional approach that could be used to solve this problem would be to simply subtract the unwanted component, monitored on its own channel, from the required signal. However, this would only be possible if the separately recorded noise signal does not differ from the noise component of the physiological signal of interest. That is, it must be recorded with no time delay, no difference in scaling, and not be filtered.

We have developed an experimental method which enables the removal of unwanted waveforms using system identification techniques (Kearney & Hunter, 1981) and does not require that the two waveforms be similar. The approach has been implemented for a specific problem and consists of the following procedures: (1) The impulse response between the Input Signal (ECG_1 from electrodes placed on the chest) and the Output Signal (EMG plus ECG_2 from electrodes placed over a respiratory muscle) is identified. (2) The impulse response is convolved with the Input Signal to create the Predicted Output. (3) The Residual Output (respiratory EMG-ECG) emerges when the Predicted Output is subtracted from the Output Signal.

The Residual Output is thus an EMG signal which is only minimally marred by ECG. Data collected and analyzed in this manner are shown below; Figure 1a is the original Output Signal including both EMG and ECG and Figure 1b is the modified output signal (= Residual Output) showing negligible ECG. The original ECG (Fig. 1c) is included for comparison.

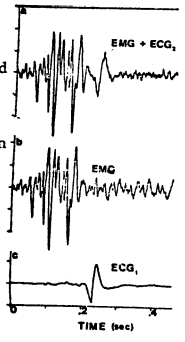

Fig.1

Kearney, R.E. & Hunter, I.W. (1981). APS - A package for the analysis of physiological systems. 14th Canadian DECUS Symposium, Montreal.

222.11 CHRONIC EXPOSURE TO 60-HZ ELECTRIC FIELD ENHANCES RECOVERY FROM FATIGUE IN MAMMALIAN SLOW-TWITCH MUSCLE. R. A. Jaffe, B. L. Laszewski* and D. B. Carr*. Biology and Energy Systems Depts., Battelle Memorial Institute, Pacific Northwest Laboratory, Richland, WA 99352.

Reports in the literature have suggested that mammalian and avian nervous systems can be affected by exposure to extremely low frequency (ELF) electromagnetic radiation. In a study of electrical substation workers (Sazonova), strength-duration curves for the adductor pollicis muscle appeared to show a significant increase in excitability following a working day exposure to high-voltage ELF fields. Experiments in the present study were designed to detect possible effects of chronic exposure (30 days) to 60-Hz electric fields (100 kV/m; unperturbed field strength) on neuromuscular function in the rat.

The exposure system is capable of simultaneously exposing 144 rats, housed in individual plastic (lexan) cages, to uniform vertical, 60-Hz electric fields. Extensive tests have been made, and document that the exposure system is free of corona discharge and ozone formation and that the animals do not receive spark discharges or other shocks in the housing system during exposure to electric fields. The system is free of significant levels of harmonic distortion and vibration. Electric field measurements showed that the electric field strength within a cage varies by about 7%, due to the effects of the lexan cage material, and that the differences among fields in different cages are less than 3%. The effective field strength was about 65 kV/m, a reduction caused by mutual interaction (shielding) among adjacent animals during exposure.

Isometric force transducers were attached to the tendons of the plantaris (predominantly fast-twitch) and soleus (predominantly slow-twitch) muscles in the urethan-anesthetized rat. Square-wave stimuli were delivered to the distal stump of the transected sciatic nerve. Several measurements were used to characterize neuromuscular function, including: twitch characteristics, chronaxie, tetanic and post-tetanic potentiation, and fatigue and recovery. The results from three independent series of experiments are reported. Only recovery from fatigue in slow-twitch muscles was consistently and significantly affected (enhanced) by electric-field exposure. This effect does not appear to be mediated by field-induced changes in either neuromuscular transmission or in the contractile mechanism itself. It is suggested that the effect may be mediated secondary to an effect on mechanisms regulating muscle blood flow or metabolism.

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222.12 ARCHITECTURE OF CAT HINDLIMB MUSCLE: IMPLICATIONS FOR FUNCTIONAL SIGNIFICANCE. R.D. Sacks* and R.R. Roy* (SPON: A. Herrera). Neuromuscular Research Lab., Dept. of Kinesiology, UCLA, L.A., CA 90024

Since it is known that a muscle's architecture dictates to a major degree the force and velocity properties of that muscle, this aspect of 24 major cat hindlimb muscles was determined. Muscle wet weights, muscle lengths, fiber lengths, and angle of pinnation were used to calculate physiological cross-sectional area (PCA). Average sarcomere lengths were obtained for selected flexor, extensor and adductor muscles. Within a given muscle, the fiber length to muscle length ratio was essentially the same in all regions for almost all muscles. The calculated PCA of the knee extensors was similar to that of flexors (16.43 cm^2 vs 16.83 cm^2) whereas for the ankle extensor muscles it was more than two times greater than the major flexors (15.61 cm^2 vs 7.61 cm^2). Ratios of wet wt./predicted maximal tetanic tension for each muscle was calculated using the value 2.3kg/cm^2 for specific tension. This ratio provided a convenient means to compare the priority of muscle force vs muscle length-velocity for a give mass of muscle. For example, the mean value of this ratio for FDL was 0.96 vs 0.39 for its synergist FHL. Therefore, although these two muscles serve the same function, it appears that the FDL is designed for force production whereas the FHL is designed for velocity. The adductor femoris is unique in that it has a wide range of fiber lengths but they are in proportion to the length of that portion of the muscle from which they are obtained. Assuming the entire muscle to be biochemically homogeneous, the fibers at the distal end, which are three times longer than at the proximal end, must contract three times faster in order for all fibers to participate in maximum force production. This difference is resolved by considering the architectural features: the fibers at the distal end have 3 times as many sarcomeres in series and consequently contract 3 times faster. Therefore, it is evident that the architectural features can affect the speed of shortening of a muscle as much as its intrinsic biochemical properties. These results suggest that the complementary nature of the biochemical and morphologic features of muscles and muscle groups seem to minimize the complexity of the neural control required to produce effective movements.

222.13 MYOFIBRILLAR GEOMETRY OF SINGLE MUSCLE FIBERS AS REVEALED BY LASER DIFFRACTION STUDIES. A.F. Leung*, Y.M. Cheung* and J.C. Hwang. Dept. of Physics, The Chinese University of Hong Kong and Dept. of Physiology, Faculty of Medicine, University of Hong Kong, Sassoon Rd., Hong Kong.

Analysis was made on the intensity minima and maxima of the diffraction patterns of isolated single skeletal muscle fibers of frog. As the intensity distributions along the zero- and first order diffraction lines follow the Bessel function, a cylindrical shape for the myofibril was indicated. Myofibrillar diameters could then be predicted from studies of the intensity minima and maxima. Such calculated values of the myofibrillar diameters are in general agreement with those obtained from electron microscopic studies. When the sarcomere length was set at 2.9 μm, the calculated myofibrillar diameter increased when the muscle fiber was transferred to hypotonic solutions and decreased in hypertonic solutions. Experiments were then performed in which the sarcomere length (S) was varied by passive stretch. The changes in myofibrillar diameters at different sarcomere lengths were again in general agreement with the prediction when the volume of the myofibril (V) was assumed to be constant, (V=A). However a slight departure of the myofibrillar diameters (D) or volumes (V) from the predicted values could also be observed. The volume (V) then becomes a function of (A+BS) where B is a second constant and BS describes the departure from a constant volume. Such slight departure could be readily explained by a model in which the myofibril is assumed to be a ribbed cylinder with the ridges occurring at the Z lines. (supported in part by the Research Grants Committee, University of Hong Kong).

222.14 INTRACELLULAR LOCALIZATION OF CONCONAVALIN A (CON A) RECEPTORS IN DE-EPONIZED SECTIONS OF NORMAL AND DISEASED HUMAN MUSCLE. W. F. Odenwald*, V. Askanas, and W. K. Engel. Dept. of Neurology, Univ. of S. Calif. School of Medicine, Los Angeles, Calif. 90033

Intracellular localization of Con A receptors in glutaraldehyde-fixed muscle blocks is difficult due to the poor penetration of lectins through muscle plasmalemma. Therefore, we have developed a technique which allows direct binding of Con A to muscle cell organelles. This technique can be applied to study the binding of several different lectins to serial cross-sections of normal or diseased muscle, or other cells in order to define the profile of lectin binding to normal or pathologic subcellular organelle, and hence delinate the profile of sugar components in that organelle. One-micron-thick sections of glutaraldehyde-fixed, epon embedded human muscle were mounted on plastic cover slips, completely de-eponized with 50% saturated solution of ethanolic NaOH, rinsed with absolute ethanol and air-dried. After incubation with 0.1 M lysine-HCl in phosphate buffer, the sections were incubated in 20 and 10 ug/ml Con A in 0.1 M phosphate buffer. Then a modification of the technique of Bernhard and Avrameas (Exptl Cell Res 64, 232-236, 1971), utilizing horseradish peroxidase and DAB was applied for visualizing the sites of Con A binding. Following postfixation in 1% osmium, the sections were dehydrated, re-embedded in Epon and thin-sectioned parallel to their surface. Omitting Con A from the incubating medium, incorporating the Con A inhibitor α-methyl-d-mannoside (0.2M) in the incubation medium served as controls for the specificity of the reaction. In normal human muscle, staining was localized on the cell membrane complex (basement membrane and plasmalemma), t-tubules, sarcoplasmic reticulum, nuclear envelope and glycogen granules. Mitochondrial membranes remained unstained. In myophosphorylase deficiency, Con A binding was also strongly visualized under the muscle plasmalemma which ultrastructurally corresponded to the staining of abnormally accumulated glycogen granules, which is characteristic of this metabolic defect. In muscle fibers of familial idiopathic hypokalemic periodic paralysis, characterized by the presence of large vacuoles, which are known to be greatly dilated t-tubules, in the muscle fibers, Con A was localized on the membrane of those vacuoles and occasionally inside vacuoles bound to glycogen granules and to amorphous material. Controls were negative in all normal and diseased human muscle. Con A defines α-d-manoside (and, to lesser extent, α-d-glucoside) sugar components. This technique can now be extended to other lectins that will define other sugars to achieve at the ultrastructural level profile of sugar components in serial sections of a given organelle.

223.1 EVOKED LOCOMOTION AFTER DESTRUCTION OF DEITERS' NUCLEUS. R.M. Jell, L.M. Jordan and D.J. Ireland. Department of Physiology, University of Manitoba, Winnipeg, CANADA R3E OW3.

Recent studies have provided convincing evidence for a connection between descending vestibular outflow and the spinal stepping generator by way of direct excitatory influence on the FRA pathway to extensor motoneurons and to interneurons which mediate group IA reciprocal inhibition. The possibility that this outflow is necessary for initiation of locomotion was raised by Shik et al (Biophysics, 11:756-765, 1966) with the demonstration that some neurons in Deiters' nucleus (DN) became tonically active before the onset of locomotion evoked by electrical stimulation of the mesencephalic locomotor region (MLR), and that these neurons later became rhythmically active in synchrony with stepping. Further evidence was presented by Steeves and Jordan (Neuroscience Letters, 20:283-288, 1980) in a selective lesioning study of descending spinal tracks necessary for initiation of locomotion, showing that a region of the ventrolateral quadrant containing the lateral vestibulospinal tract (LVST) was necessary for MLR evoked locomotion. We present results which demonstrate that initiation of locomotion is not abolished by bilateral lesions of DN.

Treadmill locomotion induced in decerebrate cats by electrical stimulation of MLR was studied before and after bilateral (and, in some cats, unilateral) lesioning of DN with a hot probe using a dorsal approach. Hindlimb extensor rigidity, present before the lesion, was abolished bilaterally (or ipsilaterally) after lesioning. Hindlimb locomotion was initially abolished bilaterally (or ipsilaterally) but could be evoked with variable threshold after 2 or 3 hours. The quality of hindlimb stepping tended to be altered but ankle extensor and flexor electromyographs revealed appropriate levels and phasing of muscle activity.

These results support and extend the findings of Orlovsky (Brain Res. 40:359-371, 1972), who reported initial ipsilateral abolition of MLR evoked stepping after unilateral DN lesions, with eventual return, and strongly suggest that the reticulospinal pathways carry the descending command signals for initiation of locomotion.

223.2 EVIDENCE FOR RHYTHMIC EXCITATION AND INHIBITION OF FLEXOR AND EXTENSOR MOTONEURONS DURING FICTIVE LOCOMOTION. S.J. Shefchyk*, J.E. Menzies* and L.M. Jordan. Dept. of Physiology, Univ. of Manitoba, Faculty of Medicine, Winnipeg, CANADA R3E OW3.

Various models of the central pattern generator for locomotion have been proposed, but the details of the synaptic inputs which produce motoneuron rhythmic activity have not been determined. Previous studies have shown that the rhythmic firing of motoneurons during "fictive" locomotion is accompanied by rhythmic activity in flexor- and extensor-coupled Ia inhibitory interneurons (Feldman and Orlovsky, Brain Res. 84:181, 1975; McCrea et al. J. Neurophysiol. 44:475, 1980). This suggests that both flexor and extensor motoneurons are subject to rhythmic inhibitory input, which alone cannot account for the rhythmic activity of motoneurons (Menzies et al, Neuroscience Abst. 4, 1219, 1978). In the present study the nature of excitatory and inhibitory synaptic input to identified flexor and extensor motoneurons during fictive locomotion was examined.

Fictive locomotion was produced by stimulation of the mesencephalic locomotor region in decerebrate cats paralyzed with Flaxedil. Intracellular recordings were obtained using microelectrodes filled with 3M potassium acetate or 3M potassium chloride. Motoneurons were identified on the basis of their monosynaptic and antidromic responses to stimulation of the nerves to anterior biceps-semimembranosus, posterior biceps-semitendinosus, vastus lateralis, v. intermedius, v. medialis, sartorius, gracilis or tibialis anterior muscles. Conductance measurements obtained using a bridge circuit for injection of hyperpolarizing pulses through the recording electrode revealed the presence of synaptic input during both the depolarized and hyperpolarized phases of locomotor activity in flexor and extensor motoneurons. Intracellular chloride injection and strychnine administration (0.1 mg/kg, i.v.) revealed that these techniques could reduce or abolish the hyperpolarized phase of the locomotor cycle in all the types of motoneurons examined. The rhythmic excitatory input persisted in these experiments, and the amplitudes of excitatory postsynaptic potentials were increased. These studies establish that alternating excitatory and inhibitory synaptic input produce the depolarizing and hyperpolarizing membrane potential shifts in mammalian flexor and extensor motoneurons during fictive locomotion. The parallel effects of strychnine on the disynaptic IPSPs produced by Ia afferent stimulation and on the hyperpolarizing potentials during locomotion suggest that Ia inhibitory interneurons are the source of the rhythmic inhibition of motoneurons. It is concluded that a system of excitatory interneurons driven by or forming a part of the central pattern generator is responsible for the depolarization of motoneurons.

223.3 CAT FORELIMB MODEL PROVIDING FORCE AND EMG ACTIVITY FROM A SINGLE ELBOW EXTENSOR IN THE UNANESTHETIZED ANIMAL. D.D. ROSCOE AND M.W. KEITH*. Rehab. Eng. Cen., Case Western Reserve Univ., Cleveland, Ohio 44106.

An animal model is presented which will allow measurement of force and EMG activity of a single elbow extensor muscle in the cat forelimb to be recorded during voluntary movements in the unanesthetized animal.

Surgery was performed on the cat's forelimb using strict sterile techniques. First, the epitrochlearis muscle was completely excised. The tendons of the medial (accessory, intermediate and short portions) and long heads of the triceps muscle were then detached at their insertion on the olecranon. With these two muscle heads partially reflected proximally, the anconeus muscle was excised exposing the distal one-third of the humerus. The medial and long head tendons were reattached with sutures pulled through a small hole drilled posterior-to-anterolaterally through the humerus, just proximal to the olecranon fossa. It is critical that the tendons are reinserted into bone so that the forces generated by the medial and long heads are directed away from any adhesions formed with the lateral head during healing. The lateral head of the triceps was left intact and serves as the only extensor muscle across the elbow. Before closing the incision, the triceps were electrically stimulated and checked for activity. Only the lateral head produced elbow extension.

Two weeks post surgery, the cat was able to extend the elbow of the operated forelimb against gravity. To verify that this extension was due solely to the lateral head of triceps, the cat was anesthetized and percutaneous intramuscular electrodes were implanted in the medial and long heads. Maximal stimulation of these heads produced no elbow extension.

The animal model has been developed to evaluate a modified version of a new EMG processing technique (Hogan and Mann, IEEE Trans. Biomed. Eng. 27:382, 1980) to be used as a source of command signals in the control of powered prostheses or the control of forearm and hand movements by the quadriplegic patient using functional neuromuscular stimulation of paralyzed muscles. An electrode array will be implanted in the lateral triceps with lead wires exiting via a pyrolitic carbon transcutaneous connector located in the cat's back. Because of the surgical reconfiguration of the cat's forelimb, it will be possible to externally measure elbow extension forces produced by the unanesthetized animal during a trained task and correlate these forces to the processed EMG activity obtained from the functionally isolated elbow extensor. (Supported by NIHR grant G008005815).

223.4 COMPARTMENTALIZED ORGANIZATION OF FIBRE TYPES AND RECEPTORS IN THE TRAPEZIUS MUSCLE IN THE CAT. J. Keane* and F.J.R. Richmond (SPON: J. Milligan). Dept. of Physiology, Queen's University, Kingston, Ontario, K7L 3N6.

It is increasingly recognized that all muscles may not function as a single homogenous structure. Rather some muscles may be sub-divided into functional compartments, each with a different extrafusal fibre composition and a characteristic receptor grouping. One especially clear example of a compartmentalized muscle is the cat trapezius, a muscle with 3 distinct heads. One head, called clavotrapezius, originates from the lambdoidal crest and nuchal midline and inserts on the clavicle. The other two heads, acromiotrapezius and spinotrapezius both have a wide midline origin from cervical and thoracic vertebrae and insert on the scapula. In addition to confirming the separation of motor (spinal accessory) and sensory (C2 to T3) innervation to this muscle, examinations of the motoneurone organization, fibre histochemistry and receptor organization have demonstrated that each head of trapezius has unique characteristics.

Retrograde transport of horseradish peroxidase has shown that all trapezius motoneurones are located in the spinal accessory nucleus but that motoneurones supplying different muscle heads are grouped in different regions. Histochemical studies of extrafusal fibres showed that slow fibres accounted for less than 15% of the total fibre populations in clavotrapezius but about 30% and 50% of acromiotrapezius and spinotrapezius respectively. Muscle spindle densities were similar from one muscle head to another, and ranged from about 24 spindles per gram in acromiotrapezius and spinotrapezius, to about 34 spindles per gram in clavotrapezius. The form and arrangement of spindles differed in different heads. Spinotrapezius spindles usually existed singly and had an intrafusal fibre content with two bag fibres and 4 to 5 chain fibres. Acromiotrapezius and clavotrapezius spindles had more complex arrangements. In both acromiotrapezius and clavotrapezius there were circumscribed regions where spindles had a greater variation in their intrafusal fibre contents, and spindles with a single bag fibre were commonly seen. These same spindles were commonly linked with one another in complex spindle forms. Observations suggest that within the 3 heads of trapezius there may be further sub-compartments which differ in their receptor distribution and sensory innervation.

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223.5 EVIDENCE FOR AUTHENTIC CHANGES IN THE GAIN OF AN AUTOGENETIC REFLEX IN THE SOLEUS MUSCLE OF THE DECEREBRATE CAT. T. R. Nichols* (SPON: H. D. Patton). Dept. of Kinesiology, Univ. of Washington, Seattle, WA 98195

A skeletal muscle with its associated reflex apparatus, together known as the motor servo, presents a mechanical stiffness which depends upon the gains of autogenetic reflex pathways and upon nonlinearities in the motor servo (Houk, J.C., Ann. Rev. Physiol., 41:99-114, 1979). The latter mechanism obtains in the case where stiffness varies with the initial or the mean force exerted by the muscle. It has been shown that, for a given central state, stiffness depends upon force but rather less upon muscle length (Feldman, A.G., Neurosci. 5:81-90, 1980). Therefore, authentic changes in reflex gain are indicated if the changes in stiffness occur independently of a change in force.

Static force-length trajectories were obtained from the soleus muscle in the decerebrate cat. In some cases, trajectories had similar thresholds but different slopes. Average stiffnesses varied over a two-fold range over the same range of forces. Transient changes in stiffness were measured during the application of ramp stretches and releases at a variety of initial forces and lengths. The stiffness of the regulated muscle varied over a three-fold range when ramps were initiated from the same initial force and length. These results indicate that the changes in stiffness were probably not due to nonlinearities associated with the motor servo. Changes due to a progressive decline in the condition of the preparation were also excluded. In addition, the static force-length trajectories could not be accounted for by the length-tension curves of a fixed population of muscle fibers contracting at a constant firing rate except at short lengths and high forces. The ramp responses could not be accounted for by the responses of unregulated muscle (no reflex action) except at very high forces.

These results indicate "spontaneous" changes in the gains of one or more autogenetic reflex pathways which probably arise from muscle spindle receptors.

(Supported by an N.I.H. fellowship and by the Graduate School Research Fund, University of Washington)

223.6 ELECTROMYOGRAPHIC ACTIVITY PATTERNS OF EMBRYONIC CHICK HINDLIMB MUSCLES. M. J. O'Donovan*, M. W. Cooper* and Lynn T. Landmesser (SPON: M. Bak). Dept. of Biol., Yale Univ., New Haven CT. 06511.

We have recorded spontaneous and evoked electromyographic activity of several identified extensor and flexor muscles of the hip and knee in stage 34-36 chick embryos unilaterally in ovo, and bilaterally in an isolated spinal cord-limb preparation maintained in vitro. In ovo muscle electrical activity was recorded using flexible suction electrodes (tip diam. 100-150μ) inserted through a window in the shell after exposure of the appropriate muscles. Embryonic temperature was maintained at 35°± 2°C using an in ovo thermistor controlling a heating coil around the egg. Recordings in ovo revealed several different forms of muscle activity. Most frequently observed was a recurring, patterned sequence of bursts, in which activity alternated between antagonistic muscles. Each burst was typically 1-2 seconds long, and the whole sequence, lasting up to 30 seconds, was composed of several cycles of antagonistic activity. Occasionally, following such a period of phasic activity, both antagonistic and synergistic muscles co-contracted in a tonic discharge of variable duration, occasionally for as long as 1 minute. Other irregular and unpatterned activity was also seen. Patterned alternation of antagonistic muscles, could be evoked by tactile or electrical stimulation of the forelimb or electrical stimulation of the anterior cutaneous nerve in the thigh.

Evoked and spontaneous activity was also recorded bilaterally from the same group of muscles in an isolated cord-limb preparation (ICP), transected at the cervical cord, and maintained in oxygenated Tyrode at 30°C for several hours. In contrast to the in ovo behavior, ICP muscle activity consisted only of sequences of phasic alternation of antagonistic muscles that could occur spontaneously after several hours in the bath or be evoked earlier by a single electrical stimulus to the ventral surface of thoracic or lower cervical cord. Bilateral recordings revealed that identical muscles in both limbs were co-active during these sequences. The patterning and duration of activity between different muscles in the isolated preparation was similar to that observed for spontaneous alternating sequences in the intact embryo, suggesting operation of the same neural elements, which may represent the embryonic homologue of the intraspinal locomotor generator described in other vertebrates. (Supported by NIH Grant NS10666 to LTL, an MDA postdoctoral fellowship to MO'D and NIH Grant GM07527 to MWC).

223.7 EFFECT OF MUSCLE LENGTH ON FORCE-VELOCITY PROPERTIES OF CAT SOLEUS DURING MOVEMENT. S. A. Spector*, R. J. Gregor* and S. C. Bodine* (SPON: W. F. H. M. Mommaerts). Neuromusc. Res. Lab., UCLA, 90024.

The soleus (Sol) muscle's contribution to total force production during movement depends, in part, on its capacity to generate the required forces (F) and velocities (V) at the Achilles tendon. The in situ F-V properties of Sol suggest that this muscle's peak shortening velocity (Vmax) precludes its effective participation during movements requiring high rates of shortening (> 160mm/s; Spector et al., J. Neurophysiol., 44:951, 1980). Since Sol may shorten up to 15% of its resting muscle length (ML) during these movements (Goslow et al., J. Morphol., 141:1, 1973), the effect of ML on F-V properties and Vmax of this muscle is of interest, also.

To study the effect of ML, approximately 180 afterloaded tetanic contractions of Sol (n=5) were initiated from ML's varying from 85 to 110% of the length (Lo) at which peak tension (Po) was developed. Tendon displacements, F and V were determined for each contraction, and discrete F-V points were grouped according to the ML at which peak V of the contraction occurred. The linear form of Hill's equation, (Po-P)/V=P/b+a/b, was used to estimate Vmax for each ML.

The results show that average Vmax exhibits greater sensitivity to changes in ML than does Po. At 93% of Lo, Sol develops 94% of Po, whereas this muscle can generate only 82% of Vmax observed at Lo. Similarly, at 103% of Lo, Po is affected minimally (2%) while Vmax is reduced to 88% of the value at Lo (156mm/s).

The complete F-V plots at different ML's for one Sol are shown in Figure 1. The peak Vmax that can be developed for any given load is continually reduced as ML is decreased below Lo. These data indicate that during movements requiring rapid displacement of the Achilles tendon, the participation of the Sol is not only comprimised by its inability to generate significant amounts of force (due to its F-V characteristics), but also by its dependence on ML to develop sufficient shortening velocities.

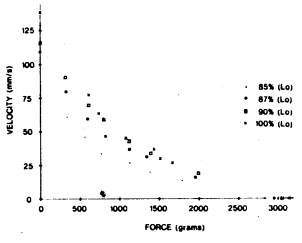

Figure 1: Force-velocity relation of Sol at different ML's.

223.8 COMPARISON OF NORMAL USE AND REFLEX RESPONSE PATTERNS OF CAT DISTAL HINDLIMB MUSCLES. L.D. Abraham, G.E. Loeb, and W.B. Marks. Lab. of Neural Control, NINCDS, NIH, Bethesda, MD 20205

In order to determine the function of individual muscles in the cat distal hindlimb, we compared reflex response patterns with normal patterns of muscle use in a variety of behaviors.

Chronic recording techniques were used to obtain EMG records from the following muscles: medial and lateral gastrocnemius (MG, LG), soleus (SOL), plantaris (PLT), flexor digitorum longus (FDL), flexor hallucis longus (FHL), tibialis posterior (TP), tibialis anterior (TA), extensor digitorum longus (EDL), flexor digitorum brevis (FDB), and peroneus longus (PL). Individual muscle length and tension were obtained with implanted transducers specially designed for and attached to the appropriate tendons. Stimulating electrodes were implanted on the medial plantar and sural nerves. Analog and video recordings were made during jumping, scratching, paw shaking, standing, and treadmill locomotion at different speeds. Reflex responses were recorded to brief single shocks occurring during treadmill walking at random points in the step cycle. Terminal experiments provided anatomical and mechanical data for individual muscles, as well as calibration of the implanted gauges.

In general, neither the normal use nor the reflex recruitment of these muscles could be accurately predicted from the classical anatomical dichotomy of flexors and extensors. Rather, the actual use of each muscle appeared governed by the specific requirements of the task and detailed mechanical specializations intrinsic to each muscle (e.g. fiber type and orientation) and extrinsic (e.g. insertions producing non-axial or joint-angle-dependent torques). For example, reflex responses of FDL and FHL were consistent with differences between these digit flexors in normal use (see Fleshman, Lev-Tov, and Burke, Soc. Neurosci. Abstr., 1981), and with differences in muscle fiber orientation. Thus FDL appears to be specialized for rapid digit flexions against low loads, while FHL is used for more static contractions against relatively large loads during extension. TP provided several examples of dissociation from its classical ankle extensor synergists MG, LG, and SOL. During scratching (which combines ankle flexion/ extension with inversion), extensor EMG was almost entirely from TP. TP activity at the end of stance during running (after activity in MG, LG, and SOL had ceased) reflected its increasing mechanical advantage as the ankle extends.

This type of kinesiological analysis provides a basis for relating patterns of connectivity and activity of motoneurons and detailed structural specializations of muscles with patterns of normal use.

223.9 SYNAPTIC ORGANIZATION IN FDL AND FHL MOTOR NUCLEI: A SEARCH FOR MECHANISMS UNDERLYING THE FUNCTIONAL DISPARITY IN STRICT ANATOMICAL SYNERGISTS. J. W. Fleshman, A. Lev-Tov*, and R. E. Burke. Lab. of Neural Control, NINCDS, NIH, Bethesda, MD 20205.

The flexor digitorum longus (FDL) and flexor hallucis longus (FHL) muscles of the cat are strict anatomical synergists that together flex the distal hindlimb phalanges and protrude the claws. However, previous studies (see O'Donovan et al., Soc. Neurosci. Abstr. 5:380, 1979 and 6:858, 1980) have shown that these muscles are clearly not functional synergists during locomotion. FHL behaves as a stereotyped antigravity extensor, with activity throughout the stance phase of stepping at all speeds. In contrast, FDL is a functional flexor, active primarily in the early swing phase, with little stance activity even during high speed locomotion. In addition, FDL exhibits non-stereotyped "facultative" burst behavior related to perturbations in individual step cycles and not found in FHL. We assume that functional differences reflect differences in synaptic organization in the overlapping FDL and FHL motor nuclei and we have begun to search for such differences using conventional intracellular recording and electrical stimulation methods in unanesthetized, spinal cats (anemic brain destruction). Mean amplitudes (±SD) of homonymous, heteronymous and total group Ia EPSPs were generally larger in FHL as compared to FDL motoneurons:

Motoneurons	FDL Ia EPSPs	FHL Ia EPSPs	Number
FDL	4.8 ± 2.6 mV	1.9 ± 0.9 mV	20
FHL	2.4 ± 1.2 mV	7.2 ± 2.9 mV	30

Plantaris (PL) Ia afferents produced small or negligible Ia EPSPs in most FDL and FHL motoneurons but PL cells received FDL and FHL Ia EPSPs, with FDL EPSPs > FHL in 16/17 cases. Polysynaptic PSPs were examined in motoneurons with membrane potentials >55 mV. Stimulation of the saphenous and superficial peroneal nerves produced mixed PSPs in which excitatory components were common and often predominant in FDL cells but small and often undetect- able in FHL motoneurons. Stimulation of the sural nerve and the ipsilateral hindlimb central plantar pad produced mixed PSPs with no consistent difference between FDL and FHL. Stimulation of the palmar pad or superficial radial nerve in the ipsilateral fore- limb produced polysynaptic inhibition in most FHL motoneurons but little or no hyperpolarization in FDL cells. The stronger monosynaptic Ia projection to FHL cells (implying stretch responsiveness) fits their more stereotyped antigravity role while the apparent sensitivity of FDL cells to distal skin excitation may be related to FDL's "facultative" action in perturbed steps, which is presumably attuned to other sensory feedback. The significance of forelimb input to FHL cells remains to be clarified.

223.10 RESPONSES TO CONTROLLED POSTURAL DISTURBANCES IN THE CAT. D. S. Rushmer and C. J. Russell, Neurological Sciences Institute, Portland, OR 97209.

Normal cats instrumented with chronic EMG electrodes were subjected to controlled postural disturbances during quiet standing. The disturbances consisted of anterior-posterior translations and independently controllable vertical movement of the supports under the four paws. EMG responses from shoulder and forelimb muscles and vertical forces from each of the four paws were recorded during the disturbances, and stick figures were constructed from videotape. EMG responses had "preferred" latencies of 15-20, 55-60, and 95-105 msec., times similar to those seen in the hindlimb of the dog (Mori and Brookhart, Am. J. Physiol. 215:339, 1968). The timing and amplitude variability of the response indicate that they are highly stereotyped. A major feature of the response to both forward and backward translation is stiffening of the elbow joint, so that most of the joint angle changes occur at the shoulder and digits. In backward translation, which loads the forelimbs, greater EMG activity is seen.

Drop of the support under one forelimb provokes a weight shift to the contralateral forelimb and ipsilateral hindlimb (Massion and Smith, Br. Res. 61:400, 1973), but without any concomitant placing response. When the paws on opposite sides of the body are raised and lowered in a reciprocal fashion, the action of the hindlimbs and forelimbs differs. Considerable stiffness is maintained in the elbow and wrist, so that most of the changes in forelimb position are effected by the raising or lowering of the scapula. In the hindlimbs, on the other hand, the pelvis moves very little, and most of the joint angle change occurs at the knee and ankle.

The results of more complex stimulus presentation, such as pairs of disturbances presented in sequence at various intervals, and unexpected change to a second type of stimulus after repetition of one type of stimulus for a number of trials, suggest that the response to each disturbance is organized independently over a wide range of inter-stimulus parameters.

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223.11 ANKLE EXTENSOR JOINT TORQUES AND MUSCLE FORCES DURING CAT, LOCOMOTION. M. R. Zomlefer. Rehab. Eng. Res. and Dev. Cntr.(153), VA Medical Center, Palo Alto, CA 94304.

Cats (n=6) were trained to walk across a force plate which recorded vertical and horizontal contact forces during the stance phase for one hindlimb. Hindlimb trajectories were also measured using a Selspot system with an effective sampling rate of about 160 Hz. A biomechanical model for the cat hindlimb was programmed into a minicomputer (HP 21-MX), and the combined force-trajectory information served as system inputs for the model. Numerical techniques were used to estimate limb segmental velocities and accelerations, and the model then predicted the net torque about the ankle joint. Acute follow-up studies were conducted to determine parametric data (masses, moments of inertia,lengths) required by the computer algorithms.

In order to verify the model's predictions, a buckle transducer was placed over the Achilles' tendon in three animals; this transducer recorded net force produced by the principal ankle extensor muscles,i.e. soleus,medial and lateral gastrocnemius, and plantaris. The computed torque values and the known geometry of the hindlimb were then used to estimate the (minimum) net ankle force required to generate predicted torque. The computed and measured net ankle extensor forces were in good agreement, usually within 10% of one another. For the walks studied, corresponding to locomotion speeds of 0.3-0.8 ms^{-1}, the inertial terms used by the algorithm proved insignificant. Hence, for slow walks, the determination of ankle extensor torque during the stance phase requires measurement of ground contact forces and limb position.

Supported by Swedish MRC 3026 and 5402 and Jasper Fellowship at the University of Montreal. Experimental work and computer modelling were conducted at Karolinska Institute (Prof. S. Grillner).

223.12 THE EFFECT OF BIOMECHANICAL PARAMETERS ON PROGRAMMING LANDING IN CATS. P.A. Reback, J.L. Smith & R.J. Gregor*. Neuromotor Control Lab & Brain Research Institute. UCLA, L.A. CA. 90024

In our previous report (Neurosci. Abst.,1980) data were presented suggesting that forelimb EMG is predetermined centrally for voluntary landing. The onset of extensor EMG activity was shown to be constant across all jump heights for both flight and landing bursts with reference to landing and not to take-off. This study discusses the kinematic and kinetic parameters of jumping, and how they may be influenced by neural control mechanisms.

Five adult cats were trained to jump down onto a force plate from a platform placed at heights of 1.2, 1.0, 0.8, & 0.6m. Ground reactive forces (GRF) and EMG of the biceps and lateral triceps were coordinated with 16mm high speed film(100 fr/sec).

The peak GRF rises with increasing jump height and doubles as jump height changes from 0.6m (29N ± 8.28) to 1.2m (63N ± 9.59). At impact, this force brings about a quick elbow flexion (F) resulting in a rapid increase in angular velocity (ω). Although the average peak ω increases with each jump height, there is a wide distribution of values from 150-1600 °/sec at each height studied. However, the times to reach maximum ω and maximum elbow flexion post-landing are independent of jump height and remain constant at 26ms and 90ms respectively.

At landing, the cat experiences a 40ms flexion torque (T) followed by an extension T that lasts for the duration of elbow flexion. As expected, the T values vary between cats, increasing with larger moments of inertia of the forearm. In the individual cat, the T about the joint is consistent across all jump heights during the landing phase. Typical ranges for peak flexion and extension T are 18-26 kgm/kg body wt and 9-12 kgm/kg body wt respectively.

The variability of the GRF and ω at a given jump height suggests that there is considerable generality in the programming of the movement that may be compensated by the inherent properties of the muscle. An interaction between neural mechanisms and inherent muscle properties in controlling landing is implied by the temporal consistency at which maximum ω and elbow flexion occur. Finally, the independence of T values to jump height, in contrast to the GRF which parallels jump height, indicates that there is a substantial transfer of angular momentum to other parts of the body. This suggests that descending pathways may be responsible for adjusting the relative position of body segments in insure adequate transfer of angular momentum.

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223.13 CHRONIC RECORDINGS OF ONGOING UNITARY ACTIVITY IN VESTIBULAR NUCLEI DURING VESTIBULAR COMPENSATION. D.W. Jensen. Dept. Otorhinolaryngology/Program in Neuroscience, Baylor College of Medicine, Houston, TX. 77030.

Guinea pigs compensate for a unilateral vestibular neurectomy relatively quickly (several days) in terms of spontaneous postural asymmetries (eye nystagmus and lateral head deviation, e.g.). However, in terms of the movement evoked directional preponderance of vestibular reflexes, the compensation is much slower (several weeks). In order to determine the time courses of long-term changes in the mean rates of ongoing discharges in central vestibular and reticular areas during these compensations, guinea pigs are being implanted with arrays of up to 10 chronically indwelling fine wire microelectrodes. Multiple and single unitary activity is biotelemetered to data acquisition equipment via a miniature FM transmitter, or by direct wire. The mean rate of ongoing discharges is monitored in the alert animal before and after a unilateral vestibular neurectomy.

Two animals have been analyzed. Ongoing activity levels in all positively identified vestibular nuclear loci recorded from thus far have remained depressed to less than half of their prelesion levels for the first 48 hours of compensation. During this time, 98% of the postural asymmetry abatement occurs.

These results and others will be discussed, especially with respect to the working hypothesis that activity levels in the vestibular nuclei are expected to be reflective of movement-evoked responses, and their compensation, and not reflective of asymmetrical postural tonus and its compensation.

223.14 RESPONSE PATTERNS OF CENTRAL VESTIBULAR NEURONS TO MODULATED OTOLITH INPUT. R. H. Schor, A. D. Miller, and V. J. Wilson. Rockefeller Univ., New York, NY 10021.

Using roll tilt, responses of single vestibular neurons and EMGs of neck and forelimb extensor muscles were obtained from 23 decebrate cats. These animals previously had all six semicircular canals rendered insensitive to angular acceleration by being plugged with bone dust. Stimulation consisted of various combinations of sinusoidal roll tilt, covering a frequency range of 0.01 - 2 Hz, providing a modulated otolith stimulus. Vestibulospinal neurons were identified by antidromic activation from the vestibulospinal tracts in the cervical cord; units were also tested with electrical stimulation of the entire labyrinth.

Below 0.1 Hz, the muscle responses are in phase with tilt, with forelimb extensors most active during side-down tilt, neck extensors during side-up; at higher frequencies of tilt, significant phase lags develop in these reflexes (Schor and Miller, J. Neurophysiol., in press). Neural responses could be classified on the basis of two criteria -- at low frequencies of tilt, maximum firing could occur during side down or side up tilt (the alpha and beta responses of Duensing and Schaefer). In addition, the behavior of the phase of the neural response over the frequency range of 0.01 to 2 Hz was usually either flat, and in phase with position, or showed a phase-lagging response similar to that observed in neck and forelimb muscles (M-like in table).

	Alpha		Beta		Other
	Flat	M-like	Flat	M-like	
All	51	1	9	38	29
Vestibulospinal	30	1	6	22	14
Monosyn. from lab.	21	0	4	14	16
Not monosynaptic	10	0	2	11	2

The two most prominent response patterns were flat alpha responses and muscle-like beta responses. The distribution of neurons shown to project to the spinal cord was similar to the entire population's. Similarly, units receiving a monosynaptic input from the labyrinth (receptor unknown) as well as those without such input are present in these classes. The phase-lagging beta responses are appropriate for contributing to the reflexes observed in the ipsilateral neck and contralateral forelimb; the role of vestibulospinal neurons with flat alpha responses awaits further study. (Supported by NASA grant NSG-2380, NIH grant NS02619, and PHS Fellowship 5-F32-NS06128).

223.15 CIRCLING INDUCED BY INJECTION OF GABA AND GABA ANTAGONIST PICROTOXINE INTO THE SUPERIOR COLLICULUS. C. Geula* and D. Asdourian. Lab. of Biopsychology, Wayne State University Department of Psychology, Detroit, MI. 48202.

Lesions of the Superior Colliculus have been shown to lead, among other things, to ipsiversive circling. Recent anatomical evidence has shown an extensive projection from Substantia Nigra Pars Reticulata to the deeper layers of the Superior Colliculus(SC), and biochemical findings suggest that GABA is the neurotransmitter involved in this projection. The aim of the present study was to investigate the role of GABA in SC-induced circling behavior. Intracranial injections of GABA, the GABA antagonist Picrotoxine or isotonic saline were made into the SC. Injections of GABA induced ipsiversive circling while injections of Picrotoxine induced contraversive circling. In both cases assymetry was also present. Saline had no effect upon circling behavior. The results suggest that GABA activity in the SC may be involved in motor assymetry.

223.16 TETRAHYDROCANNABINOL POTENTIATION OF RESERPINE-INDUCED CATALEPSY. D. E. Moss, S. N. Deyo*, J. Rogers, and S. B. McMaster*. Psychobiochem. Lab., Psychology Department, University of Texas at El Paso, El Paso, TX 79968, and A. V. Davis Center for Behav. Neurobiol., The Salk Institute, San Diego, CA 92138.

Delta-9-tetrahydrocannabinol (THC), a substance found in marihuana, potentiated reserpine-induced catalepsy in rats as measured in the "bar test" which times how long rats will stand with their forepaws on a horizontal bar 9 cm high. The rats were pretreated with 7.5 mg/kg reserpine (RES) or a placebo ip 24 hours before the tests for catalepsy in all experiments. The subjects were also pretreated with THC (1 to 10 mg/kg) or placebo by gavage 1, 2, 5, 12 or 24 hr before the bar test. THC, which produced no catalepsy by itself, produced a dramatic dose-dependent potentiation of RES-induced catalepsy with a more than 20 fold increase observed at 10 mg/kg THC ($p < .001$). The THC effect was fully developed by 1 hr after THC administration and was completed by 12 hr. The THC/RES catalepsy was slightly enhanced by the cholinesterase inhibitor physostigmine salicyclate (0.5 mg/kg) ($p < .05$), slightly but not significantly reduced by the anticholinergic scopolamine HBr (2 mg/kg), but almost completely blocked by ethopropazine (30 mg/kg), an anticholinergic antiparkinsonian drug ($p < .01$). THC/RES catalepsy was completely unaffected by naloxone (1 mg/kg).

Because the new phenomenon reported here shows that THC has important motor effects in RES-induced catalepsy which is an animal model of Parkinson's disease, it may be that THC or a similar drug can be used to increase the therapeutic potency of RES in hyperkinetic motor disorders. RES was one of the first drugs used successfully in the treatment of various choreiform disorders (Kempinsky et al., Neurol. 10, 38, 1960) and has been shown to be of benefit in tardive dyskinesia (Soto et al., Dis. Nerv. Sys. 32, 680, 1971; Villenuve and Boszormenyi, Lancet 1, 353, 1970) and in the treatment of Huntington's disease (Klawans, Eur. Neurol. 4, 148, 1970). Although the mechanism by which THC produces this dramatic interaction with RES is unknown at this time, receptor binding and other pharmacological experiments are being conducted in order to elucidate this phenomenon. It is hoped that the results of these experiments can lead to increased effectiveness in drugs used for the treatment of choreiform disorders as well as provide a behavioral model that can be used to identify neurochemical actions of THC that have important functional significance.

Supported by NINCDS/MBS Grant RR08012-9 to D. E. M. and a gift from the Moss family.

223.17 INVOLVEMENT OF NUCLEUS INTERPOSITUS IN ACTION TREMOR. R. J. Elble, M. H. Schieber, and W. T. Thach. Depts. of Anatomy and Neurobiology and Neurology and Neurosurgery, Washington U. Sch. of Med., St. Louis, MO 63110

A rhesus monkey was trained to insert its hand, fingers extended, into a wedge-shaped manipulandum and to track a visual display by angulating the wrist. Hold-ramp-hold and rapid alternating movements were performed under various uniform torque, viscous, and inertial loads. Microelectrode recordings were obtained from cerebellar nuclei, C7 and C8 dorsal root ganglia, and forearm muscles. Prior to CNS recording, a fine 11-13 Hz action tremor was present in the wrist movements and particularly the extensor movements under extensor loading. This tremor was associated with phase-locked bursting in the extensor muscle electromyograms (EMGs). During the first thirty days of recording from the cerebellar nuclei, this tremor exhibited a five-fold increase in amplitude while its frequency dropped to 5-7 Hz. With inertial loading, the mean tremor frequency dropped as low as 2.5 Hz, and with large viscous loads, the tremor was abolished. Using auto- and cross-spectral analyses, coherency and phase (timing) relationships were computed for tremor-related activity in the EMGs, spindle afferents, and interpositus neurons. The EMG tremor bursts in flexors and extensors were 180 degrees out of phase with respect to each other, led the peak muscle stretch by approximately 66 degrees, and were so timed that the resulting muscle tension from the EMG burst would be expected to enhance the tremor. Two spindles were studied, one flexor and one extensor. Each was modulated by the tremor. Their modulations were 180 degrees out of phase with respect to each other and had phase leads of 150 degrees with respect to peak muscle stretch. Thus, the EMG bursts followed the spindle bursts by approximately 39 msec. By applying torque pulses in opposite directions, 6 of 14 tremor-related interpositus (IP) neurons could be classified as responding to stretch of either the extensor or the flexor muscles. The tremor bursts of these neurons had an average phase lead of 51 degrees with respect to peak muscle stretch; their bursts followed spindle bursts by approximately 46 msec and EMG bursts by roughly 7 msec. Post-mortem histologic examination showed only faint gliotic electrode tracks in cerebellar nuclei (some into brainstem) without larger lesions, suggesting that these may have caused the change in tremor. In sum, tremor of 15 degrees amplitude or less produced nearly simultaneous bursts of forearm EMG and IP neurons. Since the slight damage in the vicinity of these neurons must have altered the tremor, IP neurons may play a role in feedback suppression of tremor.

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223.18 LINGUAL VELOCITY AND DISPLACEMENTS IN SPEECH CONTROL. D.J. Ostry*, E. Keller*, A. Parush*, and F. Côté* (SPON: P. Braun). Departments of Psychology and Linguistics, McGill University and Université du Québec à Montréal, Montreal, Quebec,

Displacement, velocity and duration of tongue movements were examined to assess constraints on speech movement control. A computerized ultrasound recording system monitored vertical tongue dorsum displacement during the production of simple speech sounds. Three normal subjects produced self-paced sequences of non-meaningful consonant-vowel pairs, involving linguo-velar constriction in the posterior portion of the upper speech tract (e.g., /kaka/ or /gaga/). The sequences were produced at two speech rates, either with alternate vowels lengthened, or with all vowels given equal length.

The data were obtained using a unidirectional pulsed echo ultrasound transducer, placed in a vertical orientation below the chin along the midline of the jaw. The transducer was held in fixed position with respect to the hard palate by a head harness. The position of the tongue dorsum and a simultaneous acoustic signal were sampled at a one-msec rate. The tongue dorsum samples were pooled, providing an effective bandwidth of 15 Hz, and B-spline functions were derived for estimations of displacement, velocity, and acceleration.

Differences in displacement, velocity and duration of movement were obtained as a function of vowel length. Values for all three measures were greater for long vowels than for short vowels. Correlational analyses examined relations on a within-subject basis between maximum velocity on tongue lowering, distance and time to reach maximum velocity from linguo-velar contact, and distance and time from maximum velocity to the point of greatest distance from the velum. Subjects produced reliable correlations between maximum lowering velocity and distance from point of closure to the point of maximum velocity ($p < .01$ in all cases). In contrast, maximum lowering velocity was not related to any measure of movement duration. Thus peak velocities were greater for longer movements, but were not systematically related to duration. In addition, distance was correlated with movement duration only for the portion of the movement beyond the point of maximum lowering velocity. Findings are interpreted in the context of a system in which duration is a consequence rather than a specification of coordinated movement control for speech.

224.1 VITAL FREEZING OF HUMAN SCHWANN CELLS (HScC) FOR A CULTURAL FUTURE. J. V. Lawrence*, V. Askanas, W. K. Engel. Dept. of Neurology, Univ. of S. Calif., Sch. of Med., Los Angeles, California 90033.

Culturing of diseased HScC provides unusual opportunities for distinguishing between dysschwannian and dysneuronal human neuropathies, for studying biochemical and ultrastructural expression of defects in primary dysschwannian neuropathies (Neurol., 31:128, 1981, Ann. Neurol. in press) and for therapeutic trials in culture. We have recently described a technique for culturing of HScC and their histochemical and ultrastructural properties (Arch. Neurol., 37:329-337, 1980). To optimally utilize the irreplaceable patient material, we have now developed a technique for vital freezing both biopsied human sural nerve and human cultured schwann cells in dimethyl sulfoxide (DMSO) that permits long-term storage in liquid nitrogen and subsequent recovery for culturing. Some other human cells can be successfully frozen in 8% DMSO and 10% fetal calf serum (FCS) and later cultured; however, freezing of HScC required substantial modification of the freezing medium because use of that standard medium resulted in no survival of human schwann cells. The new vital freezing medium that we have used successfully is composed of 6% DMSO, 30% FCS, and 10% chick embryo extract in medium F-14. Any higher concentration of DMSO or lower amount of FCS caused decreased longevity of subsequently cultured human schwann cells and induced morphologic changes. Another important factor in successful freezing of HScC (not required for fibroblasts, lymphocytes, and myocardial cells) is slow freezing, during which the temperature drops 1°C per minute (from +25 to -60). Before slow vital freezing, the biopsied sural nerve was cleaned of epineurium and other connective tissue visible under dissecting microscope and cut into $1mm^3$ pieces, which were placed in sterile ampoules containing freezing medium. Previously cultured HScC, before slow vital freezing, were trypsinized in 0.25% trypsin for 15 min., the trypsin removed, and the cells placed in ampoules containing freezing medium. After freezing, the ampoules were stored in liquid nitrogen. In schwann cell cultures established after slow vital freezing, either in the nerve or in dissociated cells, the growth characteristics, phase-contrast, histochemical, and ultrastructural appearance of HScC were identical to the schwann cells in primary cultures of the same specimens. Vital freezing of diseased sural nerve biopsies and cultured HScC makes it possible to establish a tissue bank which provides the opportunity for studying those cells at virtually any time and also makes them available for the investigators in distant laboratories, thereby providing maximum utilization of the valuable, often irreplaceable, patient material.

224.2 NEURONAL SURVIVAL IN CNS CELL CULTURES ENHANCED BY ANTIMITOTIC DRUGS. Richard W. Burry, Department of Anatomy, College of Medicine, The Ohio State University, Columbus, Ohio 43210.

Dispersed cell cultures of the CNS contain both differentiating neurons and continually dividing non-neuronal cells. The purpose of this study was to evaluate several antimitotic drugs for their effect on neuronal survival.

Using a dispersed cell culture system of the rat olfactory bulb, several antimitotic drugs were tested to determine which would enhance neuronal survival. The olfactory bulb from two day old rats were dispersed mechanically with a Pasteur pipet and plated in Hams F_{12} with 24 mM K^+ at a density of $8X10^{-5}$ cells per 35mm dish. The drugs were added at 2 days in vitro (DIV) for 5 days at the following concentrations: methotrexate (MTX) 10^{-6}M, cytosine arabinoside (Ara-C) $8X10^{-6}$M, bromodeoxyuridine (BUdR) 10^{-5}M, and flurodeoxyuridine (FUdR) 10^{-4}M. MTX and FUdR were used in medium without thymidine. Cultures were examined every 7 days with a phase microscope and micrographs were taken.

In cultures without drugs the non-neuronal cells underwent continuous proliferations while most of the neurons died by 14 DIV. BUdR greatly reduced the number of non-neuronal cells and also allowed neurons to survive in large aggregates. By 91 DIV however, there was a return of a limited non-neuronal proliferation. Ara-C made a more permanent block of the non-neuronal cell through 91 DIV, but in the large aggregates of neurons there was a more refractile cellular debris than in BUdR treated cultures. FUdR, like Ara-C greatly reduced the number of non-neuronal cells but FUdR did not allow many neurons to survive. MTX did slow some of the non-neuronal cell growth, but like FUdR killed most of the neurons.

In summary, this study showed that BUdR reduced the number of non-neuronal cells and allowed maximum survival of neurons. BUdR incorporated before mitosis acts after mitosis in the daughter cells causing incorrect transcription of DNA to RNA. Ara-C greatly reduced non-neuronal cells with minimal effect on neurons. Ara-C inhibits nucleic acid incorporation into DNA of cells preparing for mitosis. MTX and FUdR reduced the number of non-neuronal cells and were also toxic to neurons. Both MTX and FUdR inhibit the synthesis of thymidine in cells preparing to duplicate their DNA for mitosis. It is concluded that BUdR and Ara-C, because they act later in mitosis than MTX and FUdR, are the antimitotics of choice in the olfactory bulb cell culture system. (Supported by a NINCDS Grant, NS-15894).

224.3 HYALURONIC ACID AFFECTS THE BEHAVIOR OF CULTURED NEURAL CREST CELLS. L. Luckenbill-Edds and J. Carrington*. Dept. Zoology & Coll. Osteopathic Med., Ohio Univ., Athens, OH 45701.

The extracellular matrix has been implicated in the migration and differentiation of neural crest cells in vivo (LeDouarin et al., 1975, Weston et al., 1978). We have tested the influence of hyaluronic acid (HA), a component of extracellular matrices in the early embryo, on the migration and morphological differentiation of neural crest cells in vitro. We cultured neural crest cells from neural tubes of 2-day Japanese quail embryos either in the presence of HA (5 µg/ml, Sigma type IV), or in control medium (CON) without added HA. Medium included Eagle's MEM, 10% heat-inactivated horse serum, 2mM HEPES buffer. The cultures were grown on gelatin-coated Nunc dishes and covered with a cellulose dialysis membrane (M. Derby, pers. commun.). Tubes were removed 16-19 h after explantation to eliminate contamination from neural tube cells. Cellular morphology was examined with an inverted phase contrast microscope. Migration of crest cells in paired CON and HA cultures was assessed in two ways: 1. the distance the population had migrated during the first 16-19 hours after explantation was measured for 50 cultures, 2. the total area of the culture was measured daily for 40 cultures. Since the total number of cells in a sample of 7 HA and CON cultures declined between days 1 and 3, it appears that proliferation does not contribute significantly to population outgrowth. In CON cultures, cells at first appear mesenchymal-like and migrate away from the origin where the neural tube had been located. Then, the majority of cells in CON cultures aggregate into clusters of small, phase bright cells which send out processes. Some small phase bright cells do not aggregate, but remain as single cells with branching processes. On the other hand, HA cells tend to remain mesenchymal-like, and migrate significantly further than CON cells, beginning with the first 16-19 h after explantation and continuing for the life of the culture. Few aggregates of small, phase-bright cells appear in HA cultures, and when they do, their appearance is delayed compared with CON cultures. The effect of HA is reversible, for if it is removed after 3 days of culture, the mesenchymal-like cells transform into small phase bright cells with processes as in CON cultures. On the basis of these results, our working hypothesis is that HA inhibits or delays the differentiation of mesenchymal-like neural crest cells into neurons.

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224.4 TOXICITY OF ORGANOTIN COMPOUNDS ON MAMMALIAN CENTRAL NERVOUS SYSTEM CELLS IN CULTURE. D. W. Schulz*, R. L. Thomas*, A. R. Kolber and R. S. Dyer. Chemistry and Life Sciences Group, Research Triangle Institute, Division of Neurotoxicology. U. S. Environmental Protection Agency, Research Triangle Park, NC 27709.

Several of the organotin compounds are known to induce pathological conditions such as convulsions, hyperagression, and locomotor difficulties. At the cellular level, triethyltin (TET) causes edema of CNS white matter, decreases in conduction velocity, and inhibition of oxidative phosphorylation, while trimethyltin (TMT) brings about neuronal damage specific to certain brain regions, most notably the hippocampus.

In an effort to establish an in vitro model of neurotoxicity, dissociated fetal mouse CNS cells were grown in culture and exposed to different concentrations of TET or TMT for varying durations. Cadmium (Cd), a known cytotoxin, was used as a positive control. The cells were explanted at day 13-17 in utero from either spinal cord, cortex, cerebellum, or hippocampus and maintained until functional differentiation was complete. The resulting mixed cultures contained morphologically identifiable astrocytes, oligodendrocytes, and neurons. Viability of the cultures was assessed by cell growth kinetics while morphological data was obtained using light and electron microscopy. Additional parameters monitored included changes in ATPase activity and alterations in normal electrophysiological behavior.

Each of the three organometallic compounds tested had a characteristic pattern of morphological toxicity. TMT at lower doses (10^{-6}M) selectively affected neurons, with marked changes seen in the structures of both dendritic processes and somas. TET induced formation of vacuoles in both glial and neuronal populations, while Cd caused a breakdown of all cell types. These effects were dose- and time-dependent and were reversible for TMT only. Inhibition of ATPase activity was observed following incubation with TET at concentrations as low as 3×10^{-7} M. Neurophysiological changes such as decreased resting membrane potential and depressed spontaneous activity were often noted following chronic or acute exposure to both tin compounds, but these generally occurred only after some of the above morphological changes were obvious.

The results of this in vitro study correspond well with data from whole animal experiments and suggest that a tissue culture system may provide a successful short-term method for assessing the neurotoxicity of suspected agents.

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224.5 DEVELOPMENT OF CILIARY AND CHOROID NEURONS IN LONG-TERM CELL CULTURES. E. Rieske*, M. C. Kitzes*, L.-H. Liaw*, G. W. Kreutzberg* and M. Berns* (SPON: R. Wuerker). Max Planck Inst. Psychiatry, Munich, F.R.G., and Dev. Cell Biol., University of California, Irvine, CA 92717.

Cell culture conditions are described which allow maturation and long-term survival of differentiated ciliary and choroid neurons of chick embryo ciliary ganglia. Dissociated cells were cultured in medium containing 9-15 day old chick embryo eye and heart extract. The cultures were kept up to 6 months in vitro.

Initially, all cells are isolated from each other. During the first week a monolayer of non-neuronal cells is developed on top of which the neurons regenerate a fiber network. Neuron somata are 5 to 10μm in size, contain eccentric nuclei, abundant mitochondria and free ribosomes but only scant rough endoplasmic reticulum (RER). They reaggregate into small groups, their fibers being arranged into fiber bundles. At this stage neither neuron somata nor fibers are enveloped by glial cells. Axo-somatic puncta adherentia and synapses with clear and dense core vesicles and post-synaptic membrane thickening appear after 2 days.

During the 2nd and 3rd week almost all neuron somata are enveloped by satellite cells, and the nerve fibers by Schwann cells. Intracellular injection of Lucifer yellow shows that the neurons are typically unipolar.

After 4 weeks populations of small and large neurons become obvious. Small neuron perikarya, presumably choroid cells, are 10-15μm in size, have centrally located nuclei and dispersed RER. Large neuron somata, presumably ciliary cells, are about 30-45μm. Their centrally located nuclei are surrounded by abundant mitochondria, Golgi complexes and lysosomes. The RER is located at the perinuclear region. Neuronal size and ultrastructure indicate that choroid and ciliary neurons persist in vitro in spite of apparent absence of target cells.

Intracellular activity and responses to long lasting pulses of depolarizing current show that, at all stages, neurons are either silent or discharge repetitively for several min. after penetration. Neurons in cultures younger than 10-15 days have resting membrane potentials between -40 mV and -52 mV and typically are unresponsive to direct stimulation. Neurons in cultures older than 10-15 days show resting membrane potentials from -40 mV to -65 mV. Whether silent or spontaneously active, the neurons fire repetitively to long lasting high intensity pulses, as do ganglion cells in situ. The number of evoked discharges may decline with repeated current pulse delivery until only a single discharge occurs at the onset of the depolarizing stimulus.

Occasional transneuronal dye transfer after intracellular injection of Lucifer yellow may be indicative of gap junctions.

224.6 IMMUNOHISTOCHEMICAL CHARACTERIZATION OF PRIMARY CULTURES OF FETAL MOUSE SPINAL CORD. D.L. Begley*, E. Serrano*, B. Ranson*, J. DeVellis, Y-Berwald Netter*, and L.F. Eng. Dept. Path. and Neurol., VA Med Cen and Stanford U. Sch. Med., Stanford CA. 94305; Dept. Anat., UCLA, Los Angeles, CA. 90024; College of France, 75005 Paris.

The immunohistochemical PAP technique of Sternberger was employed to characterize cells in primary culture. These cultures were plated from dissociated spinal cord and dorsal root ganglia from 13 and 14 day fetuses. The cultures were analyzed at 3 and 4 weeks in vitro when neurons exhibit mature electrophysiological properties. Rabbit antibodies to the following were utilized: 1) glial fibrillary acidic (GFA) protein -- specific to astrocytes (Eng et al, J. Histochem. Cytochem. 27:513, 1978); 2) tetanus toxin-specific to neurons (Dimpfel et al, Naunyn Schmiedeberg's Arch. Pharmacol. 290:329-333, 1975); and 3) glycerol phosphate dehydrogenase (GPDH) -- specific to oligodendrocytes (Leveille et al, Brain Res. 196:287-305, 1980).

Each antiserum stained a specific cell population. GFA stained cells of three morphologies: 1) large flat polygonal cells with oval nuclei (astroblasts); 2) star-shaped cells with round nuclei and multiple processes (mature astrocytes); and 3) cells with large cytoplasmic cavities (intermediate form). Tetanus toxin stained cells of conventional neuronal morphology -- round cell bodies with large nuclei relative to cytoplasm and long branching processes. GPDH stained perinuclearly cells with 2 morphologies: 1) small bipolar cells with round nuclei surrounded by rims of cytoplasm and 2) small unipolar cells also with round nuclei surrounded by rims of cytoplasm with irregular outpouchings. Cells not stained by any of the antisera were always present in the cultures.

This study demonstrates the usefulness of immunocytochemistry and cell specific antibodies in characterizing primary tissue cultures with a heterogeneous population.

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224.7 THE CULTURE OF ADULT HUMAN MUSCLE FOR BIOCHEMICAL INVESTIGATION. R.B. Moore*, M. Merickel, and S.H. Appel (SPON: A. Coats) Dept. of Neurology, Baylor College of Medicine, Houston, TX 77030

We have been interested in the biochemical characterization of adult human muscle using primary cultures with the goal of studying abnormalities in muscle disease. The primary culture of dissociated cells from adult muscle has several problems which are not necessarily encountered with embryonic or neonatal muscle: 1) Inaccuracy in the determination of the initial plating density results in cultures which develop in a non-uniform manner; 2) Low yield of undifferentiated cells greatly limits the number and kind of experiments which can be designed; 3) Heterogeneity of cell types confuses the interpretation of results.

To overcome these difficulties, we have investigated the following procedures. First, cultures of uniform plating density were obtained by replating after about 10 days in culture. There is little or no debris, the cells are large and they can be enumerated with greater precision. The plating efficiency of cells following this replating procedure was calculated to be greater than 90%. Cells from different muscle specimens that were replated at a density of 2.0×10^4 cells per 35mm dish developed similarly and fusion began between day 8 and day 10 after replating. Second, the number of cells to be used for an experiment was increased by growing them in a low calcium (100 to 200 μM) medium. This medium permits cell proliferation while preventing cell fusion. Myotubes which had formed after replating or after being grown in low calcium appeared to retain their basic properties since their resting membrane potentials and action potential parameters were not significantly different from myotubes which had formed in original cultures. Third, preliminary experiments using a discontinuous gradient of Percoll have demonstrated that a 36 to 54% interface contained a fraction of cells that were free of debris and which appeared to form more multinucleate myotubes following fusion than cells which were not placed on the gradient.

We intend to use these techniques to produce sufficient quantities of uniform cultures from both normal and diseased muscle for comparative biochemical analyses. (Supported by the Kleberg Foundation and the Muscular Dystrophy Association)

224.8 CELL CULTURE OF DISSOCIATED RAT SPINAL CORD AS AN ASSAY SYSTEM FOR TROPHIC AND TOXIC SUBSTANCES. Ken Vaca* and Stanley H. Appel. Dept. of Neurology, Program in Neurosciences, Baylor College of Medicine, Houston, Texas 77030.

Substantial evidence exists from in vivo preparations for effects of peripheral target tissues on the differentiation, growth and maintenance of spinal cord (SC) neurons. Cell culture affords a methodology for assaying the molecular basis of these effects. Several variables in culture conditions may be critical to the sensitivity of such an assay system. Mechanically dissociated SC from 13-14 day rat embryos was grown in Dulbecco's Modified Eagle Medium supplemented with 10% horse serum at a plating density of 2.5×10^5 cells/cm^2. Coculture with fused rat myotubes has only marginal (0-20% increase) effects on the ability of SC cells to accumulate and acetylate ^{3}H-choline, unless the proliferative SC cells are inhibited with 10^{-5}M fluorodeoxyuridine (FUdR), in which case muscle induces a 2-3 fold increase in ^{3}H-acetylcholine (ACh) synthesis after 5-7 days coculture. Muscle-conditioned medium can cause a modest increase in neurite outgrowth in standard medium, but can prevent complete degeneration in prolonged (2 wks or more) SC cultures treated with FUdR. Dialyzed muscle extract (MX, see Smith & Appel, this volume) had no effect on ^{3}H-ACh synthesis in standard medium, but typically caused a 30-60% increase in FUdR-treated cultures. On a poly-lysine (PLYS) or poly-ornithine (PORN) substrate, MX enhanced the tendency of the cells to aggregate and extend neurites. No aggregating effect is seen on collagen (Calf skin or rat tail) where SC cells cluster spontaneously, although neurite extension is enhanced. Thus only under certain culture conditions can SC cultures be optimally used to assay for a muscle trophic substance, which may be produced or substituted for by proliferative cells endogenous to SC.

SC cells can be grown in Bottenstein & Sato's defined medium (PNAS 76: 514-517) on a collagen substrate but start to deteriorate after a few days on PLYS or PORN. Better attachment is observed if the cells are plated in standard medium prior to switching to defined medium. SC cells can also flourish in a combination of 50% defined medium and 50% human CSF. MX enhances neurite outgrowth and ACh synthesis under both conditions. Human serum can be substituted for horse serum in standard medium, although its marked proliferative effect requires careful management with FUdR. Similar culture conditions which provide a sensitive assay for trophic factors also provide a convenient assay for toxic substances of clinical relevance.

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224.9 CYCLIC MORPHOLOGICAL CHANGES OF GLIAL CELLS IN CELL CULTURES OF RAT BRAINS. K. Meller* and M. Waelsch*. (SPON: H. Eckert). Arbeitsgruppe Experimentelle Cytologie, Ruhr-Universität Bochum, 4630 Bochum, Fed.Rep.Germany.

The use of allohydroxyl-l-proline or cytosine-arabinoside and uridine in primary cultivation of isolated cells of rat brain cultures allows the separation of viable glial cell populations for long periods of cultivation (in excess of a year). During this period of cultivation it was found that glial cells undergo cyclic morphological changes.

Typical astroglial cells become flattened and form an "epithelial" monolayer. Two weeks or more later dark and small cell populations of glioblast-like cells appear between the monolayer cells. These cells divide rapidly and originate new groups of astroglial cells that are dispersed in the monolayer. This newly originated astroglia has short prolongations and becomes well differentiated after one to two weeks. In freeze-etching preparation the long prolongations of astroglial cells form typical junctional contacts, that cannot be demonstrated in any other phase of the cycle. Serum free medium causes a disappearance of the epithelial phase.

It is suggested that glial cells in the culture undergo a continual renewal and that this process is similar to the glial differentiating process in the embryonic matrix of the proliferative regions of the CNS.

224.10 CLONED THYMUS CELL LINES WITH NEURONAL PROPERTIES STIMULATE T_k ACTIVITY IN THYMOCYTES. K. Bulloch[1], R. Langman* and M. Cohn*
Division of Neuroimmunology, Department of Neurology, SUNY-Stony Brook, N.Y.11794, Developmental Biology Lab, Salk Institute for Biological Studies, LaJolla, Ca. 92138.

A study was carried out to evaluate the non lymphoid cell populations in the thymus that promote T cell maturation. To accomplish this goal, clone cell lines derived from the mouse thymus were characterized, and tested for their ability to influence the expression of T_k effector function. Because of the endocrine nature of the thymus, clones were derived from embryonic glands of the A.TL mouse utilizing tissue culture methods developed for the selection of paraneuronal cells. Two approaches were used. The cells were either subjected to treatment with the ethyl mercurial compound, thimerosal, or to the slow adaptation selection procedure. The resultant clones were then characterized as to their neurosecretory and immunological properties. In order to assess neurosecretory properties, voltage dependent channels were pharmacologically evaluated using a $^{22}Na^+$ flux assay. Histological techniques were also employed to assess the neurosecretory morphology of the cell lines. To evaluate immune function thymic and non-thymic clones were irradiated and incubated with thymocytes in the presence of con A. T_k activity was then assessed by a chromium release assay. Thymus clones selected by these methods were found to express either class A or class B "neuronal" voltage dependent channels. In addition, several of the clones expressing neuronal properties were capable of stimulating T_k activity in syngeneic but not allogeneic thymocytes. The presence of neuronal properties in thymic cell population involved in immune function is consistent with the literature. Recently, direct CNS parasympathetic (Bulloch, K. and Moore, R.Y., Anat. Rec., 196:25A, 1980) as well as sympathetic (Bulloch, K. and Loy, R., Soc. Neurosci. Abstr., 6:69, 1980) projections to the murine thymus have been described. Furthermore, both cholinergic and catecholaminergic transmitters have been shown to influence immune responsiveness. The current study begins to identify the cellular elements involved in thymic immune function and works in progress to establish a role for the autonomic nervous system in its regulation.
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224.11 ACTIVITY AND LOCALIZATION OF 2′,3′-CYCLIC NUCLEOTIDE 3′-PHOSPHODIESTERASE IN NERVE CELL CULTURES H.W. Müller*, P.A. Clapshaw*, and W. Seifert (SPON: V. Braitenberg). Friedrich-Miescher-Laboratory, Max-Planck-Institute, Tübingen, W.Germany.

Previously, we have isolated 2′,3′-cyclic nucleotide 3′-phosphodiesterase (CNPase, EC 3.1.4.37) from bovine brain white matter (Clapshaw, Müller, Seifert - J. Neurochem., June 1981) and characterized the enzyme as a glycoprotein consisting of two polypeptide chains with molecular weights of approx. 53,000 and 56,000 (Müller, Clapshaw,Seifert - J. Neurochem., June 1981).

In order to understand the biological function of CNPase, we have measured and localized CNPase activity in various neuronal and glial cell lines and in fibroblasts as well as in primary cell cultures of rat CNS.

CNPase occurs in every investigated nerve cell line and in fibroblasts. Changes in enzyme activity of the cell lines can be correlated with different states of the cell cycle , growth, and differentiation. CNPase is stimulated in serum starved cultures compared to cells actively proliferating in the presence of fetal calf serum. The enzyme activity of the cells can be further increased in the G_1 state of the cell cycle by serum addition which provokes the reinduction of DNA synthesis and resumption of mitotic cycling.

The subcellular distribution of CNPase was examined in neuronal B104 and in astroglial C6 cells. Direct immunofluorescence staining with FITC-conjugated rabbit anti-CNPase antibodies indicates a discrete and specific intracytoplasmic location of CNPase. The enzyme could not be detected in the cell nucleus, at intracellular fibrillar structures, or at the cell surface. During cell fractionation CNPase activity seems to be bound to endoplasmic reticulum membranes or related membranes as monitored by marker enzyme activities.

CNPase is not present in primary neurons from rat hippocampus in culture. However, the enzyme activity could be detected in mixed glial cultures from rat brain containing astrocytes and oligodendrocytes. As indicated by immunofluorescence microscopy CNPase is not present in astrocytes but seems to be located on the cell surface as well as within the cytoplasm of the oligodendrocytes.

224.12 A COMPARISON OF ACETYLCHOLINE METABOLISM IN NERVE GROWTH FACTOR-TREATED AND UNDIFFERENTIATED PC12 CELLS. W.P. Melega* and B.D. Howard (SPON: R. Ng). Dept. of Biol. Chem., UCLA Med. School, Los Angeles, CA 90024.

PC12 is a clonal line of rat pheochromocytoma. PC12 cells synthesize acetylcholine and dopamine, store each in separate granules, and secrete each by a Ca^{2+}-dependent process. Upon exposure to nerve growth factor (NGF) the cells extend neurites. The undifferentiated and NGF-treated cells are similar with respect to choline transport. Each type of cell takes up choline by a carried mediated system (Km = 12 μM) that is Na^+ independent and insensitive to hemicholinium. Both types rapidly convert much of the accumulated choline to acetylcholine and slowly load the newly synthesized acetylcholine into granules. NGF-treated cells contain 3-4 times more total acetylcholine and granule acetylcholine per mg of protein than do undifferentiated cells. Similarly, NGF-treated cells contain 3-4 times more newly synthesized acetylcholine and load 3-4 times more of the newly synthesized acetylcholine into granules; they also release a greater percentage of the newly synthesized acetylcholine upon exposure to 55 mM K^+. Both types of cells bind quinuclidinyl benzilate (QNB) with similar affinities, but NGF-treated cells have 30% more QNB binding sites per mg of cell protein. Supported by USPHS grant NS 12873.

224.13 ENUCLEATION OF PC12 CELLS. R.A. Nichols, C.E. Chandler* and E.M. Shooter. Dept. of Neurobiology, Stanford University Sch. of Med., Stanford, CA 94305.

Previous studies have shown that nuclei isolated from the rat pheochromocytoma cloned cell line, PC12, bind nerve growth factor (NGF) in a specific and saturable fashion. When PC12 cells are cultured in the presence of NGF, the number of nuclear receptors increases over a period of days. During this time the cells internalize NGF, some of which is transported to and accumulates in the nucleus, paralleling the increase in NGF binding sites. The PC12 cells also grow neurites in response to NGF. The question then arises as to what role the nucleus plays in this phenomenon.

We have been able to enucleate PC12 cells, exploiting their ability to adhere tightly to an extracellular matrix (ECM) elaborated by bovine corneal endothelial cells. The enucleation was effected using a centrifugation technique with cells on ECM-coated plastic discs in the presence of cytochalasin B (CB) in culture medium. Detachment of 40-50% of the CB-treated cells was observed following centrifugation, although no cells detached during centrifugation without CB treatment or with CB treatment but no centrifugation. Of those cells remaining after centrifugation of CB-treated cells, 80-90% were enucleated ("cytoplasts") as evaluated by staining with either Giesma or Methyl green following fixation, or with acridine orange-ethidium bromide without fixation.

PC12 cells cultured on ECM displayed little or no neurite outgrowth (0-1%) without NGF. However, in the presence of 50 ng/ml NGF, 20-40% of the cells possessed neurites after 20 hrs. If the cells were treated with CB, this figure dropped to 10-20%. When CB treated cells were centrifuged and then cultured without NGF, little or no outgrowth was seen (0-5% of cells with neurites) and cell survival was poor. Finally, in CB-treated, centrifuged cultures maintained in the presence of NGF, 5-15% of the cells possessed neurites. Within this population the proportion of cytoplasts with neurites was similar to the proportion of nucleated cells with neurites.

The present data suggest that enucleation of PC12 cells does not prevent them from developing neurites in response to NGF, at least over a short time period. On the other hand, there is evidence suggesting that the presence of NGF in the nucleus of PC12 cells parallels the transcriptionally-dependent neurite outgrowth response. These two sets of data can be reconciled by postulating that the nucleus represses the neurite outgrowth mechanism and that either the presence of NGF in the nucleus or enucleation removes this negative control.

Finally, the present data suggest that the interaction of NGF with its receptor is required for neurite outgrowth in enucleated cells.

224.14 PEPTIDERGIC NEURONS FROM DISCRETE HYPOTHALAMIC NUCLEI IN LONG TERM TISSUE CULTURE. M. Dennis*, and M. Chrétien* (SPON: J. Lund). Protein and Pituitary Hormone Laboratory, Clinical Research Institute of Montreal, Montreal H2W 1R7, Canada.

Enzymatically dissociated cells were prepared from arcuate, paraventricular and supraoptic nuclei dissected from late foetal or early postnatal rat hypothalamus and cultured separately on poly-lysine coated glass coverslips. Cell attachment required the presence of 10% fetal calf serum during the first two days of culture, but long term growth and neuronal development was achieved in a serum-free defined medium (SFM) introduced on the third day. The morphology of cells in culture was followed by light microscopy which revealed the appearence after several days of cells with typical neuronal features and their progressive maturation up to 5 weeks in vitro, the longest period investigated. Two neuron types were observed; small (10-15 µm), oval, phase-bright cells with one or two processes and large (> 20 µm) multipolar neurons. Non-neuronal cells exhibiting typical astrocyte and oligodendrocyte morphologies were also present, but the proliferation of these cells was limited in SFM. Preliminary results indicate the presence of immunoreactive material corresponding to the neuropeptides characteristic for each region, i.e. β-endorphin in arcuate nucleus cultures and possibly neurophysin in cultures from paraventricular and supraoptic nuclei. Cultures derived from all three regions incorporated tritiated phenylalanine and leucine into tissue protein and released radiolabeled products into the medium during 16 hour pulse incubations. Two dimensional gel electrophoresis and autoradiography revealed a broad size and charge distribution of the biosynthetic products. Attempts to identify specific neuropeptides among the newly synthesized proteins will be discussed. (Work supported by MRC PG-2).

224.15 Long-term growth and selection of carotid body chemoreceptor cells in culture. Anne E. Schaffner and Mark C. Fishman, Laboratory of Developmental Neurobiology, N.I.C.H.D., N.I.H., Bethesda, MD 20205.

In order to evaluate the biochemical and physiological mechanisms of sensory transduction we have developed a system for long-term maintenance of carotid body chemoreceptor cells in primary cell culture. Carotid bodies from embryonic rats were dissected from the carotid bifurcation, enzymatically dissociated with trypsin and collagenase, and plated in collagen-coated dishes. Growth medium consisted of F12 enriched with additional amino acids (F14), 10% fetal calf serum and 80 U/L insulin. Cultures were maintained in a humidified atmosphere of 5% CO_2 and 95% air. Small epithelioid cells, exhibiting the morphology of Type I chemoreceptor cells, grew in isolation and in small clusters resembling the glomerular-like structures seen in vivo. Other cell types including fibroblasts and bipolar cells (Type II cells) were also present. The glyoxylic acid fluorescence histochemical method revealed intense catcholamine fluorescence in Type I cells growing in isolation and in clusters. Fluorescence also revealed long, slender processes on some Type I cells. Dramatic enrichment of the cultures for Type I cells was achieved by omitting the essential amino acid tyrosine (TYR) from the growth medium. This medium allows survival of only those cells containing tyrosine hydroxylase, an enzyme that catalyzes the conversion of phenylalanine to tyrosine (1). Only a few nonfluorescent cells survived in the absence of TYR. Type I cells remained viable for periods up to 7 weeks in culture during which time they continued to exhibit intense catecholamine fluorescence. Sympathetic neurons from the superior cervical ganglion (SCG), which provide efferent innervation to the carotid body, could be cocultured with Type I cells in growth medium lacking TYR if nerve growth factor was added. Growth and catecholamine fluorescence of Type I cells were unaffected by a 1 week incubation in atmospheres low in O_2 (5% O_2) or higher in CO_2 (10% CO_2). Steroids (10^{-6}M dexamethasone) were not necessary for the survival of Type I cells as they are for SIF cells from the SCG (2). This system will allow analysis of primary sensory transduction mechanisms of carotid body chemoreceptors and the nature of the synaptic transmission between these receptor cells and neurons.

1) Breakefield, X.O., and Marshall W. Nirenberg (1974) PNAS 71:2530-2533.
2) Doupe, A.J., P.H. Patterson, and S.C. Landis (1980) Soc. Neurosci. Abstracts 6:409.

224.16 ORGANOTYPIC CULTURES OF DORSAL ROOT GANGLIA FROM ADULT CATS. N. R. West. Depts. of Anatomy and Pharmacology, SUNY Upstate Medical Center, Syracuse, New York 13210.

Most nerve tissue culture models employ embryonic tissue as their starting material and commonly from chick eggs or mammals with short and easily timed gestation periods such as mice and rats. In anticipation of studies requiring direct correlations with in vivo work, it was desirable to develop a culture model of sensory neurons from cats. Due to the difficulty and expense of obtaining timed fetuses in this species and in that Murray and Stout (AM. J. Anat. 80:225, 1947) and several others have demonstrated that adult nerve tissue can be successfully explanted for culturing, it was considered reasonable to attempt establishing our model from adult cats.

Ganglia in sexually mature cats of unknown age are removed from the lumbar region of animals being used for spinal cord electrophysiological experiments. The dorsal root ganglia (DRG) are transferred quickly to ice cooled minimum essential medium supplimented with 600 mg% glucose and 50 ng/ml β-NGF (courtesy of Dr. R. W. Stach) in which the ganglia are then stripped of their perineureal capsule. After digestion with collagenase and trypsin the softened DRG are transferred to a small volume of explanting medium where any residual capsule is removed. They are then placed in a petri dish containing three drops per culture of medium where the DRG are gently teased into small clumps of neurons. The resulting suspension is pipetted into collagen coated dishes and maintained (Bunge and Wood, Brain Res. 57:261, 1973) without antibiotics but with 50 ng/ml β-NGF in the medium.

For the first four days in vitro (DIV) the axon stumps appear to swell along their length indicative of unwrapping of their myelin while at the cut tips the axons per se swell to form large bulbs. On 4 DIV supporting cells begin to migrate away from the clumps. Concommitantly, a large number of neurons undergo degeneration which continues for a week or more. During this time those neurons which are going to survive retain a fairly healthy appearance. These start to send out sprouts by 7 DIV, the new growth not coming from the cut axon tips. Myelinization starts within the clumps as early as 14 DIV and gradually continues out the growing processes. Growth continues until a state analogous to confluency is reached from which point there are only gradual changes and an occassional neuron degenerating.

Beyond their ability to survive axotomy and explantation, it is significant that at least a population of adult DRG neurons seems to be responsive to if not dependent upon NGF. This dependency upon NGF, plus neuron electrophysiological parameters and immunohistochemical affinities are being characterized.

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224.17 ASTROGLIA PROVIDE A TEMPLATE FOR CEREBELLAR CELL POSITIONING IN VITRO. M.E. Hatten and R. Liem*. Dept. of Pharmacology, New York University Medical Center, New York, NY 10016

Indirect immunocytochemical staining with antisera raised against purified glial filament protein was used to study cell interactions between astrocytes and neurons dissociated from developing mouse cerebellum. During the first five days in vitro more than 99% of all processes present in cerebellar cultures were glial in origin. After that time, unstained processes appeared.

Stained astroglial processes formed a network which served as a template for cerebellar neurons. The time course of association of postnatal cerebellar neurons with astroglial processes was studied by the PAP method and by time-lapse video recordings. At plating, the cells were "rounded up" and monodisperse. Astroglial processes were extended after 4-6h in vitro, and after this time phase-bright, neuronal cells rapidly associated with the glial fibers.

Neurons could be removed from the network of astroglial process by a gentle stream of air from a Pasteur pipette mounted on a micromanipulator. The characteristic morphology of cerebellar astroglia was lost in the absence of cerebellar neurons. Purified cerebellar neurons reassociated with glial processes when added back to the glial process network. Supported by NIH grants NS-15429 to MEH and NS-15182 to RL.

224.18 SPONTANEOUS AND INDUCED DIFFERENTIATION OF NEUROBLASTOMA X GLIOM HYBRID CLONE NG 108-15: EFFECTS ON GANGLIOSIDE PATTERNS. R.L. Schnaar and N.M. Dahms*. Dept. of Pharmacology and Experimental Therapeutics, The Johns Hopkins University, Baltimore, MD 21205.

Differentiation of NG 108-15 cells in culture was quantitated using choline acetyltransferase (CAT) specific activity as a biochemical marker. The cells spontaneously differentiated (in the absence of added inducers) as the cell density increased. The CAT specific activity rose from 150 to over 400 pmol/mg protein-min as the cells approached confluence. The differentiating agents dibutyryl cyclic AMP (Bt_2cAMP, 1 mM) or a combination of PGE_1 (10 μM) and theophylline (1 mM) caused morphological differentiation (increased neurite extension) and increases in the CAT specific activity to 380 or 520 pmol/ mg-mi respectively after 72 h, regardless of the cell density. The "mock" differentiating agent, sodium butyrate (1 mM) caused morphological differentiation but only modest increases in CAT specific activity (to 240 pmol/mg-min after 72 h), and inhibited the density-dependent increase found with control cells.

Gangliosides extracted from cells grown under the various conditions described above were assayed by quantitative thin layer chromatography. All cells contained four gangliosides: monosialogangliosides GM_3, GM_2, and GM_1, and the disialoganglioside GD_{1a}. As the cell density of control cells increased, the predominant ganglioside GM_3 dropped from 35.9% (of the lipid sialic acid) to less that 19%, while GM_2 increased from 11.4% t 21.4% and GD_{1a} increased from 34.5% to 44.8% (GM_1 remained relatively constant). This shift towards more complex gangliosides was even more marked in the PGE_1-theophylline treated cells: GM_3, 12.3%; GM_2, 19.1%; GM_1, 17.2%; and GD_{1a}, 51.4%. The "mock" differentiating agent sodium butyrate caused no increase in GD_{1a}, but did cause a decrease in GM_3 (to 12.7%) and a marked increase in GM_2 (to 44.8%). The ganglioside pattern of the Bt_2cAMP-treated cells was intermediate between that of the PGE_1-theophylline and sodium butyrate treated cells.

These results demonstrate that: 1) Increased cell density causes spontaneous biochemical differentiation of NG 108-15 cells. In previous studies comparing differentiated to "undifferentiated" cells consideration must be given to the cell density of the controls. 2) Biochemical differentiation (spontaneous or induced) causes a marked decrease in ganglioside GM_3, and an increase in the more complex gangliosides GM_2 and GD_{1a}. 3) Mock differentiation causes only the GM_2 to increase without the concomitant increase in GD_{1a}. The biochemical basis for these changes, and their significance to the differentiated phenotype are matters for further investigation. Supported by NIH Grant #HD 14010, and National Foundation-March of Dimes Grant #5-302. NMD is supported by NIH Training Grant #GM 07445.

224.19 PURKINJE CELL MATURATION IN TISSUE CULTURE:AN ULTRASTRUCTURAL STUDY. N.K. Blank* and F.J. Seil (SPON: W.R. Woodward). Neurology Research, V.A. Med. Ctr. and Dept. of Neurology and Pathology, Univ. of Oregon Health Sci. Ctr., Portland, OR 97201.

Mature Purkinje cells from cerebellar explants have been shown to have many ultrastructural features similar to those found in vivo. Some investigators have also described several disimilarities, including: 1) persistence of numerous Purkinje cell somatic spines 2) the lack of relatively complete astroglial envelopment 3) an increased ratio of Purkinje cell dendritic spine to parallel fiber ratio 4) the presence of many unattached Purkinje cell dendritic spines and 5) the prominence of perineuronal extracellular space. The purpose of this study was to determine if these morphological differences were characteristic of in vitro development or were related to suboptimal culture conditions.

Cerebellar explants derived from newborn Swiss Webster mice were maintained in Maximow assemblies and fed nutrient medium twice weekly. Explants from 16-24 days in vitro were fixed in mixed aldehydes and prepared for ultrastructural studies using standard methods. Cerebellar cortex was surveyed and Purkinje cells and their dendrites were identified. The somata were relatively smooth, were ensheathed by glial processes and lacked spines. Purkinje cell dendrites were also surrounded by glial processes. Only minimal numbers of unattached dendritic spines were present, while the majority of the spines contacted parallel fibers in a 1:1 relationship. Perineuronal extracellular space was minimal. In suboptimal explants, such as in granuloprival cultures resulting from exposure to cytosine arabinoside, numerous Purkinje cell dendritic spines were found to be unattached to presynaptic elements and glial ensheathment of Purkinje cells was absent. In other suboptimal cultures, multiple dendritic spines were in contact with a single axonal terminal. The results of this study suggest that cultured Purkinje cells may attain a state of development resembling their in vivo counterparts. The previously reported atypical in vitro features were evident in suboptimal culture conditions in which complete Purkinje cell maturation was not attained. (Supported by the Veterans Administration).

224.20 IN VITRO DIFFERENTIATION OF A STEM CELL LINE FROM A RAT NEUROTUMOR RT4 TO DIFFERENT CELL LINES WITH NEURONAL AND GLIAL PROPERTIES

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Rat peripheral neurotumor RT4 was induced by injection of neurospecific carcinogen ethylnitrosourea into newborn rat. Four morphologically distinct cell types were derived from RT4: RT4-AC, RT4-B, RT4-D, and RT4-E. Further, single cell clones of RT4-AC can generate the other three cell types (RT4-B, RT4-D, and RT4-E5) (cell type conversion). Karyotype analyses show that chromosome numbers of RT4 family are close to normal rat diploid cells. Biochemical and immunological analyses of mass populations of each of these cell types in cultured cells show the following results. The stem cell (RT4-AC) and RT4-D produce glial property markers, S100 and GFA proteins, but cell types RT4-B and RT4-E do not. The stem cell also shows a small but significant neuronal-specific response to veratridine on voltage-dependent Na^+-influx. Cell types RT4-B and RT4-E show a clear response of voltage-dependent Na^+-influx to veratridine as neuronal cells. Na^+-influx of RT4-AC, RT4-B, and RT4-E are greatly stimulated by scorpion toxin. In contrast glial type RT4-D does not show any response of voltage-dependent Na^+ influx to veratridine in the presence of scorpion toxin. These results indicate that (a) a population of the stem cell expresses both neuronal and glial properties. (b) RT4-B and RT4-E express a neuronal property and (c) RT4-D express glial properties. Therefore, cell type conversion of stem cell type to RT4-B and RT4-E seems to be a differentiation towards neuronal cells, and cell type conversion of RT4-AC to RT4-D to be a differentiation towards a glial cells in culture in vitro. In the process of cell type conversion at least two glial properties are segregated and expressed together in glial type cells RT4-D but not in neuronal type cells RT4-B and RT4-E (conversion coupling). RT4-AC and glial type RT4-D are tumorigenic cells, on the other hand neuronal type RT4-B and E are not tumorigenic. Therefore, tumorigenicity is coupled with glial property.

225.1 CHARACTERIZATION OF GLUTAMATE TRANSPORT INTO CULTURED GLIOMA CELLS. Robert A. Waniewski and David L. Martin. Toxicology Institute, Div. of Labs and Research, New York State Dept. of Health, Albany, NY 12201.

The accumulation of glutamate by glial cells in the nervous system suggests the involvement of these cells in the regulation of extracellular levels of this putative neurotransmitter. We have examined the properties of ^{3}H-glutamate uptake with LRM-55 cells, a glioma cell line derived from a rat spinal cord astrocytoma (Martin and Shain, J. Biol. Chem., 254:7076, 1979). Cells were grown in 24-well Falcon tissue culture dishes and fed Ham's F12 medium supplemented with 2.5% fetal calf serum. For uptake experiments, cells were washed and switched to a HEPES-buffered Hank's solution (pH 7.3) and incubated at 37°C. Time course experiments revealed a rapid accumulation of ^{3}H-glutamate reaching a maximum at 30 minutes. This maximum was followed by a decline in intracellular ^{3}H levels indicating rapid metabolism and release of metabolites. To estimate the initial velocity and saturation kinetics of ^{3}H-glutamate uptake, experiments were performed with incubation times of 5 minutes. Glioma cells were found to accumulate ^{3}H-glutamate primarily by a high affinity component with an apparent K_m of approximately 35 μM and a V_{max} of 5.4 nmoles/mg protein/minute. A low affinity component, found not to be saturable at substrate concentrations of up to 5mM, contributed minimally to the total uptake of ^{3}H-glutamate.

The transport system was found to be very specific for glutamate. In competition studies, all tested analogs of glutamate and aspartate were required in at least 10-fold excess of glutamate to produce any inhibition of uptake. L-cysteine sulfinic acid, L-aspartic acid and L-cysteic acid were the most potent competitors, reducing ^{3}H-glutamate uptake by 50% at approximately 50 times the concentration of glutamate. L-cysteine, D,L-homocysteic acid, D-glutamate and D-aspartate were of intermediate potency as competitors. Those analogs having little or no inhibitory effect on glutamate uptake include: N-methyl-D,L-glutamate, N-methyl-D, L-aspartate, kainic acid, D,L-alpha-methylglutamate, D,L-aspartate beta-hydroxamate, 2-amino-3-phosphonopropionic acid and asparagine. Taurine, glycine and isoproterenol were tested at concentrations of up to 10mM and were found to have no effect on high affinity ^{3}H-glutamate uptake. Diazepam inhibited uptake by only 20% with a 2 hour preincubation at 0.1 mM.

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225.2 GLUTAMINE SYNTHETASE INDUCTION IN C-6 GLIOMA CELLS. W.J. Nicklas and E.T. Browning, Departments of Neurology and Pharmacology, Rutgers Medical School, Piscataway, N.J. 08854.

Brain glutamine synthetase (GS) is localized primarily in glial cells and may function to inactivate neuroexcitatory glutamate and provide a non-excitatory transport form for the carbon structure of glutamate, i.e. glutamine. Past work (J. Neurochem,30, 955) reported a low concentration of GS in C-6 glioma cells. Present studies show increased GS in C-6 cells with increasing culture passage or after treatment with dibutyryl-cyclic AMP (dbcAMP). Cells obtained from the American Type Culture Collection at passage 34 express low activities of GS (0.2-0.4 umole/mg protein/hr) 8-13 passages thereafter. Between passage 13 and 24 the activity rises to approximately 1 umole/mg protein/ hr. Cellular protein/DNA ratio rose from 13 to 18 over the same culture interval suggesting that the cells increase in size with increasing culture passage. DbcAMP (1mM) increased GS at both low and at high culture passage and also increased cellular protein/DNA. The maximum enzyme activity observed was 1.5 umole/mg protein/hr, comparable to that of rat brain homogenate in the same experiment. HPLC analysis indicated that 90% of the added dbcAMP remained in the medium after 2 days in culture. 8-Bromo-cAMP also increased GS. Butyrate (0.1 mM) did not increase GS activity. Lactate dehydrogenase activity was increased 2- to 3-fold by dbcAMP but was minimally altered by culture passage. Cycloheximide inhibited the rise in GS and lactate dehydrogenase activity induced by dbcAMP. The dbcAMP effect on cells at low passage was undetectable 3 hr after drug addition, detectable at 8 hr, reached a maximum at 48 hr after which it remained essentially constant. In conclusion, GS appears to be inducible in C-6 cultures and to reach activities observed in brain tissue under certain conditions of culture passage and drug treatment. (Supported by NS 08436, Rutgers Medical School GRS Funds, and CMDNJ Fdn.)

225.3 COUPLED DESENSITIZATION OF BETA ADRENERGIC RECEPTOR ADENYLATE CYCLASE AND INCREASED GLUCOSE UPTAKE IN C6 RAT GLIOMA IN VITRO N. Shitara*, F. Hirata*, C.J. Cummings*, P. E. McKeever*, B. H. Smith, S. Schmidt* and P.L. Kornblith*.(SPON. B.Smith)Sur. Neur. Br., NINCDS and Lab. Clin. Med, NIMH, Bethesda,MD 20205

Glucose uptake by both neurons and glia in the central nervous system may involve modulation by a direct coupling of the neurotransmitter receptor system to the glucose transport system. To study this possibility in glial cells the rat C6 cells are known to have beta-adrenergic receptors which are coupled to adenylate cyclase.

When C6 glioma cells were stimulated with 10^{-6}M isoproterenol, uptake of ^{3}H-deoxyglucose decreased by 50% in the first 30 minutes and then increased by 200% up to 150 minutes (compared to unstimulated control). This increased glucose uptake appears to be mediated through β-adrenergic receptors, since the order of potency of various catecholamines is isoproterenol > epinephrine > norepinephrine. In addition, the d-isomer of isoproterenol was much less active than the l-isomer and the β-adrenergic antagonist, propranolol, but not the α-adrenergic antagonist, phenoxybenzamine, inhibited the increase in glucose uptake. The intracellular cAMP levels under constant stimulation by β-agonists were used as a standard marker of adenyl cyclase uncoupling. Uncoupling occurred at 30 minutes at the same time that the 2-deoxyglucose incorporation rate increased.

The above data give evidence for an association between glucose uptake and β-adrenergic receptor stimulation.

225.4

WITHDRAWN

225.5 VIMENTIN PHOSPHORYLATION: NOREPINEPHRINE DEPENDENT PHOSPHORYLATION IN C-6 GLIOMA CELLS. E.T.Browning Dept. of Pharmacol, Rutgers Med. Sch.Piscataway, N.J. 08854.

Vimentin forms the major subunit of a cytoskeletal intermediate (10 nM) filament in cells of mesenchymal origin including astrocytes and C-6 glioma cells. Vimentin undergoes phosphorylation on a small fraction of its copies; previous work demonstrated a 2-fold increase in vimentin phosphorylation following norepinephrine(NE) treatment of intact C-6 glioma cells (Mol. Pharmacol, 18, 427; J. Cell. Biol., in press). C-6 cells were equilibrated with ^{32}Pi for 4 hr then exposed to NE for 5' and the cellular phosphoproteins resolved by 2-dimensional gel electrophoresis as in past studies. Addition of actinomycin D to incubation fluid during equilibration of cells with ^{32}Pi decreased the ^{32}P-vimentin occurring under control conditions yet increased the increment in phosphorylation produced by NE. In the presence of actinomycin D the proportional change in phosphorylation induced by NE increased to 4.4-fold. Peptide mapping was used to further study the norepinephrine dependent phosphorylation of vimentin. Surface cultures were exposed to ^{32}Pi and NE as before. Cells were extracted with 0.5% NP-40, 2 M NaCl and residues containing vimentin were sonicated and vimentin solubilized with SDS-urea-lysine-$ZnCl_2$. Vimentin was purified by slab gel electrophoresis, cut from the gel and peptide mapped with Staph. aureus strain V_8 protease in a second gel. With increasing protease concentration vimentin cleaved into major discrete ^{32}P-peptides of 25,000 then 11,000 daltons. The labeling of ^{32}P-vimentin fragments derived from control and NE treated cultures suggested the presence of more than one phosphorylation site in the protein in that proportional increases in phosphorylation of individual peptides exceeded the increases observed in the intact vimentin. Again, increases were larger if actinomycin D was present during equilibration of cells with ^{32}Pi. Present evidence indicates that NE dependent phosphorylation can produce a 4-fold increase in phosphorylation of vimentin and suggest that the NE dependent phosphorylation site is distinct from another site which is phosphorylated under control conditions in the absence of actinomycin D.(Supported by NS 08436 and CMDNJ Fdn.)

225.6 ELECTROPHYSIOLOGY OF AND SENSITIVITY TO FUROSEMIDE AND MK473 OF Cl^- TRANSPORT IN PRIMARY ASTROCYTE CULTURES. H.K. Kimelberg and H. Hirata, Div. of Neurosurgery, Albany Medical College, Albany, New York 12208.

Astrocytes growing in primary culture show normal membrane potentials of -70 to -80mV at external K^+ concentrations of 4.5 mM K^+. These cultures were prepared from the dissociated cerebral hemispheres of 3 day old rats. Changes in external K^+ concentrations produced a close to Nernstian response in the membrane potential with a slope of up to 57mV per 10-fold change in $[K^+]_o$ for values of $[K^+]_o \geq 4.5$. The extrapolated value for $[K^+]_i$ was 130mM. In contrast, complete replacement of external Cl^- by isethionate or gluconate had either no detectable effect or resulted in a depolarization of up to 3mV. As previously reported (Neurosci. Abst. 6:548, 1980) tracer studies with $^{36}Cl^-$ showed that it rapidly enters these cells, reaching a steady state level after 10-20 min which represents around 0.15µmoles Cl^-/mg protein. Based on this steady state value and estimates of cell volume of 3 to 4µl/mg protein, the internal concentration of exchangeable Cl^- is 40 to 50mM. This is about 4 to 5-fold higher than expected from equilibration with a -70mV membrane potential. Addition of the anion $Cl^-/HCO_3^-/OH^-$ exchange inhibitor SITS (4-acetamido-4'-isothiocyanostilbene-2,2'-disulfonate) inhibited the initial rate of uptake of $^{36}Cl^-$, but had little or no effect on the steady state level. In contrast, furosemide inhibited both the initial rate of uptake and the final steady state level of $^{36}Cl^-$. Furosemide also partially inhibited uptake of K^+ as measured with $^{86}Rb^+$. Addition of furosemide also resulted in cell shrinkage, presumably due to loss of KCl, and caused intracellular acidification as measured with ^{14}C dimethyloxazolidine-2,4-dione. However, furosemide had no detectable effects on the measured membrane potential. Inhibition of Cl^- steady state levels was also found after addition of an indanyloxyacetic acid derivative of ethacrynic acid, MK473 (DCPI or [6,7 dichloro 2 cyclopentyl-1-2 methyl 1-oxo-5 indanyl] oxy acetic acid) which also inhibited $^{86}Rb^+$ uptake. In contrast SITS had no effect on $^{86}Rb^+$ uptake and its inhibition of Cl^- transport was not additive with furosemide. These results suggest that active accumulation of Cl^- in astrocytes occurs and is due to an electrically neutral coupled cation-Cl^- co-transport system involving at least K^+, which is inhibited by both furosemide and indanyl derivatives of ethacrynic acid but not by SITS. The function of the SITS and furosemide-sensitive anion exchange system may thus be primarily one of the intracellular and extracellular pH control, perhaps secondary to active accumulation of Cl^-. (Supported by NINCDS grant 13042 and a grant from Merck, Sharp and Dohme, who also supplied MK473).

225.7 A MONOCLONAL ANTIBODY TO OLIGODENDROCYTES. W.W. Peng*, R. Cole* and J. de Vellis (SPON: A. Castiglioni, Jr.). UCLA School of Medicine, Los Angeles, CA 90024.

A monoclonal antibody was obtained which distinguishes oligodendrocytes from astrocytes in rat brain primary cultures. This monoclonal antibody, named O1la, is a product of the fusion of S194/5.XXO.BU.1 myeloma cells and spleen cells from a mouse which was previously immunized with pure oligodendrocytes. Primary culture of dissociated rat brain cerebral cells prepared from 0 to 1-day-old rat pups. Oliogodendrocytes were identified as phase dark cells, resting on top of a bed layer cells of astrocytes. The overlying oliogodendrocytes were separated from the bed layer cells by mechanical shaking. The remaining astrocytes and purified oliogodendrocytes were then plated and grown on microtest plates or cover slips. The cultures were used for this study within two weeks. Antibody O1la was originally detected by ^{125}I-protein A binding assay on pure oliogodendrocyte primary cultures and cloned by limiting dilution method. Immunofluorescence experiments indicated that antibody O1la does not bind to primary astrocytes, normal fibroblasts, or C6 glioma cells, but it does bind specifically to oliogodendrocytes in purified cultures as well as mixed cultures of astrocytes and oligodendrocytes.

Since glycerol-3-phosphate dehydrogenase (GPDH) is a known biochemical marker for oligodendrocytes, a double labeling experiment of O1la antibody and anti-GPDH was performed on the same primary culture using fluorescein and rhodamine coupled second antibodies. The results demonstrate that antibody O1la and anti-GPDH bind to the same population of cells. Thus, from the morphological identification and the double labeling experiment, we conclude that the monoclonal antibody O1la is specific for oligodendrocytes in rat brain primary cultures. The distribution of O1la antigen in the rat brain and its relation to brain development and myelination will be studied. (Supported by NIH and DOE)

225.8 MONOCLONAL ANTIBODY TO A RAT GLIAL CELL ANTIGEN. E. Tiffany-Castiglioni* and J. de Vellis. Lab. of Nuclear Medicine and Radiation Biology, Univ. of California, Los Angeles, CA 90024.

A monoclonal antibody was obtained by the hybridoma technique which binds to an antigen of 1-3 week old cultured rat glial cells, C6 rat glioma cells, and glial fibers in rat brain tissue. The hybrid cell line which secretes the antibody arose from the fusion of mouse myeloma cells and spleen cells from a mouse injected with human myelin basic protein. The hybrid was detected by the binding capacity of its spent culture medium to an antigenic site in 2-3 week old cultures of glutaraldehyde- or paraformaldehyde-fixed neonatal rat oligodendrocytes using a protein A binding assay. The antigen, designated G23, was also present in aldehyde-fixed C6 cells as well as bed layer cells of neonatal rat cortex cultures from which oligodendrocytes had been removed by shaking. Astrocytes make up most of the bed layer population, though stem cells capable of differentiating into either glial cell type may also be present. Anti-G23 did not bind to fixed fetal rat skin fibroblasts or to live cells. Indirect immunofluorescence studies with FITC-labeled rabbit antibody to mouse IgG showed extranuclear binding of anti-G23 to the cell bodies of fixed (paraformaldehyde 4%, 24 h) oligodendrocytes and C6 cells, though a small percentage (<10%) of these cell types did not label. Anti-G23 also labeled most phase dark cells and some bed layer cells in 10 d and 22 d old primary cultures from neonatal rat cortex fixed 24 h, and pure cultures of the bed layer cells showed patchy staining along some cell bodies. Skin fibroblasts did not bind the antibody, nor did primary cultures or bed layer cells fixed for less than 2.5 h. The pattern of immunofluorescence in fresh and fixed (4% paraformaldehyde, 5 d) cryostat sections of 2-3 week neonatal rat brain was compared with that of anti-glial fibrillary acidic (GFA) protein. In sagittal sections of cerebellum, anti-G23, like anti-GFA, labeled Bergmann glial fibers and cells in the white matter which had small cell bodies and numerous processes. The fibers labeled by anti-G23 were finer and shorter than those labeled by anti-GFA. Both antibodies labeled more strongly in fixed than fresh-frozen tissue. In fixed and fresh coronal sections of the corpus callosum, anti-GFA labeled cells of similar shape to those seen in cerebellar white matter. However, anti-G23 detected cells of this shape only in fixed tissue. We conclude that G23 is an intracellular antigen found in glia subpopulations, notably oligodendrocytes and perhaps stem cells. Anti-G23 may have arisen in response to a contaminant of the original MBP inoculum.

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225.9 FURTHER EVIDENCE RELATED TO A POSSIBLE MACROGLIAL CELL INVOLVEMENT IN THE TRANSFER OF ^{3}H-PROLINE-LABELED MOLECULES ALONG FIBER TRACTS IN THE CENTRAL NERVOUS SYSTEM OF THE CAT. PART II. K.J. Berkley, N. Contos and H.H. Molinari. Dept. Psychol., Fl. St. Univ., Tallahassee, FL 32306 (KJB, NC) and Dept. Anat., Albany Med. Col., Albany, N.Y. 12208 (HHM).

Results obtained previously in our laboratory demonstrating that ^{3}H-proline (^{3}H-pro) is preferentially incorporated into the macromolecules of macroglial cells suggest that macroglial cells may be involved in the transfer of ^{3}H-pro-labeled molecules along fiber tracts. The light microscopic autoradiographic work to be reported here and in an associated abstract (Part I, Contos et al., 1981) extends these investigations.

Two studies were done to characterize the nature of the ^{3}H-pro-labeled material. (1) ^{3}H-pro was injected into the dorsal column nuclei (DCN) together with puromycin, a reported blocker of protein synthesis. No labeling occurred either in DCN or in its terminal targets. (2) The ^{3}H-pro labeling pattern was compared with that of ^{3}H-hydroxyproline (^{3}H-hyp), which is an amino acid found abundantly only in fibrous proteins such as collagen. The ^{3}H-pro and ^{3}H-hyp labeling patterns in DCN and its terminal targets were identical. These results suggest that the ^{3}H-pro in these experiments has been incorporated into a macromolecule that may be related to basement membrane collagen.

Three studies were done to characterize the transfer process. (1) ^{3}H-pro was injected directly into fiber tracts (internal capsule, internal arcuate). Dense labeling occurred over glial cells extending several mm in both directions along the length of the tracts. (2) ^{3}H-pro was injected in DCN subsequent to an injection of kainic acid, a reported neurotoxin. Labeling still occurred over glial cells in DCN, but no labeling was observed over terminal targets of DCN such as the inferior olive and thalamus. (3) ^{3}H-pro was injected in DCN together with colchicine, which is reported to interfere with axonal transport associated with microtubules. Labeling occurred not only over glial cells in DCN, but also over the inferior olive. Taken together, the results of these three experiments strongly support the involvement of glial cells in the transfer of ^{3}H-pro-labeled macromolecules along fiber pathways. The results with kainic acid and colchicine, however, indicate that this transfer probably involves a complex interaction between the glial cells and their associated neurons.

Supported by NSF grant BNS 79-03424.

225.10 FURTHER EVIDENCE RELATED TO A POSSIBLE MACROGLIAL CELL INVOLVEMENT IN THE TRANSFER OF ^{3}H-PROLINE-LABELED MOLECULES ALONG FIBER TRACTS IN THE CENTRAL NERVOUS SYSTEM OF THE CAT. PART I. N. Contos, H.H. Molinari and K.J. Berkley. Dept. Psychol., Fl. St. Univ., Tallahassee, FL 32306 (NC, KJB) and Dept. Anat., Albany Med. Col., Albany, N.Y. 12208 (HHM).

Previous electron microscopic autoradiographic work in our laboratory has demonstrated that when ^{3}H-proline (^{3}H-pro) is injected into the dorsal column nuclei (DCN), it is preferentially incorporated into the macromolecules of macroglial cells not only within the vicinity of the injection site, but also along the internal arcuate fibers projecting from DCN to its terminal targets. In contrast, when ^{3}H-leucine is injected into DCN, it is incorporated by neurons as well as glial cells in DCN, and the axoplasm of the internal arcuate fibers is more densely labeled than the associated myelin and glial cells. These results suggest that macroglial cells may be involved in the transfer of ^{3}H-pro-labeled molecules along fiber tracts. The light microscopic work to be reported here and in an associated abstract (Part II, Berkley et al., 1981) extends these investigations.

In order to examine the generality of ^{3}H-pro's incorporation pattern in the nervous system, injections of ^{3}H-pro were made in different nuclei located at several levels along the neuroaxis (e.g., cerebral cortex, thalamus, cerebellar cortex, reticular formation, lateral cervical nucleus, dorsal horn of spinal cord and dorsal root ganglion). Invariably, glial cells were densely labeled at the injection site. The density of neuronal labeling was usually less than that of the glial cells, but varied with site. For example, dorsal root ganglion cells were as densely labeled as the oligodendrocytes associated with its fiber processes, but neurons in the ventrobasal complex of the thalamus were considerably less densely labeled than their associated glial cells. In the lateral cervical nucleus, the large neurons were sparsely labeled compared to the glial cells.

It is concluded that the dense incorporation of ^{3}H-pro into macromolecules located in macroglial cells is a generalized feature of ^{3}H-pro's incorporation pattern throughout the nervous system, whereas the neuronal incorporation of ^{3}H-pro appears to vary from site to site. The basis for this variance is not yet understood.

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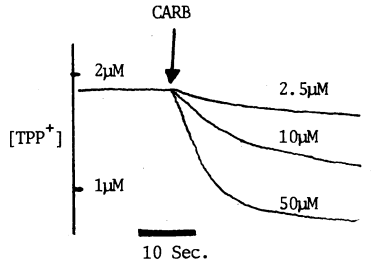
226.1 LIGAND-INDUCED CONFORMATIONAL CHANGE OF THE CHOLINERGIC POST-SYNAPTIC MEMBRANE. S. Hestrin*, C.G. Davis*, A.S. Gordon, I. Diamond, and J.I. Korenbrot. Depts. of Neurology and Physiology, Univ. of Calif., San Francisco, CA 94143.

We have found that the cholinergic agonist carbachol induces partitioning of the hydrophobic ion TPP^+ (tetraphenylphosphonium) into AChR-enriched membranes isolated from the electroplax of Torpedo californica. The concentration of free TPP^+ in solution was measured with a TPP^+ selective electrode (which gave a Nernstian response down to 5 x 10^{-7}M TPP^+). The concentration of free TPP^+ in a Ringer's solution containing 2uM TPP^+ and AChR-enriched membranes (~10uM α-bungarotoxin binding sites) was continuously monitored. Addition of carbachol to the solution resulted in a decrease of the concentration of free TPP^+ to 1um. It was found that this response was specific for AChR since preincubation with α-bungarotoxin prevented any detectable decrease. The decrease of free TPP^+ was not the result of a change in transmembrane potential because it occurred with symmetric ionic solutions across the membrane and, in addition, was not affected by 0.1% digitonin. In the range of 1uM to 1,000uM carbachol the time constant of the initial rate of decrease was 100 seconds to 2 seconds respectively.

We conclude that binding of cholinergic ligands to the AChR induced thermodynamic changes of the post-synaptic membrane. The slow time course of the TPP^+ partitioning indicates that it is responding to events which are secondary to the conductance increase.



226.2 INFLUENCE OF PH ON THE INTERACTIONS OF DRUGS WITH THE NICOTINIC RECEPTOR-ION CHANNEL COMPLEX. Robert S. Aronstam. Department of Pharmacology, Medical College of Georgia, Augusta, GA 30912

Perhydrohistrionicotoxin (H_{12}-HTX) and phencyclidine (PCP) block neuromuscular transmission as a consequence of interactions with sites outside the nicotinic receptor (i.e., the ACh binding site) per se. While the nature of these sites is not clear, these compounds have been termed "ion channel blockers" because of their specific effects on the time course and conductances of end plate ion channels.

Specific binding of 2 nM [^{3}H]PCP to receptor-channel complexes in Torpedo membranes is negligible below pH 5.6 and is maximal between pH 6.4 and 9.2. A similar pattern is observed with the methiodide derviative of [^{3}H]PCP. In the presence of receptor agonists (acetylcholine (ACh), carbamylcholine and anatoxin-a) the extent of 2 nM [^{3}H]PCP binding is increased 5-8 fold. Under these conditions [^{3}H]PCP binding is negligible below pH 5.6, increases steadily from pH 5.6 to a maximum at pH 9.2, and decreases to about 70% of maximum by pH 11.0. While the binding of [^{3}H]H_{12}-HTX to the ion channel is also increased in the presence of receptor agonists, the binding is constant between pH 6.8 and 9.2. No specific [^{3}H]H_{12}-HTX binding could be detected below pH 5.6 and binding decreased above pH 9.2 to 60% of maximum at pH 10.0. It would appear that in the presence of receptor agonists sites or interactions are available for [^{3}H]PCP in its uncharged form (the pK_a for PCP is 9.42 at 25°, Maayani and Gross, Fedn. Proc. 39:30, 1980). In the absence of receptor agonists, [^{3}H]PCP and [^{3}H]PCP-MeI interact with a limited number of sites which are qualitatively different.

[^{3}H]ACh binding to the receptor was measured by equilibrium dialysis. Binding was totally depressed at pH 5.2 and rose to a broad peak between pH 7.6 and 9.6.

When the membranes were held at various hydrogen ion concentrations for one hour before measuring [^{3}H]ACh binding at pH 8.0, an irreversible denaturation of binding sites occurred below pH 6.4; preincubation at pH 5.2 resulted in complete inhibition. [^{3}H]PCP and [^{3}H]H_{12}-HTX binding was similarly affected by exposure to low pH. In contrast, the inhibition of all receptor and ion channel binding by previous exposure to pH levels between 9.2 and 11.0 was completely reversed upon lowering the pH. The similar senstivity to pH of ion channel and receptor binding indicates a close relationship between the sites.

Supported by NIH grant DA-02834.

226.3 ULTRAVIOLET LIGHT-INDUCED CROSSLINKING OF NONCOMPETITIVE BLOCKERS TO THE TORPEDO MARMORATA ACETYLCHOLINE RECEPTOR. R.E. Oswald and J.P. Changeux. Neurobiologie Moléculaire, Institut Pasteur, 25 rue du Dr. Roux, 75015 Paris, France.

The acetylcholine receptor (AcChR) of Torpedo electroplaque is composed of four subunits having apparent molecular weights of 40 000 (α), 50 000 (β), 60 000 (γ), and 66 000 (δ). These polypeptide chains seem to contain binding sites for AcCh, an ion channel specific for cations, and a binding site(s) for noncompetitive blockers of the response to AcCh. The AcCh sites are carried at least in part by the α subunit, and studies with the covalent blocker, 5-azido-trimethisoquin, suggest that the binding site for these compounds is carried by the δ chain (Oswald, Sobel, Waksman, Roques & Changeux, FEBS Lett., 111, 29-34, 1980; Saitoh, Oswald, Wennogle & Changeux, FEBS Lett., 116, 30-36, 1980). The portion of the molecule comprising the ion channel is unknown but may be associated with the site for noncompetitive blockers.

We have found that reversible noncompetitive blockers can be attached covalently to the AcChR by ultraviolet irradiation at 254 nm. UV irradiation of the AcChR in the presence of (^{3}H)-trimethisoquin, (^{3}H)-phencyclidine, (^{3}H)-perhydrohistrionicotoxin, (^{3}H)-chlorpromazine, or (^{3}H)-meproadifen resulted in the covalent labeling of the receptor (observed on SDS gels) in a manner consistent with the reversible binding of noncompetitive blockers. That is, the labeling was enhanced by cholinergic agonists (and some antagonists) and was inhibited by other noncompetitive blockers. Trimethisoquin, phencyclidine, and perhydrohistrionicotoxin specifically labeled the δ chain, whereas meproadifen labeled both the α and δ chains and chlorpromazine labeled all four receptor subunits. These data indicate a contribution of the δ chain to the binding site(s) for noncompetitive blockers and suggest that either other receptor subunits contribute to this binding site or that other subunits contain additional binding sites.

This work was supported in part by grants from the Muscular Dystrophy Association.

226.4 RELATIVE POTENCIES AND CHANNEL PROPERTIES INDUCED BY CYCLIC NICOTINIC AGONISTS. C.E. Spivak* and E.X. Albuquerque. (SPON: J.E. Warnick). Dept. of Pharmacol. & Exp. Ther., University of Maryland School of Medicine, Baltimore, MD 21201.

Several cyclic nicotinic agonists were assayed by contracture of the frog's rectus abdominis muscle and compared in terms of their receptor channel properties. The mean channel lifetime (τ) and the mean channel conductance (γ) were computed by Fast Fourier analysis of endplate current noise generated when the agonists were applied to frog sartorius endplates by iontophoresis. Natural anatoxin-a (AnTX-a) was found, by rectus abdominis assay, to be 2.5 times as potent (95% confidence interval, 2.2 to 2.9) as the synthetic, racemic mixture. Although AnTX-a is an agonist at least as potent as ACh (Spivak et al., Mol. Pharmacol. 18:384, 1980), these findings suggest that the unnatural enantiomer may be a weak inhibitor of the receptor ion channel complex. Arecoline methiodide (ArMeI), which resembles AnTX-a by its conjugated carbonyl group, induces a shorter γ than does ACh or AnTX-a. Thus at -90 mV and 22 °C, τ for ArMeI was 0.41 msec whereas τ for ACh was 1.6 msec; at -75 mV and 10 °C, τ for ArMeI was 1.4 msec whereas τ for ACh was 4.0 msec. The slopes of the plots of ln τ vs membrane potential were indistinguishable for these two compounds at both 22 °C and at 10 °C. Average γ's were also indistinguishable (~ 20 pS). The tricyclic agonist cytisine was found, by the rectus abdominis assay, to be 0.088 times as potent as racemic AnTX-a (95% confidence interval, 0.076 to 0.104). Noise analysis of endplate currents produced at 22 °C showed that mean γ induced by cytisine was about 70% of that induced by ACh when data were pooled. Paired responses (N = 6), however, to ACh and cytisine delivered succesively by a double barrelled pipet indicated no difference. On the other hand, γ induced by cytisine was significantly less ($P < 0.05$) by both types of analysis, being about 60% of that induced by ACh. (±)-muscarone, at 10 °C, induced significantly shorter τ's than did ACh at 10 °C. At -90 mV, τ for muscarone was 1.6 msec vs 4.0 msec for ACh. The voltage sensitivity of τ induced by muscarone was indistinguishable from that of the ACh-induced τ. The γ induced by muscarone was about 75 % of that induced by ACh. These cyclic agonists, which are more nearly rigid than the acyclic ones, hold more promise of discovering how parameters of structure in agonists modify fundamental responses in the receptor ion channel complex. (Supported in part by USPHS grant NS-12063 and U.S. Army Research Office grant DAAG-29-78-G-0203.)

226.5 OPEN ACETYLCHOLINE (ACh)-GATED CHANNELS BLOCKED BY CHLORISONDAMINE UNDERGO A TRANSITION TO A BLOCKED-CLOSED STATE. C. Lingle. Biology, Brandeis University, Waltham, MA 02254.

This report shows that chlorisondamine (CHLOR) blocks ACh-activated responses by a channel block mechanism. Regions of the gml muscle of the lobster, Panulirus interruptus, were voltage clamped. Synaptic currents were examined with an extracellular focal electrode placed at single synaptic sites.

Block by CHLOR of ACh currents is favored by hyperpolarization. During repetitive ACh iontophoretic responses in CHLOR, following a voltage step in the hyperpolarizing direction several pulses are required before a steady state block is obtained. Latency after voltage step prior to the first response in a series does not affect initial response magnitude. Thus, the block requires exposure to ACh. When steady-state block is reached at -140mV, intervals up to 3 min. between iontophoretic responses produce no recovery from the block. Similarly, steps in voltage from -140mV to -80mV for intervals up to 3 min. before return to -140mV do not produce recovery from the steady state blocked level. If a voltage step from -140mV to -80mV is followed by at least one ACh pulse prior to return to -140mV, the next response elicited at -140mV is unblocked.

Ejc decays are single exponentials in the presence and absence of CHLOR. $5x10^{-6}$M CHLOR shortens the ejc decay rate from 32 ms to 13 ms at -140mV and 18 to 14 ms at -80mV at 11C. In CHLOR, ACh-induced currents during voltage jumps show a slow exponential decay. Despite variations in agonist concentration between different jumps the time constant of slow decays was 2.19 ± 0.33 s at -140mV and 11C. A reaction sequence which minimally accounts for the results is:

$$A+R \underset{\alpha}{\overset{K_{12}}{<-->}} AR \underset{b^*}{\overset{\beta}{<-->}} AR^* + CHLOR \overset{f^*c}{<-->} AR^*C \overset{k_c}{<-->} ARC \overset{K_{56}}{<-->} A+RC \quad (k_o)$$

where A is agonist; R, receptor; AR^*, open channel; AR^*C, blocked-open channel; ARC, blocked-closed channel; and rate constants or equilibrium constants are as indicated. Transitions from RC to R do not seem to occur. Measured decays thus represent the rate of establishment of equilibrium among 6 states as manifested in transitions between open and closed states. The modification of ejc decay in CHLOR is interpreted to be a function of f^*c, the apparent forward blocking rate, about $10^7 M^{-1}s^{-1}$ at -140 and increased with hyperpolarization. If, during long voltage jumps, transitions between AR^* and AR^*C are assumed to be near steady-state, the slow relaxation reflects $1/k_c+k_o$, the time constant of the process establishing equilibrium between the blocked-open and blocked-closed states at a given membrane potential. Supported by a MDA postdoc, NSF BNS-78-15399, the McKnight Foundation (to E. Marder).

226.6 END-PLATE CURRENTS ARE PROLONGED AT REINNERVATING NEUROMUSCULAR JUNCTIONS. THOMAS M. ARGENTIERI* AND JOSEPH J. McARDLE. (SPON: H. SAPRU) DEPARTMENT OF PHARMACOLOGY NEW JERSEY MEDICAL SCHOOL - CMDNJ, NEWARK, NEW JERSEY.

The deep peroneal nerve was crushed approximately 1.2 cm from its entrance into the extensor digitorum longus muscle (EDL) of female Wistar rats. Under this condition, functional reinnervation begins nine days after nerve crush (McArdle and Albuquerque J. Gen. Physiol., 61:1, 1973). Animals were sacrificed 14-15 days after nerve crush and the EDL, along with a 1.5 cm length of attached nerve, was removed. The nerve-muscle preparation was then set-up for in vitro recording (20-22°C) of synaptic activity. End-plate potentials (EPP's) recorded in cut fiber preparations, were found to be prolonged at regenerating synapses. Specifically, the $T_{½}$ of EPP decay increased from a control value of 1.90±0.49 msec. to 7.00±1.87 msec. (mean ± S.E.M.). Several factors in reinnervating muscle may be responsible for this abnormality: 1) an increase in the time constant of the muscle membrane, 2) the reduction of acetylcholine esterase (AchE) activity after denervation 3) changes in the channel environment which in turn may alter channel kinetic properties, and 4) channel changes which result in altered channel life time. To exclude the influence of post synaptic cable properties, a voltage clamp technique was used to analyze the end-plate current (EPC). The $T_{½}$ decay of EPC's was also found to be prolonged during synaptogenesis from a control value of 1.52±0.18 msec. to 6.8±1.56 msec. at a holding potential of -60 mV. Experiments were also performed to study the role of AchE at regenerating synapses. EPC's were further prolonged when preparations were superfused with 1.0 μM neostigmine-bromide. This data suggests that 14-15 days following nerve crush, functional levels of AchE do exist, however, its role in regulating EPC life time remains to be quantitated. It is known that changes in membrane fluidity at the site of the acetylcholine receptor channel will alter the decay of the EPC's (Gage et al, Life Sci. 14:2277, 1974). Experiments to study the effect of denervation on membrane composition and fluidity will be discussed. (Supported by NIH Grant NS-11055-08).

226.7 REDUCTION OF CHOLESTEROL CONTENT OF MUSCLE INCREASES THE END-PLATE RESPONSE TO ACETYLCHOLINE. J.J. McARDLE AND A.J. D'ALONZO.* LABORATOIRE DE NEUROBIOLOGIE CELLULAIRE, CNRS, 91190 GIF-SUR-YVETTE, FRANCE AND DEPARTMENT OF PHARMACOLOGY, CMDNJ, NEWARK, NEW JERSEY, 07103.

Katz and Thesleff (J. Physiol. 137:267, 1957) demonstrated that the response of the muscle end-plate (EP) to acetylcholine (ACh) is directly proportional to the electrical resistance of the sarcolemma. The objective of this study was to take advantage of this relationship to increase the ACh sensitivity of the EP. To do this, animals were treated with 20,25 diazacholesterol (DC) which inhibits cholesterol synthesis. Rats received 300 mg/Kg (p.o.) of DC on days 0 and 7. Between days 20 and 25, the extensor digitorum longus muscle (EDL) was removed. Gas liquid chromatography revealed that cholesterol content of the EDL decreased from 247.5±41.1 (mean ±SEM) to 137.0±11.6 mg/g of muscle. This was associated with an increase of membrane resistance from 711±33 to 1698±163 Ω/Cm2 which was due solely to a decrease of chloride conductance from 1401 to 570 pS. Because of its anatomical advantages, the deep thoracic muscle of mice was assayed for ACh sensitivity. This muscle responds to DC similarly to the EDL. Mice were injected with 50 mg/kg (s.c.) of DC at 4 day intervals and sacrificed 4 days after the 6th injection. The EP sensitivity to ACh was increased from 2062 to 3553 mV/nCoul as a result of this treatment. This finding suggests that synaptic efficiency is increased in response to reduction of the cholesterol content of muscle. The implications of this observation for neuromuscular pathology and neurobiology in general are under investigation. (Supported by Le Fondation De L'Industrie Pharmaceutique Pour La Recherche and NIH grant NS 11055-08).

226.8 "HIDDEN" α-BUNGAROTOXIN BINDING SITES IN RAT SKELETAL MUSCLE IN VIVO. A. Pestronk, Dept of Neurology, Johns Hopkins Sch. of Med. Baltimore, MD. 21205

The distribution of acetylcholine receptors (AChR) on the surface membrane of skeletal muscle can be demonstrated by the binding of α-Bungarotoxin (α-BuTx). In innervated muscle α-BuTx binding is located mainly at neuromuscular junctions. After denervation extrajunctional α-BuTx binding increases markedly. We have found that treatment of rat skeletal muscle with saponin reveals additional "hidden" α-BuTx binding sites.

Soleus muscles from 10 wk old rats were removed, teased into longitudinal strips and fixed in 2% formaldehyde. Available surface AChR were then measured using an ^{125}I-α-BuTx binding technique. "Hidden" α-BuTx binding sites were demonstrated by incubation of muscles for 5 min in 0.5% saponin before exposure to ^{125}I-α-BuTx.

Fixed muscles showed an average of 1.5 ± 0.3 x 10^7 AChR per neuromuscular junction (NMJ). This was not different from the figure of 1.6 ± 0.3 x 10^7 AChR/NMJ found in fresh unfixed fibers from the same muscles. Treatment of fixed muscle with saponin revealed an additional 0.9 ± 0.2 x 10^7 α-BuTx binding sites localized in segments of muscle containing NMJs. Autoradiography revealed that these sites were located at and near the area of the NMJ but over a longer length of the muscle fiber. These binding sites were specific since preincubation with unlabeled α-BuTx inhibited all NMJ binding of ^{125}I-α-BuTx in control and saponin treated muscles. Blockade of surface AChR in vivo had no effect on the "hidden" α-BuTx binding sites. 5 days after injection of 5 μg of α-BuTx into the soleus muscle junctional AChR were reduced to 0.3 ± 0.2 x 10^7/NMJ while the "hidden" α-BuTx binding sites were unchanged at 0.9 ± 0.2 x 10^7/NMJ.

In NMJ-free segments of innervated muscle there were low levels of surface AChR and hidden α-BuTx sites. Extrajunctional AChR averaged 1.7 x 10^3/μm of muscle fiber compared with 1.1 x 10^3 hidden α-BuTx sites/μm. After denervation both extrajunctional AChR and "hidden" α-BuTx sites rose substantially. After 4 and 7 days of denervation there were, respectively, 16 x 10^3 and 21 x 10^3 AChR/μm of muscle fiber. In the same muscles hidden α-BuTx sites rose to 16 x 10^3 and 31 x 10^3/μm.

These results show that saponin treatment of young adult rat skeletal muscles reveals "hidden" specific α-BuTx-binding sites. The distribution of the α-BuTx sites along the length of the muscle fiber parallels that of surface AChR in both innervated and denervated muscle. It is tempting to speculate that these sites are similar to the internal AChR found in skeletal muscle in vitro (Fambrough, D.M. et al., J Cell Biol. 76:237, 1978).

226.9 α-BUNGAROTOXIN BINDS TO DEVELOPING CILIARY GANGLION NEURONS BUT NOT AT SYNAPSES. Michele H. Jacob and Darwin K. Berg. Dept. of Biology, Univ. of Calif., San Diego, La Jolla, CA 92093.

A continuing controversy exists as to whether α-bungarotoxin (Bgt 2.2) binds to neuronal acetylcholine (ACh) receptors as it does to ACh receptors in muscle and electric tissue. We and others have shown that Bgt 2.2 does bind to ciliary ganglion neurons in situ and in culture but it does not block ACh receptor function. We now report that horseradish peroxidase-labeled Bgt 2.2 (HRP-Bgt 2.2) binds specifically to membranes of functionally innervated neurons in embryonic ciliary ganglia but not at synaptic sites.

Ciliary ganglia from 14-, 16-, and 20-day old chick embryos were incubated with 10^{-7} M HRP-Bgt 2.2, reacted for HRP activity, and processed for electron microscopy. In examining the ciliary cell population, dense reaction product was observed coating the membranes of small processes which emerge from the soma in the region of preganglionic innervation. Less dense labeling was occasionally associated with the smooth membrane portions of the perikaryon. In contrast, the pre- and postsynaptic membranes of clearly identifiable synapses were not reacted even in the immediate vicinity of heavily labeled structures. Synaptic regions were characterized by a strictly parallel arrangement and thickening of the pre- and postsynaptic membranes, a widening of the cleft, an enhanced postsynaptic density, and an accumulation of clear synaptic vesicles adjacent to the presynaptic membrane. HRP labeling represented specific binding of HRP-Bgt 2.2 in that it was completely blocked by incubation of the ganglia in either 10^{-5} M Bgt 2.2 or 100 μM d-tubocurarine and 1.4 mM hexamethonium prior to and along with HRP-Bgt 2.2.

We have previously reported that another neurotoxin, Bgt 3.1, from B. multicinctus venom does block ACh receptors on ciliary ganglion neurons and that, in a separate action, Bgt 3.1 can also induce the internalization of Bgt 2.2 bound to the neuron surface. Exposure of HRP-Bgt 2.2 treated ganglia to 5×10^{-7} M Bgt 3.1 for 1 hr at 37° lead to a reduction in the amount of label associated with the ciliary cell plasmalemma and an increase in the number of reactive smooth-membraned vacuoles in the cytoplasm, suggesting that internalization of bound HRP-Bgt 2.2 had been induced.

Though the Bgt 2.2 binding site does not appear to be associated with synaptic ACh receptors at early times, it does appear to be a specific component with a restricted distribution in the neuronal membrane. It will be important to determine whether the sites do become associated with synapses at later developmental stages and whether these observations apply to choroid neurons in the ganglion as well. (Supported by NIH grant NS12601 and by the Muscular Dystrophy Association.)

226.10 INTRAMEMBRANE PARTICLES OF CULTURED EMBRYONIC MUSCLE AND THEIR RELATIONSHIP TO ACETYLCHOLINE SENSITIVITY. P. C. Bridgman*, S. Nakajima, A. S. Greenberg*, Y. Nakajima. Dept. Biol. Sci., Purdue Univ., West Lafayette, Indiana 47907.

Dissociated muscle cells derived from developing Xenopus laevis (st 15-16) somites were grown in culture for 3 hours to 3 days. Cells were tested for sensitivity to acetylcholine by quantitative iontophoresis, fixed and processed for freeze-fracture. After fracturing, the tested cells were reidentified in the platinum replicas, and an average particle size distribution was constructed.

Cells first gained sensitivity to acetylcholine within 3.5 to 10 hours of culturing. The cells during this time period would correspond to stage 19-24 in the embryo. The average level of acetylcholine sensitivity nearly tripled in the next 9 to 15 hours and then increased more slowly up until the third day of culture. Cells with sensitivity to acetylcholine had higher dispersed particle densities than cells without sensitivity, and the difference was limited to large particles with diameters between 8 to 20 nm. During the initial quick increase in acetylcholine sensitivity, the density of dispersed 8-20 nm particles also increased suggesting that at least some of these individual particles may represent acetylcholine receptors. Aggregates of large particles which are thought to represent hot spots on older cells[1] were not yet observed during this time period. Thus, in order for acetylcholine sensitivity to appear, putative acetylcholine receptor particles do not have to be in an aggregated form.

(1) Peng, H. B. and Nakajima, Y., PNAS 75, 500-504.
(Supported by Grants NS-10457 and NS-08601).

226.11 MYASTHENIC ANTIBODIES INDUCE DISPERSAL OF ACHR CLUSTERS IN CULTURED RAT MYOTUBES. S. Bursztajn, S.B. Elias*, J. McManaman* and S.H. Appel, Dept. of Neurology, Baylor College of Med., Houston, TX 77030.

Antibodies from sera of myasthenia gravis (MG) patients bind to acetylcholine receptors (AChR) and accelerate the rate of degradation of AChR in cultured myotubes. We have investigated the effect of MG antibodies (IgG) on the distribution of AChR on the surface of non-innervated rat myotubes. We have determined that AChR clusters are dispersed following incubation with MG antibodies within 6 h. of exposure. Seven day old cultures were incubated with α-bungarotoxin (α-BTX) conjugated to tetramethylrhodamine (TMR) to saturate all AChR present at time 0 (T_0). The cultures were washed to remove free α-BTX-TMR and replaced with culture media containing control IgG (5 mg/ml) or MG IgG (5 mg/ml). Sister cultures were incubated with the antibodies at 37°C for 0, 5 min., 1 h., 3h., and 6h., and then fixed in 2% formaldehyde. Clusters were visualized with a fluorescent microscope and quantitated. 92 + 6% of clusters present at T_0 remained intact after 6 h incubation with normal IgG. In cultures incubated with MG IgG, the clusters rapidly became dispersed such that after 5 min. 81 + 6%; 1 h 57 + 4% and 6 h 7 + 3% of the fluorescence present at T_0 were visualized as clusters. These effects were reversibly inhibited by incubating cultures at 4°C. To quantitate the total degradation of AChR that occurred during the incubation, sister cultures were simultaneously labeled with ^{125}I-α-BTX and treated similarly with normal IgG and MG IgG. MG IgG accelerated degradation of AChR producing a loss of approximately 50% of AChR after 6 h. However, at this time MG IgG induced the disappearance of nearly all AChR clusters. Since the loss of AChR clusters (93%) is much greater than the loss of total AChR (50%), these results suggest that: 1) AChR clusters are preferentially degraded by MG IgG, and/or 2) AChR clusters are dispersed by MG IgG. Since AChR degradation follows first order kinetics, it seems unlikely that one population of AChR is preferentially degraded over another. The appearance of small fluorescent speckles of AChR in myotubes incubated with MG IgG favors the second explanation. These microclusters have previously been seen by others and presumed to be due to cross-linking of AChR on the myotube surface. The results presented suggest that at least some of the microclusters originate in the larger clusters and that cluster formation may be the result of protein-protein interaction and not a structure fixed by the cytoskeleton (Supported by the Klieberg Foundation).

226.12 BLOCKING OF ACETYLCHOLINE INDUCED CHANNELS BY A MONOCLONAL ANTIBODY AGAINST THE BINDING SITE IN CULTURED CHICK MYOBALLS. G.Goldberg*, Y.Lass, D.Mochly-Rosen*, S.Fuchs. Dept. of Physiol. & Pharmacol. Sackler School of Medicine, Tel-Aviv University, Ramat-Aviv, Israel and Dept. of Chem. Immunol. Weizmann Inst. of Science Rehovot, Israel.

Muscle weakness in myasthenia gravis is known to be associated with an autoimmune response to muscle acetylcholine (ACh) receptor.

Studies based on the use of radioactively labelled alpha bungarotoxin have shown a reduction in the number of functional ACh receptors in myasthenia gravis.

Electrophysiological studies have shown a reduction in the miniature end plate potentials in neuromuscular junctions obtained from myasthenic patients and in experimentally induced myasthenia.

We have studied the effect of a monoclonal antibody directed against the cholinergic binding site of the ACh receptor in acute experiments, in which the antibody was applied to chick muscle cells during standard electrophysiological measurements in-vitro. We found a time and dose dependent reduction in ACh sensitivity of the muscle cells. The blocking action of the antibody could be explained by a reduction in the ACh induced channel conductance.

226.13 PURIFICATION AND SUBUNIT COMPOSITION OF A PUTATIVE ACETYLCHOLINE RECEPTOR FROM DROSOPHILA MELANOGASTER. T. Schmidt-Glenewinkel*, T.R. Venkatesh* and L.M. Hall (SPON: S.A. Cohen). Dept. of Genetics, Albert Einstein College of Medicine, Bronx, NY 10461.

A putative nicotinic acetylcholine receptor has been purified from the central nervous system of Drosophila melanogaster. The receptor was solubilized from membranes prepared from Drosophila heads using 1% Triton X-100 and 0.5 M sodium chloride. The solubilized extract was passed through an α-cobratoxin-Sepharose 4B affinity column and eluted with 0.2M carbamylcholine-1% Triton X-100 containing buffer. The eluted fraction was passed over a lentil lectin-Sepharose 4B affinity column, washed with 1M sodium chloride-1% Triton X-100 and eluted with 2% α-methylmannopyranoside in the presence of 1% Triton X-100. Subsequently this receptor fraction was applied to a small affinity column consisting of α-cobratoxin-Biogel A 15m. The column was washed with 1M sodium chloride-1% Triton X-100 containing buffer and Triton X-100 was replaced with 0.1% deoxycholate. The receptor was eluted with 1M carbamylcholine-0.1% deoxycholate. The final purification of the receptor fraction was 3700-fold with a specific activity of 2.7 μmoles ^{125}I-α-bungarotoxin binding sites/g protein. The purified receptor was labeled with ^{125}I and analyzed on sodium dodecylsulfate (SDS) - polyacrylamide gels.

Membrane-bound receptor was photolabeled in situ with the 4-methylazidobenzoimidate derivative of ^{125}-I-α-bungarotoxin and was also analyzed by SDS-polyacrylamide gel electrophoresis. From the comparison of the two gel profiles it appears that the receptor is composed of four subunits with molecular weights of 48,000, 57,000, 64,000 and 78,000 daltons. A polypeptide of 102,000 daltons, also detected on the gels, could be shown by peptide mapping to be a dimer of the 64,000 dalton subunit. A sedimentation coefficient of ~9S was found for the receptor by sucrose density gradient centrifugation. The molecular weight of the receptor as determined by gel filtration in Triton X-100 containing buffer was found to be 460,000 daltons. A second peak with a sedimentation coefficient of 13.4S and a molecular weight of 880,000 daltons was also observed and might represent a dimeric form of the receptor. (Supported by grant #1126AR1 from The Council for Tobacco Research U.S.A., Inc. and NSF grant BNS 80-41293.)

226.14 ALPHA-BUNGAROTOXIN LABELLING OF AN IDENTIFIED CENTRAL SYNAPSE. Hall,D.H., Day,J.W., Hall,L.M., Bennett,M.V.L. Dept. of Neurosci., Albert Einstein Col. of Med., Bronx, New York 10461.

The South American hatchetfish, Gasteropelecus, has 10-14 myelinated giant axons postsynaptic to the Mauthner fibers in the medulla. In light microscopic examination of thick sections, these axo-axonic synapses appear as distinctive gaps in the heavy myelin coat investing each fiber (Model, et al., Br. Res., 45:288,1972). Previously reported data indicate that this synapse is cholinergic: 1) curare reversibly blocks transmission, and 2) acetylcholine reversibly desensitizes the synapse (Spira, et al., J. Cell Biol., 47:199a,1970).

We have found that alpha-bungarotoxin specifically binds to receptors in the hatchetfish medulla. We performed binding assays on tissue from medullas, each known to contain several dozen giant synapses. Homogenates of fresh and of formalin-fixed tissue (4%, 16 h) were tested for binding of ^{125}I-alpha-bungarotoxin, with or without high levels of d-tubocurarine. Both fixed and unfixed tissue showed a significant binding of toxin which was blocked by curare.

Autoradiography revealed that ^{125}I-bungarotoxin specifically labels giant synapses in the medulla. We used several methods to apply radiolabelled toxin: 1) in vivo pressure injection into the fourth ventricle, 2) in vivo subependymal injection and 3) 24 h soaking of formalin-fixed medullas. With each method, after incubation with toxin the tissue was washed in saline and fixed in glutaraldehyde, followed by embedding, sectioning, and autoradiography. Individual giant synapses were identified in bright field or phase contrast microscopy, and then examined in darkfield for the presence of silver grains. With darkfield illumination there is some interference from light scattered by the osmicated myelin of the Mauthner fibers and giant axons. Fixation with lower concentrations of osmium eliminated this interference, and still allowed identification of individual synapses. With each method of toxin administration we found clusters of silver grains at all identified synapses. Control experiments, done with curare and ^{125}I-toxin added simultaneously, showed no specific binding at the Mauthner fiber-giant fiber synapse. Thus we have further evidence that the hatchetfish giant synapse is cholinergic.

Supported by NRSA NS-06082 to JWD, NSF grant BNS 80-41293 to LMH, and NIH grant NS-12627 to MVLB.

226.15 FAST NICOTINIC AND SLOW MUSCARINIC POTENTIALS IN MYENTERIC NEURONS. T. Tokimasa*, K. Morita* and R.A. North. Neurophysiology Lab., Dept. of Pharmacology, Loyola Univ. Stritch Sch. of Med., Maywood, Illinois 60153.

Acetylcholine (ACh) was applied by iontophoresis onto the soma membrane of neurons in the myenteric plexus of the guinea-pig ileum. Low ejection currents (<40 nA, 5-20 ms) evoked short latency depolarizations of rapid time course closely similar to the fast excitatory postsynaptic potential (e.p.s.p.). Larger ejection currents (>40 nA, 1-2 s) evoked long latency (>500 ms) depolarizations of slow time course (total duration up to 30 s). The fast ACh potential was associated with a conductance increase, reversed its polarity at -15 mV and was abolished by hexamethonium (200 μM), and in these respects was identical to the fast e.p.s.p. The slow ACh potential was associated with a conductance decrease, reversed its polarity at -90 mV and was abolished by hyoscine (1-10 nM). A similar slow depolarization was also observed with iontophoretic application of oxotremorine. When added to the perfusion solution, oxotremorine (100 nM - 1 μM) and methacholine (300 nM - 10 μM) caused concentration dependent depolarizations which were also accompanied by a conductance decrease, and reversibly abolished by hyoscine (10 nM). At even lower concentrations, both muscarinic agonists reversibly reduced the amplitude of the fast e.p.s.p. without affecting the fast ACh potential. This action was reversed by hyoscine (1 nM). The results indicate that activation of muscarinic receptors on myenteric neurons causes both potassium channel inactivation on the soma membrane, and presynaptic inhibition of ACh release.

226.16 MUSCARINIC AUTORECEPTORS CONTROL ACETYLCHOLINE RELEASE IN SYNAPTOSOMES FROM RAT HIPPOCAMPUS. M. Marchi*, P. Paudice* and M. Raiteri (Spon: J.M. Lauder). Institute of Pharmacology and Pharmacognosy, University of Genova, Via al Capo di S. Chiara 5, Genova, Italy.

The existence of presynaptic autoreceptors modulating acetylcholine (ACh) release from central cholinergic nerve endings was investigated utilizing rat hippocampal synaptosomes in a superfusion system. The presence of exogenous acetylcholine, carbachol or oxotremorine in the superfusion medium produced a dose-dependent inhibition of the release of ^{3}H-ACh elicited by 15mM KCl in synaptosomes prelabeled with tritiated choline. Acetylcholine seems to be the most active agonist tested at the presynaptic receptors, whereas nicotine was found to be ineffective. The inhibition of ^{3}H-ACh release was antagonized by atropine or scopolamine which, as expected in superfused synaptosomes did not cause, per se, the increase of ^{3}H-ACh release found in slices or in incubated synaptosomes. Interestingly bethanechol, which is a very effective agonist at muscarinic postsynaptic receptor in the peripheral nervous system was actually inactive on the ACh autoreceptors in the central nervous system.

Our results indicate that cholinergic nerve terminals in the rat hippocampus possess autoreceptors of the muscarinic type for the control of acetylcholine release.
(Partially supported by Grant CT 80.00549.04 from Italian CNR).

226.17 ACETYLCHOLINE-EVOKED CURRENT IN NEUROBLASTOMA CELLS HAS THREE COMPONENTS. Eiji Kato*, Fred N. Quandt* and Toshio Narahashi (SPON: Charles A. Berry). Dept.of Pharmacol., Northwestern Univ. Med. Sch., Chicago, IL 60611.

The mouse neuroblastoma cell line N1E-115 is sensitive to iontophoretic application of acetylcholine (ACh). The membrane potential response to ACh was composed of three phases: An initial fast depolarization was followed by a transient hyperpolarization which in turn was followed by a secondary slow depolarization lasting approximately 30 sec. The initial fast depolarization phase was blocked by 10 μM d-tubocurarine, but not by 0.1 μM atropine. Both the hyperpolarization and the slow depolarization were blocked by 0.1 μM atropine, but not by 10μM d-tubocurarine, and only these two phases were evoked by iontophoretic application of the muscarinic agonist methacholine.

Under voltage clamp conditions, an initial fast inward current, a transient outward current, and a secondary slow inward current were recorded in response to ACh application. These phases of current corresponded in time to the three phases of the membrane potential response. The initial fast inward current increased in amplitude by hyperpolarizing the membrane and decreased by depolarization. The mean value of reversal potential for the initial current was estimated to be -1 mV. The outward current increased in amplitude by depolarization, decreased by hyperpolarization and reversed its polarity at -67 mV (mean). This reversal potential was dependent on the potassium concentration in the external solution. When the potassium concentration was increased from 5.5 mM to 10 mM, the reversal potential was shifted in the direction of depolarization by 13 mV (mean). Decreasing the external potassium concentration from 5.5 mM to 0.5 mM caused a shift of the reversal potential in the direction of hyperpolarization by approximately 40 mV. The slow inward current increased in amplitude by hyperpolarization and decreased by depolarization. The reversal potential was approximately +20 mV.

It was concluded that the initial fast inward current is mediated by a nicotinic receptor similar to that in the endplate and postsynaptic membranes of the sympathetic ganglia. Both the outward current and the slow inward current are mediated by a muscarinic receptor. The outward current represents an increase in the membrane permeability to potassium, and the slow inward current appears to be carried by sodium and potassium. (Supported by NIH grant ES 02330).

226.18 CORRELATION BETWEEN ACETYLCHOLINE SENSITIVITY AND ALPHA-BUNGAROTOXIN BINDING AT NODOSE NEURONS IN CULTURE, E. Cooper*(SPON: K. Ruf). Dept. of Physiol., McGill Univ., Montreal, Quebec H3G 1Y6.

Dissociated neurons from newborn rat nodose ganglia (an autonomic sensory ganglion) express functional acetylcholine (ACh) receptors when grown in tissue culture. These ACh receptors are similar to nicotinic receptors of principle neurons in autonomic ganglia; the depolarizations have a fast time course (relative to muscarinic receptors), and are reversibly blocked by either hexamethonium or curare (10^{-5}M) and are unaffected by atropine (10^{-6}M). The proportion of neurons which express these nicotinic receptors depends on the culture conditions: 80-90% of neurons grown in the absence of non neuronal cells express functional ACh receptors, however only 10-30% of neurons grown in the presence of muscle (cardiac or skeletal from newborn rats) express these receptors.

Further studies on the expression and regulation of the nicotinic receptor sites in these nodose neurons would be facilitated if there were irreversible ligands for these receptors. There is some uncertainty whether alpha bungarotoxin (α-BTX), which is a useful marker for nicotinic receptors at the vertebrate neuromuscular junction, binds to neuronal ACh receptors. For example, the toxin does not appear to block ACh depolarizations of nodose neurons in culture. The question addressed in this report is whether α-BTX binding can be used as a marker for those cultured nodose neurons which express ACh sensitivity, even though the α-BTX binding site and the ACh receptors may not be identical.

The experiments consisted of combined physiological and radioautographic studies on identified neurons in culture. First the ACh sensitivity of neurons was determined by recording the membrane potential with conventional microelectrode techniques while pressure ejecting ACh (10^{-4}-10^{-3} M) onto the cell bodies; sensitive neurons depolarized 10-30 mV, insensitive neurons showed no change in membrane potential. 10-20 neurons were examined per culture and their locations were identified on photographic montages of the cultures. The cultures were subsequently incubated with I^{125} α-BTX, fixed, and processed for radioautography. The identified neurons were then examined for the presence or absence of silver grains.

The results of the preliminary experiments suggests that there is a good correlation between ACh sensitive neurons and α-BTX binding, as well as, between ACh insensitive neurons and the lack of silver grains. It appears that even though α-BTX does not block ACh depolarizations its binding may still serve as a useful marker for ACh sensitive neurons in nodose ganglion cell cultures. (Supported by MRC of Canada).

226.19 DIFFERENTIAL REGULATION OF ACETYLCHOLINE RECEPTORS IN CULTURED AVIAN RETINA. Robert G. Siman and William L. Klein. Dept. of Neurobiology and Physiology, Northwestern Univ., Evanston, Il. 60201.

Both muscarinic and nicotinic cholinergic receptors have been found in embryonic and adult chicken retina (Vogel and Nirenberg, 1976, PNAS 73, 1806-1810; Sugiyama et al., 1977, PNAS 74, 5524-5528). We present evidence obtained using retina cells grown in primary aggregate cultures that changes in cholinergic stimulation regulate muscarinic but not nicotinic receptor concentration.

Muscarinic receptor sites, monitored by the specific binding of 3H-(l)-QNB, decreased up to 76% in cultures given muscarinic agonists. Chronic agonist exposure did not alter receptor affinity for 3H-(l)-QNB. The muscarinic specific agonist oxotremorine, at 1 uM for 18 hours, induced a 67% loss of binding that was blocked by co-incubation with 10 nM atropine, a muscarinic specific antagonist. The mixed muscarinic-nicotinic cholinomimetic carbachol caused a maximum decrease of binding within 24 hours. In contrast to the large decrease found in muscarinic binding, levels of nicotinic receptors showed no change under identical conditions of stimulation. Membranes from cultures grown 48 hours in medium containing 1 mM carbachol or 100 uM nicotine bound the same amount of 125I-alpha-bungarotoxin (125I-BTX) or 3H-bromoacetylcholine (3H-BAC) as membranes from control cultures not grown in nicotinic agonist. Nicotine-displaceable 3H-BAC and 125I-BTX binding appeared to occur at related sites. Treatment of dithiothreitol-reduced membranes with 1 mM unlabelled BAC for 5 minutes, followed by extensive washing, blocked 100% of specific 125I-BTX binding. No blockade occurred in unreduced membranes. Similarly, 3H-BAC bound irreversibly to dithiothreitol-reduced membranes but not to unreduced preparations or to those reduced and then alkylated with N-ethyl-maleimide.

The rate of muscarinic receptor loss is biphasic and approximates the sum of two exponentials. For the receptor loss induced by 1 mM carbachol, the first phase had a half-time of 0.8 hours, while the second had a half-time of 3.9 hours. The first phase of the receptor loss was completely blocked by the Na-K ionophore gramicidin D. Receptor sites appeared at a calculated rate of 3.6 fmol/mg protein/hour.

Our data show that cholinergic stimulation readily alters muscarinic receptors but has little or no effect on nicotinic receptors. This differential regulation may reflect different roles played by these receptors in adaptation to altered physiological activity.
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226.20 Cholinergic Agonist-Induced Down Regulation of Neuronal α-Bungarotoxin Receptors. A. Messing* (SPON: J. Q. Trojanowski). Div. of Neuropath., Univ. of Penn. Sch. of Med., Philadelphia, PA 19104.

In view of the controversy over the significance of the αBTX binding component in neuronal membranes, I sought to determine whether this receptor could be down regulated by cholinergic ligands. Dissociated monolayer cultures of chick ciliary ganglion neurons were prepared from 8-day embryos as described previously (Messing, A. and Kim, S.U., Brain Res., 208:479, 1981). Neuronal survival in these cultures typically exceeded 10,000 neurons per ganglion and cultures were used between days 4-8 in vitro. Surface binding of ^{125}I-αBTX was determined at 22°C on intact cells, and non-specific binding (in the presence of 10 μM native toxin) never exceeded 2% of the total.

^{125}I-αBTX bound to these neurons with a K_D=2.5x10^{-10} M and a Hill coefficient of n=1.01. The cholinergic ligands carbachol, nicotine and d-tubocurarine inhibited the rate of ^{125}I-αBTX binding with IC_{50}'s of 270 μM, 0.5 μM and 0.5 μM, respectively. When cultures were pre-exposed to 1 mM carbachol at 37°C, washed and then assayed for remaining αBTX sites, αBTX receptor number decreased by 25% during the first hour of agonist exposure and then remained constant through 4 hrs. of agonist exposure. Exposure for longer periods of time (1-6 days) caused an additional slower loss of receptors to a maximum of 70% below controls. The rapid loss of receptors during 60 min. of agonist exposure occurred without a change in affinity for ^{125}I-αBTX as determined by Scatchard analysis. Nicotine (3 μM), but not d-tubocurarine (100 μM), also caused a loss of 25% of the surface α-BTX receptors after 60 min. at 37°C. Both the carbachol- and nicotine-induced ^{125}I-αBTX receptor losses were prevented when the exposure took place at 4°C; receptor loss was also prevented by preincubation with 100 μM d-tubocurarine followed by coincubation with carbachol and d-tubocurarine. None of the treatments described above had any effect on neuronal number or neuronal morphology.

Cholinergic ligands clearly interact with the αBTX receptor in these neurons, and this receptor seems capable of down regulation by agonists but not by the antagonist, d-tubocurarine. These data are consistent with the hypothesis that αBTX binds to the neuronal acetylcholine receptor, despite its apparent failure to block transmission as described by others. The rapid loss of 25% of the surface αBTX receptors during the first hour of agonist exposure is in marked contrast to the results seen in muscle (Noble, M.D., Brown, T.H. and Peacock, J.H., PNAS, 75: 3488, 1978; Gardner, J.M. and Fambrough, D.M., Cell, 16:661, 1979) (supported by USPHS grants NS-05572-17 and NS-07064-01).

226.21 IBOTENIC ACID LESIONS OF THE HIPPOCAMPUS RESULT IN A LOSS OF MUSCARINIC RECEPTORS LINKED TO PHOSPHOLIPID TURNOVER. Stephen K. Fisher*, Kirk A. Frey* and Bernard W. Agranoff. Neuroscience Laboratory, University of Michigan, Ann Arbor, MI 48109.

In a previous study (Brain Res. 189, 284-288, 1980), we observed that destruction of the cholinergic afferent input (fornix/fimbria) to the hippocampus reduced neither the number of muscarinic receptors present in hippocampal nerve ending fractions, nor did it alter a functional correlate of this receptor: muscarinic-agonist stimulation of phosphatidic acid (PhA) and phosphatidylinositol (PhI) labeling from $^{32}P_i$. This result suggested that the muscarinic receptors linked to phospholipid turnover in hippocampal nerve endings are present on cholinoceptive rather than on cholinergic structures. To further investigate this possibility, we have used the neurotoxin, ibotenic acid, to selectively destroy intrinsic neurons in the hippocampus. Male guinea pigs received 3 x 10 µg ibotenic acid injections in one side of the hippocampus. Twelve days later, completeness of the unilateral injections was evaluated histologically. Following ibotenic acid lesion, the specific activity of glutamate decarboxylase in hippocampal nerve ending fractions was reduced by 30 ± 7%, and [^{3}H]quinuclidinyl benzilate binding by 42 ± 6% ($p<0.005$). While there was a loss in yield of nerve endings (23 ± 4%), activity of basal phospholipid labeling from $^{32}P_i$ computed per mg protein from lesioned hippocampus was not reduced. There was, however, a marked reduction in carbachol-stimulated labeling of PhA (91 ± 9% in the control vs. 52 ± 7% in the lesion, $p<0.005$) and in that of PhI (31 ± 5% in the control vs. 19 ± 4% in the lesion, $p<0.02$). These results, taken together with those previously obtained from the fornix lesions, confirm that hippocampal muscarinic receptors coupled to PhA and PhI turnover are located on post-synaptic cholinoceptive structures. (Supported by NIH Grant #NS 15413. SKF was supported by NIMH Training Grant #MH 15794-01.)

227.1 RESPONSE PROPERTIES OF LAYER V NEURONS TO ELECTRICAL STIMULATION OF THE CORPUS CALLOSUM IN SLICES OF CINGULATE CORTEX. B.A. Vogt and A.L.F. Gorman. Boston University School of Medicine, Boston, MA 02118.

The corpus callosum (CC) and its termination sites in areas 24 and 29 of rat cingulate cortex were isolated in vitro and the intracellular responses of layer V neurons to electrical stimulation of the CC evaluated. The distribution of synaptic contacts formed by degenerating callosal axons with pyramidal neurons in cingulate cortex were also analyzed using a combined degeneration /Golgi-EM technique.

Layer V neurons had a resting membrane potential of 60±0.68 mV (S.E. of the mean), an input resistance of 47±4.74 megohms and produced spontaneous action potentials 50±0.5 mV in amplitude. There were three types of responses to CC stimulation of increasing strengths. First, an antidromic spike followed by an EPSP with synaptic-evoked spikes and, in some instances, a longer latency IPSP. Second, an EPSP with synaptic-evoked action potentials each with discrete stimulation thresholds, but not associated with antidromic or inhibitory activity. Third, an EPSP with an all-or-none burst of action potentials. Antidromic spikes did not have an underlying EPSP, were 0.7-1.0 msec in duration and had conduction velocities of 1.3-3.0 m/s. EPSPs had a calculated reversal potential of 0 to -20 mV and were of longer duration in anterior than in posterior neurons (50±3.57 msecs vs 26±1.56 msec) and had a higher maximum amplitude in anterior neurons (20±1.0 mV vs 11±0.79 mV). At low stimulation strengths IPSPs were observed to follow EPSPs in posterior neurons, but never antidromic spikes. During CC stimulation depolarizing intracellular currents failed to uncover IPSPs in other neurons and, at higher stimulation strengths, IPSPs were suppressed by enhanced EPSP and spiking activity.

Since greater numbers of callosal axon terminals could account for enhanced EPSP activity in anteriorally located neurons, the synaptic termination of degenerating callosal axons with layer V cells was evaluated. The apical dendrites of comparably sized pyramidal neurons formed up to four times as many synaptic terminals with callosal afferents in anterior cortex as did those with posterior neurons.

Thus, it is possible to isolate responses of cortical neurons to electrical stimulation of the corpus callosum in slices of rat cingulate cortex, and differences of the neuronal responses in anterior and posterior cortices can be partially accounted for by the number of callosal afferents they receive.

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227.2 INTRICACIES OF ACETYLCHOLINE-RECEPTOR INTERACTION DURING A MINIATURE ENDPLATE CURRENT; SIFTING OF MODELS BY MEANS OF COMPUTER SIMULATION. P. Pennefather* and D. M. J. Quastel. Department of Pharmacology, Faculty of Medicine, The University of British Columbia, Vancouver, B. C., Canada, V6T 1W5.

Virtually any model of transmitter-receptor interaction can be adjusted to predict, as a consequence of abrupt discharge of a quantum of transmitter into the synaptic cleft, a miniature endplate current (MEPC) with (1) rapid rise, and (2) a near exponential decay that is slowed by inhibiting acetylcholinesterase (AChE) and then accelerated by receptor blockade. Moreover, if parameters are chosen such that there is normally high capture of transmitter by receptor all models predict that the height of the MEPC should be insensitive to alteration of receptor density (Pennefather and Quastel, Neurosci. Abst. 5, 745). Nevertheless, certain aspects of the MEPC are difficult to mimic and can provide a basis for testing the validity of a particular model. The presumption of non-linear ACh-receptor interaction is compelled by the observation that MEPCs have a decay rate close to that of individual channels, together with indications that a high propertion of quantally-released transmitter is indeed captured. With non-linear systems it is necessary to integrate the appropriate differential equations (for diffusion, hydrolysis and ACh-receptor interaction) to determine how the system will behave. Our present FORTRAN program allows synthesis of theoretical MEPCs at up to 1 ms/min with as many as 10 states of the receptor, in multiple compartments; this permits testing a variety of models of ACh-receptor interaction. Aspects of real MEPCs that simulation shows to be sensitive to possible complexities in the system (e.g., cooperativity in binding, number of states, state transitions) and which are difficult to mimic quantitatively include (a) correlation of height and decay rate, (b) effects of exogenous agonist on the height and time course, and (c) effects of AChE poisoning and/or receptor blockade on the deviations from a simple exponential of the falling phase of the MEPC. We find that the simplest models that come close to predicting real MEPCs involve the existence of more than two ACh binding sites on the receptor (although channel opening requires the "cooperation" of only two ACh molecules) and a small but appreciable local desensitization of receptors during an MEPC.

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227.3 COMPUTER SIMULATION OF THE MAUTHNER CELL. Chris M. Wieland* and Robert C. Eaton., (SPON: A.C. Bekoff), Dept. Biol., E.P.O., Univ. of Colo., Boulder, CO 80309.

Because of its accessibility for electrophysiological analysis, the Mauthner (M-) cell of teleost fish has been utilized for a number of significant conceptual advances in synaptology. The existence of both electrical and chemical excitatory and inhibitory synapses on the cell has been helpful in elucidating detailed properties of certain synaptic interactions. Also of interest is the possibility of studying functional changes as a result of morphological development.

At present, some aspects of M-cell electrophysiology still remain inaccessible to examination with conventional techniques and another means of investigation is required. To aid in the study of synaptic interactions of the M-cell, BASIC and FORTRAN IV programs have been developed to model the electrical activity of this neuron in goldfish. Dendritic characteristics were based on the model of Furukawa (1), with biophysical characteristics of the axon hillock, initial segment, and the M-axon derived from the work of Faber and Funch (2). Mathematical representation of the dendrites and soma is based on standard cable equations while simulation of the excitable membrane is based on a four-state variable neuromime as developed by MacGregor and Oliver (3).

With these programs, biophysical and anatomical characteristics can be easily modified to simulate theoretical situations or to replicate experimental findings, such as modeling two interconnected M-cells simultaneously to simulate mutual inhibitory interactions. Since the programs are written in both BASIC and FORTRAN they can be easily adapted to most computer systems including inexpensive microcomputers.

Supported by N.S.F. grants BNS 78-10687 and 79-05770 and N.I.H. grant BRSG RR7013-80 to R.C. Eaton.

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227.4 INTERNALLY-EXPOSED POLYPEPTIDES OF THE SYNAPTIC PLASMA MEMBRANE. J.A. Babitch & D.F. Bahr*. Chemistry Department, Texas Christian University, Fort Worth, TX 76129

We previously determined the molecular weights of the major external polypeptides in the chick forebrain synaptic plasma membrane [Chiu & Babitch, J. Biol. Chem. 252 (1977) 3862-3869; Biochim. Biophys. Acta 510 (1978) 112-123]. These are membranes which have relatively low levels of carbonic anhydrase, a glial membrane marker [Parthe, J. Neurosci. Res. (1981) 119-131]. We now report the molecular weights of 4 internally-disposed synaptic membrane polypeptides.

Demonstrating that these are internally-exposed requires showing that (1) incorporation of a label occurs into polypeptides and (2) these polypeptides cannot be labeled from outside the nerve ending. To do that we incubated synaptosomes under phosphorylating conditions. Greengard and coworkers [Kreuger et al., J. Biol. Chem. 252 (1977) 2764-2773] previously suggested that the phosphopeptides they identified after such incubations might be internal synaptic components. Chick forebrain synaptosomes were incubated with histones, protein kinase and $^{32}P_i$ or γ-$^{32}P_i$-ATP either with or without apyrase. When synaptosomes were incubated with $^{32}P_i$ most of the incorporation was into soluble polypeptides. When synaptosomes were incubated with γ-$^{32}P_i$-ATP almost no labeling was observed unless protein kinase was present (when histones were labeled also) and then the bulk of the labeling was particulate. When apyrase was present, labeling from ATP dropped to levels below that of ATP alone. This indicates that protein kinase and ATP label external polypeptides (and exogenous histones), the labeling of which can be eliminated by apyrase. When synaptosomes were incubated with $^{32}P_i$ apyrase had no effect, nor did a combination of protein kinase, γ-$^{32}P_i$-ATP and apyrase.

Synaptic plasma membranes were isolated from synaptosomes incubated with $^{32}P_i$ and apyrase and plasma membrane polypeptides were separated on polyacrylamide gels. The major labeled, internally-exposed phosphopeptides have molecular weights (in thousands) of 108, 81, 69 and 55.

227.5 SPECIFIC GLYCOPROTEINS AND KAINIC ACID BINDING SITES ARE LOCALIZED AT SYNAPTIC JUNCTIONS. E. Edward Mena, Graham E. Fagg*, Alan C. Foster* & Carl W. Cotman. Dept. of Psychobiology, University of California, Irvine, CA 92717.

Synaptic glycoproteins probably play an important role in synaptic function and plasticity. The most likely glycoproteins for this function are those located specifically to the synapse. In order to identify these components synaptic junctions (SJ) were prepared from synaptic plasma membranes (SPM) by extraction with Triton X-100 and density gradient centrifugation. These SJs were enriched in the four Concanavalin A (Con A) binding glycoproteins of MWs 160,000 (I), 123,000 (II) 110,000 (III) and 95,000 (IV). We prepared crude myelin, light SPMs, SPMs, mitochondria, and microsomes and extracted them with detergent by procedures identical to those employed when preparing SJs from SPMs. Those fractions prepared from light SPMs and crude myelin contained identifiable synaptic junctions and were also highly enriched in the four Con A binding glycoproteins and the 52,000 MW postsynaptic density (PSD) protein. The SJ-like fractions from mitochondria did not contain significant levels of any of the characteristic synaptic macromolecules. However, the same fraction from microsomes, which on the basis of electron microscopy did not contain SJs, contained the 52,000 MW PSD protein to true SJs although the Con A binding glycoproteins, especially Con A-IV, were nearly absent.

The Con A binding glycoproteins of the SJ were isolated from solubilized SJs by affinity chromatography and separated by gel electrophoresis. The primary sequence of these proteins were compared by examining their two dimemsional ^{125}I-tryptic peptide maps. Con A-I, II and III had many peptides in common. The primary structure of Con A-IV was unrelated to any of the other glycoproteins. Thus, these results indicate that the Con A binding glycoproteins are markers for the SJ. Futhermore the heterogeneity that is observed among the Con A binding glycoproteins of the SJ may reside in their carbohydrate rather than protein moiety.

The SJ-like fraction prepared from crude myelin, light SPMs, and microsomes were futher analyzed for kainic acid binding sites in order to determine whether they possesed these sites and if they were distributed similiar to other transmitter binding sites. The results indicated that all SJ and SJ-like fractions contained similiar levels of binding sites for L-glu and L-asp. In contrast only those fractions that contained identifiable SJ contained high levels of KA binding sites. Thus, kainate binding sites appear more specific to synapses than L-glu or L-asp. This supports a previous suggestion that a kainate like molecule may be a neuronal modulator/transmitter.

227.6 TOPOGRAPHY AND ORGANIZATION OF SOME PROTEINS IN THE POSTSYNAPTIC DENSITY. N. Ratner* (SPON: H.R. Mahler). Dept. of Chemistry, Indiana University, Bloomington, IN 47405.

The postsynaptic density (PSD), located on the cytoplasmic side of the postsynaptic membrane of many CNS synapses, is an organelle of unknown function. Many PSD proteins have, however, been identified; among them are actin, tubulin, calmodulin and a unique "PSD protein". We have utilized a surface-specific iodination system [Markwell, M.A. and Fox, C.F. (1978) Biochemistry 17, 4807-4817] to probe the locations and accessibility of these and other PSD proteins. Actin is the major protein located on the exterior of the PSD. Other identified proteins are inaccessible to iodination unless they are first reduced with dithiothreitol. Major species that become iodinated following this treatment include PSD protein and tubulin. These results suggest that in the intact PSD these proteins may constitute a cross-linked aggregate which may be buried inside the organelle and covered by a shell of actin. These and other quantitative measurements will be used in the formulation of a model for the PSD. (Supported by research grant NS08309 from the National Institute of Neurological and Communicative Disorders and Stroke, NIH)

227.7 ANTISERUM TO TORPEDO 43K PROTEIN RECOGNIZES AN INTRACELLULAR POSTSYNAPTIC MEMBRANE PROTEIN OF THE RAT NEUROMUSCULAR JUNCTION. S.C. Froehner and V.G. Gulbrandsen*. Dept. of Biochemistry, Dartmouth Medical School, Hanover, N.H. 03755.

In addition to the acetylcholine receptor (AChR) subunits, highly-purified postsynaptic cholinergic membranes from Torpedo electric organ contain a major protein of approximately 43,000 mol. wt. (43K protein). Membrane treatments that extract peripheral membrane proteins remove the 43K protein without detectable effects on receptor function. We have shown previously by immunofluorescence on tissue sections that a rabbit antiserum directed against the 43K protein stains only the innervated membrane of Torpedo electrocytes and also recognizes a muscle antigen that is highly concentrated in the postsynaptic membrane of the rat neuromuscular junction. In these experiments, both sides of the membrane are accessible to antibodies. To determine if the 43K protein is located on the extracellular or on the cytoplasmic side of the synaptic membrane, we have examined the reactivity of anti-43K with intact muscle. Rat soleus or abductor hallucis muscles were incubated for 2 hours with either anti-AChR or anti-43K under physiological conditions. The muscles were then rinsed and cryostat sections were cut. After additional washing to remove unbound antibodies, the sections were incubated with fluorescein-labeled anti-rabbit IgG to visualize the bound antibodies and with rhodamine-αbungarotoxin to mark the endplates. Under these conditions, anti-AChR labels endplates. This finding indicates that a portion of the receptor is extracellularly exposed and also demonstrates that the synaptic region is accessible to antibodies. Little or no staining of the endplate occurs when intact muscles are incubated with anti-43K. However, if sections are reincubated with either anti-43K or anti-AChR, both antisera label the synapse. Thus, the major muscle antigen recognized by anti-43K is not exposed on the extracellular side of the postsynaptic membrane. These results are consistent with the idea that, like the Torpedo 43K protein, the muscle antigen is a peripheral membrane protein which is highly concentrated on the cytoplasmic face of the postsynaptic membrane. This work was supported by NIH grant NS 14871 and by the Muscular Dystrophy Association.

227.8 IMPULSE CONDUCTION INCREASES THE STATE OF PHOSPHORYLATION OF PROTEIN I, A NEURON-SPECIFIC PROTEIN, IN THE RABBIT SUPERIOR CERVICAL GANGLION. Eric J. Nestler* and Paul Greengard. Department of Pharmacology, Yale University School of Medicine, New Haven, CT 06511.

Protein I, a neuron-specific protein concentrated at synapses, is present in most, and possibly in all, presynaptic nerve terminals where it appears to be associated predominantly with neurotransmitter vesicles. Protein I is phosphorylated endogenously in the collagenase-insensitive region of the molecule both by a cyclic AMP-dependent and by a calcium/calmodulin-dependent protein kinase and in the collagenase-sensitive region of the molecule by a second calcium/calmodulin-dependent protein kinase. Previous studies have shown that dopamine and depolarizing agents increase the state of phosphorylation of Protein I in slices of the bovine superior cervical ganglion. These findings prompted us to determine whether stimulation of the preganglionic nerve under physiological conditions might regulate Protein I phosphorylation in the rabbit superior cervical ganglion.

The small amount of Protein I present in the rabbit ganglion (total content: 1 pmol/ganglion) necessitated the development of sensitive techniques for this study. One superior cervical ganglion from each rabbit served as the "test" ganglion and the other as the control. The preganglionic nerve supplying the test ganglion was stimulated via a suction electrode for 30 s at 10 Hz. The ganglia were then homogenized and boiled in 1% SDS; the Protein I was immunoprecipitated from the SDS extract; the precipitated Protein I was "back phosphorylated" using purified protein kinase and [γ-^{32}P]ATP; the phosphorylated samples were analyzed by polyacrylamide gel electrophoresis and scintillation spectrometry. The amount of dephospho-Protein I in stimulated ganglia was 56 $\pm$ 4% (7) [mean $\pm$ s.e.m. (N)] of that in control ganglia. In contrast, the amount of dephospho-Protein I in unstimulated ganglia was 102 $\pm$ 4% (6) of that in paired (contralateral) control ganglia. Incubating ganglia in calcium-free buffer blocked the nerve-stimulated change in Protein I phosphorylation [dephospho-Protein I in test ganglia was 103 $\pm$ 9% (4) of that in control ganglia]. The amount of dephospho-Protein I in ganglia exposed to 60 mM K^+ for 1 min was 54 $\pm$ 4% (5) of that in control ganglia. Analysis of phosphorylation sites by one dimensional peptide mapping indicated that the observed percent decreases in holodephospho-Protein I were reflected in virtually identical changes both in the collagenase-insensitive region and in the collagenase-sensitive region of the molecule.

These data demonstrate that the state of phosphorylation of Protein I is regulated by physiological activity.

227.9 CHOLINERGIC SYNAPTIC VESICLES CONTAIN AN ANTIGENIC PROTEOGLYCAN. Steven S. Carlson & Regis B. Kelly. Dept of Biochemistry & Biophysics, Univ. of California, San Francisco, Ca 94143

Antibodies raised to highly purified synaptic vesicles from fish electric organ cross-react with a cytoplasmic element of the mammalian nerve terminal, presumably the synaptic vesicle. The cross-reacting antigens are not present on the outside of a resting frog neuromuscular junction, but become exposed to the outside when the terminal undergoes exocytosis of synaptic vesicle contents (von Wedel, Carlson & Kelly (1981). PNAS 78, 1014). The antigens which are exposed on the outside of the active nerve terminal are presumably those which are inside the synaptic vesicle. We have demonstrated that electric organ synaptic vesicles contain large amounts of a material with the characteristics of a proteoglycan. It does not enter acrylamide gels. It contains protein and carbohydrate. It elutes from gel filtration columns containing SDS as a large molecular weight material between 70 and 335 kd. It binds an NP-40 detergent to wheat germ agglutinin-Sepharose 4B beads and is selectively eluted by N-acetylglucosamine. After protease digestion, a negatively charged carbohydrate is obtained with an electrophoretic mobility close to that of heparin sulfate. The proteoglycan is inside the synaptic vesicle since intact vesicles fail to bind to wheat germ agglutinin beads whereas vesicles lysed by sonication bind very effectively. The antibodies which recognize synaptic vesicles immunoprecipitate the proteoglycan. The antigens which appear during exocytosis could therefore be the proteoglycan determinants, but this has not been shown directly. The proteoglycan may play a role in packaging the contents of the synaptic vesicle. Whatever its function, the observation that synaptic vesicle proteoglycans have unique determinants suggests that their structure is functionally important.
This research was supported by NIH grants NS 09878 and NS 15927.

227.10 PARTIAL PURIFICATION OF ACTIVE ZONES OF THE PRESYNAPTIC PLASMA MEMBRANE BY IMMUNOADSORPTION. George Miljanich*, Allan Brasier* & Regis B. Kelly. Dept. of Biochem.& Biophys., Univ of Calif., San Francisco, Ca 94143

A highly specific antiserum has been previously obtained against purified marine ray electric organ synaptic vesicles (anti-SV). Anti-SV also binds to the surface of electric organ synaptosomes. Thus we have been able to use anti-SV as an affinity reagent to isolate the electric organ nerve terminal presynaptic plasma membrane (PSPM). First, synaptosomes prepared by conventional means are immunoadsorbed by anti-SV to anti-antibody-coated polyacrylamide beads. This gives a 1.5-2.5 fold increase in cholineacetyltransferase specific activity (synaptoplasmic marker), a 70 fold decrease in acetylcholine receptor specific activity (innervated postsynaptic membrane marker) and a 3 fold drop in Na/K ATPase specific activity (non-innervated postsynaptic membrane marker). 5'-Nucleotidase and cholinesterase specific activities are unchanged. Electron micrographs clearly show synaptosomes bound to beads. To enrich immunoadsorbed material in PSPM, the bound synaptosomes were hypotonically lysed and sonicated. Micrographs reveal numerous small (<250 nm), irregular vesicles and less numerous membrane sheets adherent to beads. Virtually all transferase is release. About 2% of total protein and 2% of nucleotidase activity remain bead-bound. This is not unexpected since the bulk of nucleotidase activity resides in synaptic vesicles. To monitor recovery of plasma membrane, synaptosomes were surface-labeled with ^{125}I before immunoadsorption. After lysis and sonication 10-20% of radioactivity remained bead-bound, giving a 5-10 fold enrichment in bound membrane. After lysis and sonication 50-70% of anti-SV antibody remained bound indicating a 25-35 fold enrichment of surface antigens in bound membrane. Presumably anti-SV marks the active zones of the PSPM. The presence of non-vesicle components in PSPM was shown by the properties of an antiserum raised against the PSPM preparation (anti-PSPM). Anti-PSPM can mediate the immunoadsorption of synaptosomes even after pre-adsorption with disrupted synaptic vesicles to remove anti-SV antibodies. Furthermore, anti-PSPM binds to nerve terminals in frozen microtome sections of frog neuromuscular junction just as anti-SV does. However, in contrast to anti-SV, which binds to intact terminals only after stimulation, anti-PSPM binds to intact terminals without prior stimulation. In conclusion, biochemical markers, electron microscopy, anti-SV binding and immunoadsorption, and production of a specific anti-PSPM antiserum demonstrate that we have isolated a membrane preparation which is enriched in the PSPM and its active zones. (This research was supported by NIH grant NS 09878.)

227.11 ULTRASTRUCTURAL CORRELATES OF GUANIDINE STIMULATION OF TRANSMITTER RELEASE IN SYMPATHETIC GANGLIA. Joseph J. Pysh and Scott C. Ford* Dept. of Cell Biology and Anatomy, Northwestern University Medical and Dental Schools, Chicago IL 60611.

It is well known that guanidine stimulates neuromuscular transmission by increasing transmitter release, although the mechanism is unknown. It has been proposed that guanidine increases the available store of transmitter. In previous work on sympathetic ganglia we observed that in resting terminals the presynaptic grid is not fully packed with synaptic vesicles. The purpose of this study was to determine whether guanidine increases vesicle packing at the presynaptic membrane.

The 9th sympathetic ganglia of bullfrogs were used. First, we determined that guanidine enhances synaptic transmission in amphibian sympathetic ganglia. Ganglia were removed and placed in a bath of Ringer's solution. A stimulating suction electrode was placed on the sympathetic trunk and a recording electrode was positioned on the 9th ramus. In curarized ganglia (D-tubocurarine, $4x10^{-5}M$) in which the amplitude of the postganglionic compound action potential was reduced about 80%, guanidine at 5 mM produced a four-fold increase in the size of the postganglionic potential within 30 minutes whereas a 0.5 mM concentration of the drug had no effect. Paired 9th sympathetic ganglia from bullfrogs were removed; one ganglion per pair was placed in Ringer's solution as a control and the other in Ringer's containing 0.5 mM or 5 mM guanidine. After 30 minutes, each ganglion (control or drug-treated) was rapidly frozen in a Boyne freezing machine, processed by freeze-substitution and prepared for TEM. Electron micrographs of well frozen axo-somatic synapses near the ganglion surface were taken at 57,000x and enlarged to a final magnification of 155,000x. Synaptic vesicles in a zone 500 Å deep from the presynaptic membrane were counted in random coded electron micrographs. In the control synapses, measured vesicle packing at the synaptic grid was approximately 50% of the theoretical maximum. No significant difference was detected in ganglia treated with 0.5 mM guanidine. In ganglia treated with 5 mM guanidine, vesicle packing at the synaptic grid was 18% greater, however, this vesicle density was still far from the maximum.

It appears that guanidine does not act by producing gross translocations of a few vesicles to occupy empty sites at the presynaptic grid but instead might act by allowing those vesicles already in the vicinity to approach more closely the presynaptic membrane. The observed small but significant increase in vesicle packing near the junction in guanidine-treated ganglia might reflect a closer apposition by an average of 90 Å per vesicle which could potentiate calcium's role in promoting vesicle exocytosis.
Supported by NIH Grant NS11325.

227.12 EFFECTS OF NEURONAL ACTIVITY ON THE DISPOSITION OF SYNAPTIC VESICLES. Dr. L. Maler. Dept. of Anatomy, Univ. of Ottawa, Ottawa, Ont. K1N 9S9, Canada.

We have used the electroreceptor afferents of high frequency gymnotid fish to examine the reversible effects of nerve activity upon the disposition of synaptic vesicles. Electroreceptor afferents in these fish are continuously active at frequencies in excess of 100z (Hopkins, J. Comp. Physiol. 111: 172, 1976) and terminate topographically within one layer of the posterior lateral line lobe (PLLL, Carr, Maler & Sas, submitted; Maler et al., J. Comp. Neurol. 195: 87, 1981). Both electroreceptors and their afferent nerve are readily accessible for experimental manipulation. In the normal animal electroreceptor afferent terminals contain round vesicles with a bimodal distribution of diameters; the smaller vesicles are clustered against the presynaptic membrane (Maler et al., 195: 87, 1981). Nerve activity can be eliminated by sectioning the electroreceptor afferent nerve or by placing a tetrodotoxin (TTX) cuff around it. Since electroreceptors are derived from lateral line receptors they are susceptible to the action of antibiotics such as Kanamycin and Streptomycin; the cutaneous application of these antibiotics eliminates electroreceptor activity without nerve damage. All three of the above treatments cause the vesicles to disperse from their presynaptic sites. After one day the nerve sectioned animals also show degenerative changes in their electroreceptor afferent terminals, whereas the other treatments do not alter the normal cytologic appearance of terminals. After 2-4 days the TTX cuff or antibiotic treated animals have afferent terminals containing only large vesicles or extensive cisternal elements; no small vesicles at all are present near the presynaptic membrane. The electroreceptor afferent nerve was then stimulated in both TTX cuff and antibiotic treated animals; stimulation was done at the average rate expected for the afferents in question (100-250 Hz) for a period of 30 minutes or 1 hour. After 1 hour of stimulation small vesicles were once again found to be clustered adjacent to the presynaptic membrane. These experiments demonstrate that the disposition of synaptic vesicles is under the long term control of mechanisms responsive to some aspect of nerve activity; calcium influx is an obvious candidate as a presynaptic signal of nerve activity.

227.13 COLLARED PIT: A NEWLY OBSERVED STRUCTURE POSSIBLY RELATED TO VESICLE RECYCLING AT THE SYNAPSE. Toshio Kosaka* and Kazuo Ikeda, City of Hope Research Institute, Duarte, CA. 91010.

It has been shown that a reversible, temperature-dependent blockage of neuromuscular transmission occurs in the Drosophila mutant, shibire^{ts1} (shi), and that, in accord with these physiological observations at high temperature, synaptic vesicles are depleted reversibly in neuromuscular junctions (NMJ's). Furthermore, some physiological observations indicate that in shi similar kinds of temperature-induced changes occur at synapses in the central nervous system. However, no morphological studies were done on synapses other than NMJ's. We studied the fine structure of various kinds of synapses in the mutant shi and the wild-type, Oregon-R, both at low and high temperatures. For low temperature experiments, flies were dissected at 19°C, kept at that temperature for 10-20 min. and fixed at 19°C. For the high temperature experiments, flies were dissected at 19°C and kept at that temperature for 5 min., then the temperature was raised to 30°C at a rate of 1°C/min. After 5 min. exposure to 30°C, the flies were fixed.

Synapses of the thoracic ganglion, medulla, lamina, NMJ's of the coxal and dorsal longitudinal flight muscles, and NMJ's of the circular muscles in the thoracic ventricle of the alimentary tract were observed.

At high temperature the number of vesicles varied considerably, probably dependent on the kind of neuron. Some synaptic terminals had no synaptic vesicles,others had many. In addition, a preponderance of a heretofore undescribed synaptic structure was observed in a variety of synapses at high temperature. This was a particular kind of pit arising from the plasma membrane near presynaptic sites. It consisted of a spherical head portion, about 400-600 Å in diameter, with a neck portion about 150-200 Å long and about 200 Å in diameter. This neck portion was wrapped by a cytoplasmic dense material about 100 Å thick, which looked like a "collar". Many of these pits were frequently observed to be lined up along the terminal plasma membrane. This special kind of pit with a "collar", is described here for the first time, and we would like to propose to call it a "collared pit".

The important thing is that collared pits were, although very rare, also observed in control flies. Thus, the collared pits are a normal structure in the Drosophila synapse, and increase in number in shi synapses at high temperature. Although more quantitative observations are necessary to conclude the function of the collared pits, we are speculating that they are one of the structures in the pathway of membrane recycling at synapses.

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227.14 UPTAKE OF HORSERADISH PEROXIDASE CORRELATED WITH SYNAPTIC ACTIVITY IN CRUSTACEAN MOTOR NERVE TERMINALS. C.S. Thompson* and H.L. Atwood. Dept. of Zoology, University of Toronto, Toronto, Ontario, Canada M5S 1A1.

Synaptic strength was correlated with uptake of the enzyme horseradish peroxidase (HRP) by different motor nerve terminals in the opener muscle of walking legs of crayfish Procambarus clarkii. Opener muscles were exposed and the excitatory axon isolated for stimulation. Preparations were soaked in a 1%-2% solution of HRP (Sigma Type VI) in crayfish saline for 40-60 min. The motor axon was then stimulated with 1.5 sec. trains of stimuli (30 Hz) delivered every 2 sec. After a period of rest, preparations were fixed in 2.5% glutaraldehyde, reacted with diaminobenzidine, and prepared for electron microscopy.

Virtually all of the HRP reaction product was found within synaptic vesicles. Confirming previous work, we found almost no HRP reaction product in terminals of unstimulated neurons. Labelled vesicles were observed with periods of stimulation as brief as 5 min. Nerve terminals were followed in serial sections to determine the distribution of labelled vesicles. They appeared scattered throughout the vesicle population and were not localized exclusively at presynaptic dense bodies (putative release sites). Synaptic vesicles were more diffusely distributed in stimulated than in unstimulated preparations. The percentage of labelled vesicles was fairly uniform along the length of the terminals. There is an apparent correlation between percentage of labelled vesicles and frequency of occurrence of presynaptic dense bodies along the terminals.

At low temperatures (2°C) stimulation produced large involutions of nerve terminal membrane. HRP reaction product was observed in synaptic vesicles, in other, much larger vesicles, and in irregularly shaped cisternae. When 3-aminopyridine was present and fixative applied immediately after stimulation a few coated vesicles were observed and the percentage of labelled vesicles increased.

From these results it appears that synaptic activity (amount of transmitter released) is correlated with uptake of marker. Synaptic vesicle membrane is probably recycled in crayfish motor nerve terminals as at frog neuromuscular junctions (Heuser,J.E. and Reese,T.S. J. Cell Biol. 57: 315-344, 1973). Labelled vesicles were not strictly associated with putative release sites (presynaptic dense bodies), and it is likely that uptake of marker is more widespread along the terminals than release of transmitter.

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227.15 NEURONAL SURFACE PROTEIN RELEASE ACCOMPANIES TRANSMITTER RELEASE. K.J. Sweadner and P.H. Patterson. Dept. of Neurobiology, Harvard Medical School, Boston, MA 02115.

Sympathetic neurons from the superior cervical ganglion of the newborn rat were maintained in dissociated cell culture in the absence of nonneuronal cells. Neurons loaded with ^{3}H-norepinephrine will release this transmitter when stimulated with 54mM K^+ + 5mM Ba^{++}, black widow spider venom (BWSV), the Ca^{++} ionophore A23187, or veratridine, or when treated with reagents which make the plasma membrane permeable to ions, such as the ionophores alamethicin and monensin, and p-chloromercuribenzene sulfonate (pCMBS). This chemically evoked transmitter release is accompanied by the release of a group of high MW (210, 180, 120K) acidic glycoproteins.

The proteins appear in the medium at the expense of cell-associated proteins of MW 230, 210, 200, and 180K, which are labeled by metabolic incorporation of ^{3}H-leucine or ^{3}H-fucose. The accessibility of the releasable proteins to enzymatic modification in intact cells indicates a cell surface location: 1) they are labeled by lactoperoxidase-catalyzed iodination at 0°C; 2) digestion with neuraminidase, which removes sialic acids, causes a basic shift in isoelectric point; and 3) low levels of trypsin degrade them and abolish subsequent evoked release of protein, indicating the lack of an intracellular pool such as in synaptic vesicles. The reduction in apparent molecular weight upon release suggests that the proteins are released from the surface by proteolysis, but the following protease inhibitors do not block the release: PMSF, aprotinin, chloroquine, leupeptin, Ep475, pepstatin, chymostatin, and pCMBS. The release process is selective, in that many other cell surface proteins are unaffected.

This evoked release of cell surface protein is dependent upon the presence of exogenous Ca^{++} (or Ba^{++}) in all conditions tested. It is possible to separate the release of norepinephrine from the release of surface protein by this Ca^{++}-dependence: norepinephrine can be released in the absence of exogenous Ca^{++} when the cells are treated with BWSV or pCMBS, while the proteins are not released under the same conditions until Ca^{++} is added. La^{+++} can also be used to separate transmitter release from protein release: 5mM La^{+++} evokes norepinephrine release but not protein release, and in fact La^{+++} blocks the release of protein evoked by BWSV. Both the presence of La^{+++} and the absence of Ca^{++} have been observed by others to block the recovery of motoneurons from heavy stimulation or from BWSV treatment. We propose that the evoked release of surface protein accompanies some event subsequent to transmitter release itself, such as the recapture of vesicle membrane fused with the plasma membrane during exocytosis.

227.16 STRUCTURAL DETAILS OF LIZARD NEUROMUSCULAR JUNCTIONS REVEALED BY CONVENTIONAL AND FAST FREEZE CRYOTECHNIQUES. J. P. Walrond and T. S. Reese. (Spon. H. deF. Webster) NINCDS, NIH, Bethesda, MD 20205

Fast freezing, freeze fracture and freeze substitution provide improved methods for preserving subcellular structural elements. Since fast freezing preserves well only those subcellular structures which lie within a few μm of the freezing surface, application of this technique to neuromuscular systems requires a preparation with superficially located synapses. The intercostal muscles of the lizard Anolis carolinensis meet this criterion, and furthermore provide a preparation amenable to examination by freeze fracture or freeze substitution. During development of the Anolis intercostal preparation several interesting aspects of its synaptic organization came to light. Zinc iodide-osmium staining for light microscopy revealed both singly and multiply innervated intercostal muscle fibers. Singly innervated muscles have en plaque type endings with tightly packed, interconnected boutons, while the axon terminals on the multiply innervated fibers, which are much less frequent and presumably tonic, appear in diffuse clusters. Ultrastructural studies of muscles stained for cholinesterase show that multiply innervated fibers also lack the deep junctional folds found in the postsynaptic membrane of singly innervated fibers. These characteristics permit singly innervated, twitch fibers to be recognized in freeze-fractured preparations. After conventional fixation and glycerination of the axon terminals, synaptic vesicle openings occur near the ends of paired double rows of P face particles. These "active zones" lie perpendicular to the axis of the synaptic ridge, another difference from the frog neuromuscular junction. Fast frozen, freeze fractured and freeze etched Anolis neuromuscular junctions show exceptional preservation of subcellular structural elements. In these preparations, the basal lamina running through the synaptic cleft appears as a delicate lattice of side arms emanating from a central denser lamina. In some instances the side arms appear to insert directly into the cell surface while at other points they seem to contact foot-like structures. In thin sections of fast frozen and freeze substituted preparations stained en bloc with lead octoate, the extrasynaptic basal lamina appeared as a thin backbone with periodic branches extending to the muscle cell surface. This technique also showed that this backbone is thicker in the synaptic cleft than on the rest of the muscle and that individual processes extend from it to the pre- and postsynaptic cell surfaces. We do not know yet the identity of these components or their specific relationships to certain intramembrane structures such as the postsynaptic receptors or components of the presynaptic active zone.

227.17 STRUCTURE AND DISTRIBUTION OF NEUROMUSCULAR JUNCTIONS ON TONIC MUSCLE FIBERS IN FROG. V. Verma*. NINCDS, LNNS, NIH, Bethesda, MD 20203 (Spon. David S. Forman)

There are two functionally distinct types of muscle fiber in the frog: tonic, which does not conduct an action potential but has a slow, sustained contraction; and twitch, which conducts an action potential and contracts more rapidly. The proportion of these two types of muscle fiber varies from muscle to muscle though tonic fibers are rare. However, the curalis leg muscle includes a pigmented bundle that is almost 50 percent tonic fibers. These fibers are known to be multiply innervated. Their synaptic clefts stained less intensely for cholinesterase than those in twitch fibers and they had on their surface 2-7 terminals which did not branch. The mean length of all terminals in a neuromuscular junctions was 236 ± 99 µm (N=10), and the length of individual terminals varied from 11 to 219 µm. The postsynaptic membrane at some places had irregular projections 0.4 to 0.6 µm in width and 0.1 to 0.5 µm in height, but it lacked synaptic folds. Like terminals on fast fibers, the sarcoplasm immediately underneath the postsynaptic membrane was characterized by electron opaque, fuzzy material which was irregularly partitioned to form small plaques located under membrane bulges. However, many areas of postsynaptic muscle lacked this specialization. The sub-synaptic region had few randomly distributed subneural filaments as compared to the organized bundles in the junctions of twitch fibers. The cytoplasmic organization of both types nerve ending was essentially the same; the only difference being that in twitch fiber terminals the active zones were arranged at regular intervals above each fold whereas there was no such precision in the terminals of tonic fibers. The structure of the Schwann cells also did not differ on tonic fibers but, in thin sections, cholinesterase staining in the nerve-muscle cleft was less intense. The tendency for neuromuscular junctions on tonic fibers to be spread out over the muscle fibers surface made it possible to identify them tentatively in a few favorable freeze-fracture replicas. Folds in the muscle surface did not extend beyond the edges of these terminals, increasing our confidence that they are terminals on tonic fibers. The P face of these terminals had double rows of large particles, that were similar to those at terminals on tonic fibers, and vesicle openings were occasionally found beside these rows. Thus, it appears that the presynaptic active zone, which is the structural basis for release of synaptic vesicles, is basically the same in terminals on tonic and twitch muscle fibers. Any differences in the functional properties of these terminals must depend on the shapes and deployment of active zones as well as special properties of the muscle membrane.

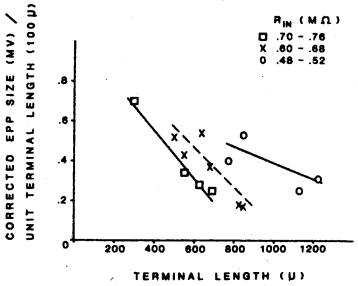
227.18 INVERSE RELATIONSHIP BETWEEN TERMINAL LENGTH AND RELEASE/UNIT LENGTH IN FROG NEUROMUSCULAR JUNCTIONS ON FIBERS OF UNIFORM INPUT RESISTANCE. B. Nudell* (SPON: A.D. Grinnell). Dept. of Biology, UCLA, Los Angeles, CA 90024.

The quantal content (m) of endplate potentials (EPPs) at frog neuromuscular junctions is known to be positively related to terminal length. However, the correlation is inexact, with wide scatter of data. We have now analyzed the EPPs of identified frog cutaneous pectoris muscle fibers, correlating release with terminal size and fiber input resistance (R_{in}). In some experiments, transmitter release was assayed by measurement of the EPPs evoked in normal Ringer and curare; in others by quantal analysis of endplate activity in low Ca^{++} Ringer. Endplates were visualized with a combined nitroblue tetrazolium (Letinsky and DeCino, 1980) and Karnovsky (1964) cholinesterase stain.

For fibers of approximately the same R_{in}, there is an inverse relationship between the level of transmitter release per unit length and total terminal length. Terminals with high levels of release/unit length tend to be shorter than do those which release relatively less transmitter/unit length. Furthermore, if the analysis is similarly restricted to fibers with nearly identical R_{in}, the total transmitter release of the largest endplates is generally no greater than that of the shorter terminals in the sample.

These findings do not contradict the overall trend of greater release and longer terminals on larger muscle fibers (with lower R_{in}). Instead, they help explain the variability in measurements of m vs. terminal length. The relationship we find is consistent with the hypothesis that, in the cutaneous pectoris, terminals are induced to grow until an adequate safety factor for transmission is achieved.

The data presented below was gathered from 14 cells of one muscle.



227.19 LONG-TERM POTENTIATION OF SYNAPTIC TRANSMISSION IN RAT SUPERIOR CERVICAL GANGLION. Donald A. McAfee and Thomas H. Brown, Div.of Neurosciences, City of Hope Research Institute,Duarte, CA 91010.

A brief period of high frequency presynaptic stimulation is known to increase postsynaptic responses to single stimuli in a number of preparations. This posttetanic increase in synaptic efficacy is transient, usually lasting for only a few seconds or minutes. However, in the hippocampus a conditioning tetanus has a long-term effect suggesting a possible role in learning and memory. We now report that long-term potentiation (LTP) also occurs in sympathetic ganglia and may be a more general property of neuronal synapses.

Isolated and desheathed ganglia were superfused with Locke solution at about 23°C. Suction electrodes were used to deliver supramaximal preganglionic stimuli to the cervical sympathetic nerve and to record the amplitude of the postganglionic compound action potential/EPSP from the internal carotid pole of the ganglion. The postsynaptic response was reduced to 50% of its maximum by curare (100 µM) in order to spare postsynaptic neurons for recruitment during increased synaptic efficacy. Atropine (2 µM) was present to block slow muscarinic responses. Postganglionic responses were elicited once a minute before and after tetanic preganglionic stimulation (20 sec at 20 Hz).

The simplest expression which describes accurately the posttetanic time course of the enhanced response consists of the sum of two exponential terms.

$$I(t) = P\exp(-t/\tau_P) + L\exp(-t/\tau_L)$$

where I(t) is a dimensionless term giving the time-dependent fractional incremental response ($I(t) = [V(t) - V_C]/V_C$, where V_C is the pretetanic control amplitude and V(t) is the posttetanic amplitude at time t). The results are summarized below. The rapid phase of this double exponential decay probably corresponds to posttetanic potentiation (PTP), since the average value of τ_P is close to the 3 minute decay time constant previously reported by Zengel, et al. (J. Gen. Physiol. 76, 213, 1980) for PTP in rabbit ganglia. By contrast, the more slowly decaying component (LTP) typically lasts for hours. (Supported by Grants BNS 79-12394 & NS 16576 and a McKnight Foundation Scholars Award).

PARAMETER	MEAN ± S.E.	RANGE	N
P	1.0 ± 0.2	0.2 - 2.0	10
τ_P	3.3 ± 0.5 min	1.2 - 5.8 min	10
L	1.2 ± 0.2	0.7 - 2.9	10
τ_L	82.2 ± 26.0 min	33.8 - 229.5 min	10

227.20 AXO-AXO-AXONIC SYNAPSES. L. E. Westrum. Depts. of Neurological Surgery and Biological Structure, Univ. of Washington, Sch. of Med., Seattle, WA 98195.

Axo-axonic synapses are typically found in CNS sensory relay nuclei and in some cases have been correlated with the phenomenon of presynaptic inhibition. In addition, vesicle-containing dendrites have been observed in synaptic glomeruli although the identification of presynaptic dendrites may be controversial in many cases. Axo-axonic synaptic contacts may involve: two axon terminals, or an axon terminal and an axon hillock, initial segment, or node (of Ranvier). I report here the unusual synaptic arrangement of three separate axonic profiles observed in the spinal trigeminal nucleus of adult cat. Profiles of axon hillocks (AH) and initial axon segments (IAS) may occasionally be seen in the neuropil. The characteristic fasicles of microtubules and cone-like emergence from a soma or large dendrite identify the AH. Membrane undercoating and fasicles of microtubules, in addition to a continuity with an AH, identify an IAS. Both AH and IAS are contacted by vesicle-containing presynaptic terminals. The most common situation is a terminal with flat synaptic vesicles (F) forming a type II, symmetrical contact with the AH or IAS. However, some of the AH and IAS profiles receive an asymmetrical, type I, contact from a terminal with round synaptic vesicles (R). In addition, a proportion of these same R terminals are postsynaptic to an F terminal, thus forming a serial synaptic complex of three separate kinds of axonic profiles: an axo-axo-axonic synapse. The F terminal lacks the usual characteristics of presynaptic dendrites. In this region the R terminal postsynaptic to an F, is usually presynaptic to a dendrite and is the primary afferent from the periphery. The origin of the F is unknown but thought to be an interneuron. Therefore, the R in the axo-axo-axonic synapse may also be a primary afferent synapsing directly on the spike generator area of the neuron and under presynaptic influence of an F synapse. The strategic position of this complex and its implications are critically relevant to the overall function of these neurons. (Supported by N.I.H. Grants No. NS 09678 and 04053. LEW is also an affiliate of the CDMRC.)

228.1 ABNORMAL β RECEPTOR FUNCTION IN BROWN ADIPOSE TISSUE OF GENETICALLY OBESE ZUCKER RATS. A. C. Sullivan*, R. O'Brien, D. Anderson*, B. E. Levin, (SPON: S. D. Cook). Dept. Pharm. II, Hoffmann-LaRoche Inc., Nutley, NJ 07110, and Dept. Neurosci., N.J. Med. School, Newark, NJ 07103.

Brown adipose tissue (BAT) accounts for up to 60% of the oxygen consumption during cold or diet-induced thermogenesis. Stimulation of this oxygen consumption in BAT is primarily under the control of the sympathetic nervous system which extensively innervates this tissue. Genetically obese Zucker rats have a decreased thermogenic capacity which does not respond to infusions of norepinephrine, suggesting receptor-effector dysfunction. Interscapular BAT of obese and lean, 4-5 mo old male Zucker rats was therefore examined to determine its role in this defect. Obese BAT pads were 4-fold heavier but had 30% less protein than lean BAT. This weight difference was due to an increased number and size of fat cells in obese BAT. Lean BAT contained $18.8 \pm 0.3 \times 10^6$ cells/pad, 77% of which were multilocular cells characteristic of BAT and 23% of which were unilocular. Obese BAT pads contained $33.4 \pm 0.7 \times 10^6$ cells, all of which were unilocular. These unilocular cells were larger in obese ($66.8 \pm 7.4\,\mu$) than lean ($48.8 \pm 2.0\,\mu$) BAT, while lean multilocular cells had the smallest diameters ($25.7 \pm 0.9\mu$). Binding of { ^{3}H } dihydroalprenalol to β adrenoreceptors of isolated BAT cell membranes produced a curvilinear Scatchard plot with a 44% increase in the apparent K_D for high affinity sites in obese (36.6 ± 4.0 nM) compared to lean (25.5 ± 1.6 nM; $p < 0.05$) BAT. The B_{max} for high affinity sites was reduced by 66% in obese$_6$ ($24.5 \pm 2.1 \times 10^{-6}$ fmol/cell) versus lean BAT ($55.4 \pm 1.8 \times 10^{-6}$ fmol/cell; $p < 0.05$), while potency for agonists in competition studies was similar between the genotypes and characteristic of the β_1 subtype. Therefore, although it is uncertain whether the morphologic abnormalities in obese BAT are the cause or effect, the reduced thermogenic capacity of obese rats can be partly attributed to a reduction of both receptor binding affinity and the number of binding sites in their BAT.

228.2 BENZODIAZEPINE RECEPTOR BINDING IN THE BRAIN OF THE SPONTANEOUSLY HYPERTENSIVE RAT. J. E. Taylor and R. M. Quock. School of Medicine, Indiana University, Evansville, IN 47732 and School of Dentistry, Marquette University, Milwaukee, WI 53233.

A number of studies have shown that neurogenic factors may be important in the initiation and development of hypertension in the spontaneously hypertensive rat (SHR). Other studies have also demonstrated that the SHR shows a marked cardiovascular hyperreactivity to anxiety and stress, which suggests that the SHR may have a functional deficiency of stress-defense mechanisms in the central nervous system (CNS). Because of the selective anxiolytic action of the benzodiazepines, it was of interest to determine whether alterations in benzodiazepine receptors were present in various brain regions of the SHR. For this purpose, ^{3}H-flunitrazepam (5 nM) binding assays were done on membrane fractions from several brain regions from the SHR and age-matched Wistar-Kyoto (WKY) normotensive controls. Specific binding was defined as that total ^{3}H-flunitrazepam bound minus that bound in the presence of 1 μM diazepam.

The binding of ^{3}H-flunitrazepam for each strain was lowest in the cerebellum and brain stem, intermediate in the thalamus/hypothalamus, midbrain, and corpus striatum, and highest in the cerebral cortex. The only region to show a significant difference in binding was the cerebral cortex, where the values were 55.3 ± 2.1 fmol/mg tissue (n=4) for the WKY and 43.4 ± 3.1 (n=4; $p < 0.02$) for the SHR cortex. Scatchard analyses of ^{3}H-flunitrazepam binding in the cerebral cortices yielded equilibrium dissociation constants of 1.76 ± 0.13 nM (n=4) for the SHR and 1.89 ± 0.12 nM (n=4) for the WKY. These data show that there is a selective decrease in the number of benzodiazepine receptor sites in the cerebral cortex of the SHR. This observation suggests that the SHR may have a diminished ability to modulate stressful and anxiety producing influences, which in turn, may contribute to the development of the hypertensive state.

228.3 CHANGES OF BRAIN SEROTONIN AND CATECHOLAMINE METABOLISM IN THE UREMIC RAT. P.J. Knott, G.O. Rankin* and K. Cressey-Veneziano*, Dept. of Pharmacology, Marshall University School of Medicine, Huntington, WV 25701

Neurological changes occur in chronic renal failure (Tyler, H.R., Am. J. Med. 44:734, 1968) suggesting changes of neurotransmission processes. Furthermore, alterations of brain serotonin (5HT) metabolism occur in experimental uremia (Siassi, F., et al, J. Nutr. 107, 840, 1977) and in advanced renal disease (Sullivan, P.A., et al., J. Neurol. Neurosurg. Psychiat. 43, 739, 1980). In the present studies, we have investigated in male Sprague-Dawley rats (350-400 g) the effects of N-(3,5-dichlorophenyl) succinimide (NDPS) a nephrotoxic agent (Rankin, G.O. Fed. Proc. 40, 675, 1981) on brain serotonin metabolism and on levels of catecholamines. Rats received NDPS (50, 100 or 200 mg/kg i.p.) or vehicle (sesame oil, 2.5 ml/kg i.p.) and brain tryptophan (TP), 5HT and 5-hydroxyindoleacetic acid (5HIAA), norepinephrine (NE), dopamine (DA) and epinephrine (EPI) levels were determined 24 and 48 h postinjection together with plasma levels of free and total TP. Plasma urea nitrogen (PUN) and plasma transaminase activity (GOT) were determined as indicators of renal and hepatic damage respectively. NDPS (200 mg/kg) increased brain TP, 5HIAA and PUN significantly. Plasma free TP was unchanged whereas plasma total TP fell and probably accounts for the elevation of brain TP and brain 5HT synthesis which only occurred in the presence or uremia. Thus, brain 5HIAA or TP correlated significantly with PUN levels ($P < 0.001$, $n = 18$) and negatively with plasma total TP ($P < 0.001$) but plasma GOT was unchanged. Further evidence that these CNS changes resulted from renal damage is shown by absence of both PUN and brain amine changes at 48 h. Brain EPI values but not NE or DA were significantly increased by NDPS at 100 mg/kg. Paradoxically within that group, brain EPI showed a strong negative correlation with PUN ($P < 0.001$). Possibly, cerebral n-methyltransferase activity is influenced directly by NDPS and indirectly by other changes occurring in uremia.

228.4 A MONOAMINE OXIDASE INHIBITOR INDUCES A TIME-DEPENDENT SUBSENSITIVITY OF DA AUTORECEPTORS. S.M. Antelman*, W.B. Orr* and L.A. Chiodo (SPON: A. Barkai). Psychobiology Program, Dept. of Psychology and Psychiatry, University of Pittsburgh, Pittsburgh, PA 15260.

We have recently demonstrated that repeated treatment with tricyclic antidepressants or electroconvulsive shock, two frequently used treatments against clinical depression, induce a subsensitivity of dopamine (DA) autoreceptors located on DA neurons in the zona compacta of substantia nigra (Chiodo and Antelman, Nature 287:451, 1980; Science 210:799, 1980). Moreover, the progressive induction of autoreceptor subsensitivity by these treatments appears to be a time-dependent sensitization process since repeated treatment is not required to produce this effect. The present study extends these findings by showing that phenelzine, a clinically effective monoamine oxidase inhibitor also induces a progressive subsensitivity of DA autoreceptors.

Male rats were treated with phenelzine sulfate (5 mg/kg, ip.) for either 2 or 10 days. Forty-eight hours after the last injection, extracellular single-unit activity of verified DA cells was measured as previously described (Chiodo et al., Brain Res., 189:544, 1980). DA autoreceptor sensitivity was indexed by the degree of inhibition of spontaneous activity produced by either the administration of a presynaptic dose of apomorphine (APO) (4 ug/kg, i.v.; Skirboll et al., Science, 206:80, 1979) or the microiontophoretic application of DA (0.1M, pH4.0; 2, 5, 10, 15, 20 nA ejection currents). As previously seen with all other antidepressant treatments, both 2 and 10 days of phenelzine injections significantly attenuated both APO and DA's ability to inhibit spontaneous DA activity relative to controls.

Moreover, in animals treated for 2 days and tested 10 days later, phenelzine produced a degree of DA autoreceptor subsensitivity that was identical to that seen in animals which received 10 days of the drug followed by two drug-free days.

These results extend our previous findings showing that antidepressant treatments induce subsensitivity of DA autoreceptors, and emphasize the importance of DA systems in the mechanism of action of such treatments. Since all these treatments appear to work by the induction of a time-dependent sensitization process it is interesting to speculate that such an effect underlies the known delay in their efficacy seen clinically

228.5 BENZODIAZEPINE ACTIVITY IN SEIZURE-SENSITIVE AND SEIZURE-RESISTANT MONGOLIAN GERBILS. P.J. Syapin, P.T. Duong* and L.A. Paul*. Dept. of Neurology, USC School of Medicine, Los Angeles, CA 90033 and Dept. of Anatomy, UCLA School of Medicine, Los Angeles, CA 90024.

Anticonvulsant activity of benzodiazepines (BZ) has been shown to be mediated by specific, high affinity receptor sites in the CNS. Alterations in BZ binding may therefore play a role in the etiology of epilepsy. Mongolian gerbils are animal models for epilepsy. We examined in vitro binding of flunitrazepam to brain membranes and behavioral responses to flurazepam in seizure-sensitive (SS) and seizure-resistant (SR) Mongolian gerbils. Specific binding of flunitrazepam to membranes thoroughly washed in phosphate buffer was similar to that reported for other species. However, differences in regional distribution were noted. Specific binding was enhanced 23-36% by 200 mM NaCl except in olfactory bulbs, which showed 11-14% enhancement. BZ receptors in Mongolian gerbils appear less coupled to GABA modulatory sites than in mice or rats. Comparisons between SSs and SRs revealed more binding sites in SS hippocampus, olfactory bulbs, and neocortex, and fewer sites in cerebellum. SS hippocampi were heavier than SR hippocampi. Binding in this region was enhanced less in SS than in SR by 200 mM NaCl while strain differences were absent for other regions.

Behavioral studies utilized cross-over design with drug-naive subjects with known seizure histories. Immediately following s.c. injection, animals were observed and rated in the open field for 14 minutes. Behavior noted included seizures, ataxia, aimless running, response to visual stimulus (pencil), and fecal boli. Flurazepam s.c. did not block seizures, probably due to the rapid onset of seizures after handling (<30 sec). Effects of the drug, however, included ataxia, aimless running, and failure to respond to the visual stimulus. Strain differences in these responses were noted. Counts of fecal boli were performed on all animals at four intervals during the test session. SSs and SRs had different scores on this measure of emotionality. Following drug, SSs had reduced numbers of boli in the open field, compared with their scores after saline, while SRs produced more than in the vehicle condition. Following the second administration of this antianxiety drug, the value of this variable for SRs was more than 3 times that for SSs.

The above pharmacological findings are consistent with results (Paul et al, Science, in press) indicating that the SS hippocampus may differ structurally from the SR. These results provide further evidence that BZ receptors may play an important role in seizures. (Supported by BSRG #2-S07-RR05356-20 and a grant from the Epilepsy Foundation of America).

228.6 PROTECTION AGAINST RESERPINE-INDUCED RIGIDITY BY BLOCKADE OF ALPHA-2 RECEPTORS. B.H. Wagner*, K.J. Kellar and R.J. Anderson, Depts. Pharmacol., George Washington Univ. and Georgetown Univ., Washington, DC.

The parkinson-like syndrome (tremor, bradykinesia, rigidity) induced in rats by high doses of reserpine is effectively reversed by a variety of drugs including the antipsychotics chlorpromazine and trifluoperazine. Since the alpha adrenergic blocking property of these drugs has been implicated as the mechanism, the purpose of this study was to examine the relative contribution of actions on alpha-1 and alpha-2 receptors in modifying reserpine-induced rigidity. Rats were pretreated with either clonidine (.04-1 mg/kg), yohimbine (1-5 mg/kg), methysergide (1 mg/kg), phentolamine (1 mg/kg) or SKF 7265 (36-144 mg/kg). Thirty min later the animals were given 20 mg/kg reserpine ip and evaluated by a blinded observer. Eleven categories were used to quantitate the parkinson-like and autonomic effects induced by reserpine. The severity of the reserpine effects in these rats was compared to the effects in vehicle pretreated animals. Yohimbine (an alpha-2 antagonist) was the most effective agent in protecting against the reserpine effects. Phentolamine and SKF 7265 (which block both alpha-1 and -2) were also effective; whereas clonidine (an alpha-2 agonist) and methysergide (a serotonin antagonist) were not. In all cases the alpha blocking drugs prevented the motor responses but did not alter the autonomic responses induced by reserpine. Although yohimbine and chlorpromazine are also effective dopamine blockers, the protection seems to be due to alpha adrenergic blockade since SKF 7265 (which has little affinity for dopamine receptors; Ki = 530 nM) was among the effective agents. Protection from the reserpine effects by alpha-2 blockers was achieved at doses which were not sedating whereas alpha-1 blockers such as chlorpromazine antagonize reserpine only at sedative doses. Relative adrenergic binding affinity of the test drugs was confirmed by in vitro binding assays in a separate set of experiments. Yohimbine and SKF 7265 were equipotent in displacing ^{3}H-clonidine from alpha-2 receptors in rat cerebral cortex membranes. SKF 7265 was approximately equipotent at alpha-1 and -2 receptors but was considerably less potent in displacing ^{3}H-spiperone from dopamine receptors in rat striatal tissue. The results show not only the efficacy of alpha adrenergic antagonists in protecting against reserpine rigidity but more importantly that the blockade of alpha-2 receptors may be the functionally important action. These results are consistent with the view that some descending motor pathways are controlled by an adrenergic mechanism and suggest that alpha-2 receptors are an important component.

228.7 EVIDENCE THAT THE CENTRAL NERVOUS SYSTEM OF THE SPONTANEOUSLY HYPERTENSIVE RAT (SHR) IS DEFICIENT IN β-ADRENERGIC RECEPTORS COMPARED TO WISTAR-KYOTO CONTROL RATS (WKR). Cohen, G. A.*, A. C. Black, Jr., and D. Sandquist. Dept. Anatomy, University of Iowa, Iowa City, Iowa 52242.

Intracerebroventricular (ICV) injection of epinephrine (EPI) causes a dose-related decrease in blood pressure and heart rate in rats. This hypotensive effect is blocked by β-adrenergic antagonists, but not by α-adrenergic antagonists. Thus it appears that the cardiodepressor effects of ICV EPI may act through a CNS β-receptor mechanism[1]. SHR and their appropriate control WKR were decapitated and their brains quickly removed, and frozen in isopentane chilled in liquid nitrogen until assay. They were analyzed for β-adrenergic receptor binding using ^{3}H-dihydroalprenolol by the method of Alexander et al.[2,3], using a subcellular fraction isolated between 800 x g and 32,000 x g.

Experimental Group	Dissociation Constant*	Receptor Number**
3 week old SHR	30.5 ± 5.4	0.31 ± 0.035†
10 week old SHR	31.8 ± 2.8	0.29 ± 0.03†
10 week old WKR	39.5 ± 5.4	0.53 ± 0.07

*Dissociation constant in nanomoles per liter ± standard error of the mean for at least 7 determinations.
**Number of receptors in picomoles per mg protein ± standard error of the mean for at least 7 determinations.
†Significantly different from 10 week old WKR (P<0.01: Student's "t" test).

Interpretation: The SHR CNS is relatively deficient in β-adrenergic receptors compared with WKR. The receptor number in the SHR CNS does not increase appreciably between 3 and 10 weeks. Since the dissociation constants for SHR and WKR are not significantly different, SHR may exhibit a reduced number of β-receptors rather than a change in the nature of the receptor itself. ICV EPI induces a hypotensive response mediated by β-adrenergic receptors. Since the relative deficiency of β-receptors in the SHR CNS occurs during the establishment of hypertension, the reduced number of β-receptors could play a role in the development of hypertension in SHR. References: [1]Borokowski and Finch, Eur. J. Pharmacol., 47:281, 1978. [2]Alexander et al., Nature, 258:437, 1975. [3]Alexander et al., Proc. Natl. Acad. Sci., U.S.A., 72:1564, 1975. (Supported by Grant HL 24351 to A.C.B.).

228.8 DECREASED ^{3}H-IMIPRAMINE BINDING CAPACITY IN PLATELET MEMBRANES FROM DEPRESSED MALES AND FEMALES. Kenneth B. Asarch*, Jean C. Shih, and Agnes Kulcsar*. School of Pharmacy, University of Southern California, Los Angeles, CA 90033.

Specific high-affinity binding sites for ^{3}H-imipramine have recently been shown to exist in human brain membranes and human platelet membranes. In order to understand the clinical implications of this ^{3}H-imipramine binding, we measured the maximal binding capacity (Bmax) and the dissociation constant (Kd) for ^{3}H-imipramine in the platelet membranes from 23 untreated male and female depressives and 16 normal males and females. Ages ranged from 19 to 58 years.

Platelet membranes for binding studies were prepared from 20 ml of blood. Specific binding, defined as that ^{3}H-imipramine binding inhibited by the presence of 100 μM desimipramine, represented 75% of the total binding at 4 nM ^{3}H-imipramine. The Bmax and Kd values were determined from Scatchard plots of the specific ^{3}H-imipramine binding.

The results indicated that the male and female depressed patient groups exhibit a 20% lower Bmax than their respective normal control groups, p = .02 (Normal males, 764 fmoles/mg protein; Depressed males, 608 fmoles/mg; Normal females, 650 fmoles/mg; Depressed females, 519 fmoles/mg). Although the male subgroups displayed a Bmax about 17% higher than their respective female control groups, the effect of sex on Bmax did not quite reach statistical significance (p = .10). The effect of depression on Kd was nonsignificant, p = .69, as was the effect of sex on Kd, p = .19 (overall mean Kd = 1.9 nM). We did not observe any correlation between subject age and either Bmax or Kd in the age range studied.

Our findings extend to males an earlier finding that the ^{3}H-imipramine binding in depressed females is lower than in normal females. The decreased ^{3}H-imipramine binding may possibly represent an unsuccessful attempt to correct some biochemical abnormality in the depressed patient. Our observed trend that men have a slightly higher binding capacity for ^{3}H-imipramine than women may help to establish a basis for the clinical observation that men respond better to antidepressant therapy with imipramine than do women.

Also, we report that a small number of patients treated with tranylcypromine, amitriptyline, or nortriptyline did not exhibit any significant changes in their ^{3}H-imipramine binding parameters. (Supported in part by Biomedical Research Support Grant Program, Division of Research Resources, NIH - BRSG #RR-05792)

228.9 MUSCARINIC RECEPTORS IN HEART FAILURE. J.W. Wells,* H.-M. Wong* and M.J. Sole. Faculty of Pharmacy and Department of Medicine, University of Toronto, Toronto, Canada M5S 1A1.

Heart failure is accompanied by abnormalities in parasympathetic, cardiovascular regulation. To probe for effects at the muscarinic receptor, we have studied the binding of (-)-N-[^{3}H]-methylscopolamine (NMS) in cardiac tissue from the cardiomyopathic Syrian hamster, a spontaneous model of congestive heart failure. Flushed hearts from 12-25 animals were dissected into three components: left plus right atria, right ventricle, and left ventricle plus interventricular septum. Tissue was homogenised and centrifuged, and the pellets were resuspended in Krebs-Henseleit buffer. Binding was assayed at equilibrium and 30° using microcentrifugation to obtain bound [^{3}H]NMS. In younger animals (150-200 days), the specific binding of [^{3}H]NMS revealed an apparently uniform population of sites (-log K_d = 9.4) in each cardiac region of myopathic hamsters and matched controls. Capacities were 50 to 120 pmol/g protein, depending upon the region and the batch of animals. Inhibition of 1 nM [^{3}H]NMS by carbachol revealed three sites characterised by high, medium and low affinity for the agonist (-log K_d = 8.0-9.0, 6.5-7.0, 5.0-5.5). Sites of medium affinity represent 45-60% of binding in all regions. Sites of high affinity are absent from the left ventricle plus septum and constitute the minor component (12-17%) in the atria of myopathic and control animals; in the right ventricle, they constitute a greater fraction in myopathic animals (12%) than in controls (<5%). As the dystrophic animals become congestive at about 220 days, various abnormalities can arise in one or more regions. (a) There is an increase in the fraction of sites with higher affinity for carbachol. (b) Atrial and ventricular receptors lose the modulatory effects of guanylylimidodiphosphate (GMP-PNP) on the binding of carbachol. In control animals, GMP-PNP increases the Hill coefficient in a manner consistent with the interconversion of sites from high to low affinity in the atria and from medium to low affinity in the ventricles. (c) There ultimately is a disappearance of receptors from some or all of the regions studied. The data indicate that heart failure in the hamster is accompanied by profound changes in cardiac muscarinic receptors. A loss of receptors would be expected to result in parasympathetic abnormalities; changes in the binding characteristics of agonists or an absence of GTP-mediated transduction could be equally deleterious. We have observed that 10 nM carbachol arrests the spontaneous beating of isolated atria from controls but has little effect on atria from some failing animals; assays with [^{3}H]NMS showed comparable levels of specific binding in both tissues. (The MRC and Heart Foundation, Canada, and the NIH)

228.10 FURTHER STUDIES OF γ-HYDROXYBUTYRATE EFFECTS ON CEREBRAL ISCHEMIA. W. D. Rausch*, N. Merkel*, C. Maruki* and M. Spatz. Lab. of Neuropath. & Neuroanat. Sci., NIH, Bethesda, Md. 20205.

Gamma-hydroxybutyrate (GHB), the naturally occurring central nervous system depressant, was shown to be effective as a preventive and therapeutic agent in experimentally induced cerebral ischemia. The beneficial effect of GHB was manifested by amelioration of ischemic brain changes in the levels of metabolites and edema as well as by the increased survival rate of the affected animals (Spatz et al. In: Pathophysiology and Pharmacotherapy of Cerebrovascular Disorders. Baden-Baden, Verlag Gerhard Witzstrock, 1980, pp. 286-289).

To elucidate further the mechanism of GHB action in cerebral ischemia we investigated the synthesis of neurotransmitters by determining the activity of tyrosine hydroxylase which has been known to be reduced by this disease process.

Mongolian gerbils subjected to 15 minutes of bilateral common carotid artery clipping, with and without various periods of clip release served as a model for cerebral ischemia and recovery. The treatment consisted of a single intravenous injection of GHB (500 mg/kg) 2 minutes prior to occlusion.

The activity of tyrosine hydroxylase was assayed by radioisotope technique (McGeer et al., Can. J. Biochem. 45, 1557-1563, 1967) in basal ganglia of GHB pretreated and untreated animals. GHB treated and untreated sham-operated Mongolian gerbils served as controls.

The level of tyrosine hydroxylase activity was found to be 33% lower in the basal ganglia of the untreated than that of GHB pretreated animals (2.97 ± .05 nmoles/mg prot./1 hr = 100%). These findings suggest that GHB modulates the ischemic decrease of tyrosine hydroxylase activity and in this way affects the synthesis of biogenic amines. Hence, it might also prevent the decrease of monoamines observed in cerebral ischemia and subsequent secondary injury to the brain.

228.11 SELECTIVE CHEMICAL AND NEURONAL LESIONS IN HIPPOCAMPUS OF TRIMETHYLTIN TREATED RATS. J.J. Valdes, C.F. Mactutus, R.M. Santos-Anderson, R. Dawson, and Z. Annau. Dept. Environ. Hlth. Sci. Division of Toxicology, The Johns Hopkins University, Baltimore, MD. 21205

Trimethyltin(TMT), belonging to a group of toxic organotins, induces selective damage in limbic structures. TMT shows a unique and selective pattern of toxicity which may be related to the neurochemical innervation of the hippocampus and therefore the present study evaluated the effects of TMT-induced lesions on the uptake of endogenous and exogenous hippocampal neurotransmitters.

Adult male Long-Evans hooded rats received three intraperitoneal injections of 0,2,3 or 4 mg/kg TMT at weekly intervals and were killed four days after the third injection for neurochemical and neuropathological evaluation. The hippocampi were dissected and the uptake of tritiated gamma-aminobutyric acid (^{3}H-GABA) and norepinephrine (^{3}H-NE) were assessed in enriched synaptosomal preparations. KT and Vmax were computed representing, respectively, synaptosomal affinity and capacity for these transmitters. Additional rats were prepared for histology: those assessed via light microscopy were perfused with saline and neutral buffered formalin, celloidin embedded, and sectioned and stained with thionin, cresyl violet or hematoxylin and eosin; those assessed via electron microscopy were perfused with paraformaldehyde-glutaraldehyde, osmicated, plastic embedded, and thin sections were examined with an H-300 Hitachi electron microscope.

No mortality or significant weight loss occurred at 2 or 3 mg/kg, but all rats given 4mg/kg lost weight and most died. Kinetic analysis of the uptake data indicated dose-dependent effects of TMT on ^{3}H-GABA uptake with little effect on ^{3}H-NE. Rats treated with 2 mg/kg had decreased affinity and increased capacity, 3mg/kg rats reversed this trend, and the few surviving 4mg/kg rats did not differ from controls in their affinity or capacity for ^{3}H-GABA. Light microscopic evaluation revealed dose-dependent ventricular dilation, damage to neurons in CA1 and CA4, glial proliferation, and sparing of neurons in hippocampal area CA3a. Electron microscopy revealed neurons in varying stages of degeneration. Those in the early stages were filled with membrane-bound dense bodies which appeared lysosomal, those in intermediate stages contained numerous vacuoles surrounding the dense bodies, and neurons in advanced stages displayed electron-dense cytoplasm and nuclei, irregular nuclear profile, and clumped chromatin. These data suggest that loss of intrinsic hippocampal GABAergic neurons and, the subsequent disruption of intrahippocampal connections may be responsible for the expression of TMT toxicity in that structure.

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228.12 DIFFERENTIAL VARIATIONS IN CENTRAL AND PERIPHERAL β-ADRENERGIC RECEPTOR SUBTYPES IN SPONTANEOUSLY HYPERTENSIVE RATS. B.B. Wolfe, P. Chatelain* and P.B. Molinoff. Dept. of Pharmacology, Univ. Colo. Hlth. Sci. Ctr., Denver, CO 80262 and Dept. of Biochem. and Nutrition, Sch. of Med., Univ. Libre de Bruxelles, Brussels, Belgium.

Differences in catecholamine levels and metabolism in various tissues obtained from spontaneously hypertensive (SH) rats compared to their normotensive controls (WKY) have previously been reported. In order to determine if there are also alterations in the β-adrenergic receptors in these rats we have measured the density and properties of the β-receptor subtypes in seven tissues obtained from SH and WKY rats. The animals were obtained from Charles Rivers and were killed at 14 weeks of age when the blood pressure of the WKY rats ranged from 98 to 115 mm Hg and that of the SH rats ranged from 140 to 170 mm Hg. Additionally, the ratio of ventricular weight to body weight was 2.79 ± 0.04 for the WKY rats and 3.36 ± 0.06 for the SH rats. There were no significant differences in the densities of β-adrenergic receptors in the atria, lung, cerebral cortex, pons-medulla or hypothalamus from these animals. However, as has been previously reported, there was a 30% ($p<.01$) decrease in the density of β-adrenergic receptors in the ventricles of the SH rats. The densities of both β_1 and β_2-receptors were also measured and this decrease was found to be exclusively confined to the β_1 subtype. In contrast to this result, the density of β-adrenergic receptors in the cerebellum of the SH rats was 70% ($p<.01$) higher than in the WKY rats. In the cerebellum of WKY rats the ratio of β_1 to β_2-receptors was approximately 5:95 and this ratio was not increased in the SH rat cerebellum indicating that most, if not all, of the increase in β-adrenergic receptor density was restricted to the β_2 subtype. Supported by the PHS (HL24353 and NS13289).

228.13 EFFECTS OF THORACIC TRANSECTION ON AMINO ACID NEUROTRANSMITTERS IN THE LUMBAR SPINAL CORD. J.D. Lane and J.E. Smith. Psychiatry Research Unit, Department of Psychiatry, Louisiana State University Medical Center, Shreveport, LA 71130.

Following mammalian spinal cord injury in the thoracic region, the hindlimbs undergo a period of flaccid paralysis (spinal shock) after which there is a gradual evolution of muscle spasticity. These events can be explained by loss of descending excitation (or descending disinhibition on polysynaptic inhibitory pathways), followed by reactive synaptogenesis which results in the progressive exclusion of presynaptic, postsynaptic and recurrent inhibitory influences, which modulated the reflexes prior to the injury. Since this inhibition is mediated by glycine (Gly) and gamma-aminobutyric acid (GABA), the evolution of spasticity should be reflected by changes in these amino acids. Adult female cats were transected at the T5 level and maintained for varying times up to twelve weeks when the clinical signs of spasticity were paramount. On the day of sacrifice, the animals were given an intravenous injection of ^{14}C-D-glucose and killed at various times post-injection. The lumbar enlargement was removed, dissected into discrete regions, and analyzed for amino acid content and utilization; and total particulate membrane preparations were evaluated for in vitro binding of tritiated GABA, muscimol, strychnine, aspartate and glutamate in the presence or absence of specific displacing agent. The transected animals were immobilized for approximately 48 hours post-operative, after which they were ambulatory. By six weeks, the animals exhibited clonus, scissoring, involuntary walking movements, extensor spasms and contralateral reflexes to painful stimuli--these signs increased in magnitude up to twelve weeks. Compared to sham-operated controls, spastic animals showed a general decrease in utilization rates of amino acids, and the evolution of spasticity was reflected by alterations in the distribution of neurotransmitter receptors. The role of excitatory and inhibitory amino acids in descending supraspinal pathways, spinal shock and hyperreflexia will be entertained. (Supported by NINCDS Grant NS 16409).

228.14 ^{3}H-SPIPERONE BINDING IS INCREASED IN DIABETIC RATS. C.F. Saller*, D. Lozovsky* and I.J. Kopin (SPON: J.C. Eberhart). Lab. of Clinical Science, NIMH, Bethesda, MD 20205.

Reductions in intrasynaptic dopamine (DA) lead to increases in DA receptor sensitivity. We have found that glucose administration suppresses the firing of central DA-containing neurons (Saller and Chiodo, Science, 210:1269, 1980) and presumably DA release. We now report that the binding of ^{3}H-spiperone, a DA receptor ligand, is increased in chronically hyperglycemic diabetic rats. Rats were given saline or alloxan (185 mg/kg, s.c.) to induce diabetes, and decapitated six weeks after treatment. Striata were removed and the specific binding of ^{3}H-spiperone to membranes prepared from these tissues was measured (Fields et al., Brain Res., 136:578, 1977). The maximum binding and dissociation constants were determined by regression analysis of Scatchard plots of the binding data. Alloxan treatment increased the maximum binding of ^{3}H-spiperone to striatal membranes by 30% ($P<0.01$) but did not alter the dissociation constant for spiperone. Similarly, the binding of ^{3}H-spiperone was increased by 35% ($P<0.05$) in rats made diabetic with streptozotocin (75 mg/kg, i.p.). Thus, the number of DA receptors, as measured by ^{3}H-spiperone binding, are increased in two types of experimental diabetes. Moreover, chronic insulin treatment which maintained normal body weight completely prevented or reversed increases in DA receptor number. The possible relationships between these findings and the behavioral and mood disorders sometimes associated with disturbances of carbohydrate metabolism in humans will be discussed.

228.15 CHRONIC, BUT NOT ACUTE, TREATMENT WITH AN ANTIDEPRESSANT DRUG CAUSES A DOWN REGULATION OF β-ADRENERGIC FUNCTION IN ASTROCYTES IN PRIMARY CULTURES. L. Hertz* and J.S. Richardson (SPON: P.V. Sulakhe). Depts. of Pharmacology and Psychiatry, Univ. of Saskatchewan, Saskatoon, Sask. S7N 0W0 Canada.

A recent unifying hypothesis for the mechanism of action for all classes of antidepressant drugs (monoamine uptake inhibitors, monoamine oxidase inhibitors and drugs which exert neither of these actions potently) is that chronic application of these drugs acts by leading to a down regulation of β-adrenergic activity in brain, as evidenced by a decreased binding of β-adrenergic ligands and a reduction of the isoproterenol induced accumulation of cyclic AMP (Sulser, Trends Pharm. Sci. 1, 92, 1979).

We have previously reported that normal astrocytes in primary cultures possess a large amount of binding sites for dihydroalprenolol (DHA), a β-adrenergic ligand, and that isoproterenol causes an increase in cyclic AMP accumulation in these cells, an effect which is partly inhibited in the presence of amitriptyline (Abstracts, Soc. Neurosci. 1980, 270.21) or doxepin (Can. J. Physiol. Pharmacol. 58, 1515, 1980). In the present work, we have looked at the effect of chronic administration of amitriptyline on DHA binding and cyclic AMP accumulation. The cultures were prepared from the brains of newborn mice and grown in 60 mm plastic tissue culture dishes without any drugs until the age of 2 weeks, at which time they were confluent and contained about .3 mg protein. From then and onwards, amitriptyline (1 or 5 μM) was added to some of the cultures whereas other cultures from the same batches were left untreated as controls. After 5 or 8 days of treatment no differences were observed in DHA binding. After at least 12 days there appeared to be a decline in the binding but the variability was extremely large. The accumulation of cyclic AMP in the absence of isoproterenol was not affected by pretreatment with amitriptyline but the isoproterenol induced accumulation of cyclic AMP was significantly inhibited (25-45%) in cultures which have been grown in the presence of amitriptyline for between 12 and 27 days, with little, if any, effect after 5 days of exposure. No major differences were found between the effects of 1 μM and 5 μM amitriptyline. The lower of these concentrations is comparable to that occurring in plasma of patients treated chronically with this drug. The present experiments therefore suggest that the effects of amitriptyline on astrocytes might play a crucial role in its mechanism of action. They do not exclude that neurons could be similarly affected but the difficulties in maintaining pure cultures of CNS neurons from rodents for more than a few weeks presently prevents comparable long-term drug studies with neurons. (Supported by grant MT-5957 from the MRC of Canada.)

228.16 CHRONIC FLUPHENAZINE TREATMENT INDUCES CHANGES IN GLUTAMATE CONCENTRATIONS AND GLUTAMATE HIGH AFFINITY UPTAKE IN ADULT RAT STRIATUM. M.T.Price, R. Haft*, and J.W. Olney, Dept. Psychiatry, Washington Univ. Sch. Med., St. Louis, MO.

The use of phenothiazine-type drugs in psychiatry is sometimes associated with adverse reactions, the most serious of which is tardive dyskinesia (TD). Drug-induced supersensitivity of striatal dopamine (DA) receptors provides a plausible explanation for reversible, but not irreversible symptoms of TD. Since glutamate (Glu) is known to have neurotoxic activity and is thought to be the excitatory transmitter released at cortico-striatal synapses, either reversible or irreversible TD symptoms might be attributed hypothetically to a phenothiazine-induced disturbance in Glu uptake processes, resulting in an accumulation of Glu at striatal synapses with consequent degeneration of post synaptic elements. The present study was undertaken to determine whether long-term fluphenazine (FP) treatment (1 or 2 mg/kg sc every 2 wks for 9 mos) is associated with any disturbances in Glu concentrations or Glu high affinity uptake kinetics in adult rat striatum.

The earliest detected effect of FP treatment was an increase in the Km of Glu high affinity uptake as measured in striatal synaptosomes. This first became evident after 2 mos of FP treatment and was most pronounced in rats subjected to the highest dose (2 mg/kg). When measured at subsequent treatment intervals up to 9 mos, Kms were consistently higher than age-matched controls (e.g., at 9 mos, controls = 4.05 ± 0.4 nmol/mg/3 min; FP-treated = 6.16 ± 0.3 nmol/mg/3 min). Vmax rose transiently but significantly at 5 mos and returned to control values by 7 mos. Glu concentrations in FP striata remained at control levels up to 5 mos, then increased to values ranging from 7-26% higher than corresponding controls at 7 and 9 months.

Activation of DA terminals in the striatum reportedly inhibits Glu release from cortico-striatal fibers; therefore, the blocking action of phenothiazines on DA receptors might be expected to facilitate (disinhibit) Glu release. In addition, since the earliest and most persistent change we detected was an increase in Km (↓ affinity) of the Glu uptake receptor, an FP-induced suppression of Glu uptake may have been operative. Thus, there are two possible mechanisms relevant to Glu neurotransmission (↑Glu release and ↓Glu uptake) which might be invoked to explain the elevated levels of striatal Glu in our 7-9 mos FP-treated rats. Since both mechanisms acting simultaneously would explain the elevated Glu levels better than either by itself, this is the interpretation we favor. Supported by USPHS grants NS-09156, DA-00259, RSA MH-38894 (JWO) and Undergrad. Neurobiol. Scholarship funded by Grass Found. (RH).

228.17 CEREBRAL CORTICAL NEUROTRANSMITTERS IN SENILE DEMENTIA OF ALZHEIMER TYPE. M. Rossor*, P. Emson*, C. Mountjoy*, M. Roth* and L. Iversen*. (SPON: M. Hanley). MRC Neurochemical Pharmacology Unit, Dept. Neurology, Addenbrooke's Hospital, Cambridge CB2 2QQ, U.K.

The reduction of choline acetyltransferase (ChAT) activity in cerebral cortex in senile dementia of Alzheimer type (SDAT) is presumed to reflect a loss of afferent terminals rather than of intrinsic cortical cells.

In order to assess the integrity of intrinsic neurones we have measured four putative neurotransmitters of the cerebral cortex, vasoactive intestinal polypeptide (VIP), cholecystokinin (CCK), somatostatin (SRIF) and γ aminobutyric acid (GABA), all of which appear to be confined to interneurones and are stable in human brain after death. The concentrations of these neurotransmitters and of the activity of ChAT in SDAT, were compared with age matched, histologically normal controls.

In agreement with previous studies a widespread cortical loss of ChAT activity was demonstrated in the SDAT group when compared with controls. The concentrations of immunoreactive VIP and CCK did not differ between the two groups. There were, however, significant losses of SRIF (47%) from the temporal cortex and of GABA from both the temporal cortex (26%) and the motor cortex (27%) in the SDAT cases. These findings indicate that cell losses in the cerebral cortex in SDAT are confined to specific neuronal populations.

229.1 EFFECTS OF MELATONIN AND THE PINEAL GLAND ON THYROID PHYSIOLOGY OF FEMALE HAMSTERS. Jerry Vriend* and Russel J. Reiter (SPON: Karl M. Knigge). The Neuroendocrine Unit, University of Rochester, Rochester, NY 14642 and Department of Anatomy, University of Texas Health Science Center, San Antonio, TX 78284.

Although effects of the pineal gland and effects of melatonin, one of the products of the pineal gland, have been reported in the male hamster (Vriend, Sackman and Reiter, 1977; Vriend and Reiter, 1977) effects in the female have not been documented. Blinding female hamsters (which is known to activate the pineal) resulted in a highly significant reduction of plasma thyroxin by eight weeks. Free thyroxin index, obtained by taking the product of thyroxin concentration and the percent T3 uptake of plasma (which measures the unsaturated binding sites in plasma), was also reduced in blind hamsters. Evidence for a role of the pineal was obtained by data showing that blinded hamsters that had been pinealectomized had thyroxin levels not significantly different from normal controls. In female hamsters under long photoperiod (14L/10D) daily melatonin injections (25µg daily for 8 weeks) given late in the photoperiod also resulted in a significant reduction of plasma thyroxin and free thyroxin index. The melatonin induced reduction of plasma thyroxin was also obtained in ovariectomized female hamsters receiving daily injections of melatonin late in the photoperiod. Equal doses of melatonin given in the morning, i.e. early in the photoperiod, had no significant effect. Paradoxically, the effects of melatonin injections were reversed by subcutaneous implants of melatonin (1mg) replaced bi-weekly. The results indicate a circadian sensitivity to melatonin injections. Furthermore the results indicate that chronic availability of milligram amounts of melatonin in subcutaneous depots prevents the depression of thyroxin levels obtained with single injections of 25µg near the end of the photoperiod. The results are consistent with the view that melatonin acts on a neuroendocrine control mechanism influencing thyrotrophin releasing hormone (TRH) synthesis or release.

229.2 CONTROL OF RAT PINEAL MELATONIN SYNTHESIS: EFFECT OF MONOAMINE OXIDASE INHIBITION ON SEROTONIN N-ACETYLTRANSFERASE ACTIVITY. T. S. King*, B. A. Richardson* and R. J. Reiter. (SPON: H.-C. Dung). Dept. Anat., Univ. Texas Health Sci. Ctr., San Antonio, TX 78284.

Pineal serotonin N-acetyltransferase (NAT) activity has generally been considered the rate-limiting enzyme in the biosynthesis of melatonin. Various studies have suggested that increased NAT activity following monoamine oxidase (MAO) inhibition was related to increased NAT substrate (i.e., serotonin) concentration (e.g., Bade et al., Arch. Pharmacol. 297: 143, 1977) rather than increased beta-adrenergic receptor stimulation as a result of elevated norepinephrine levels. We sought to determine the necessity of adrenergic receptor stimulation relative to changes in pineal NAT activity and melatonin content following MAO inhibition. Adult male Sprague-Dawley rats were housed in a light:dark cycle of 12:12 (lights on 0600 h). In the first experiment, rats were injected at 1200 h with 0.9% saline vehicle, 20 mg/kg pargyline or 20 mg/kg pargyline and 20 mg/kg propranolol. NAT activity and melatonin content were measured at various times from one to six hours after these injections. In the second experiment, rats were injected at 1200 h with 0.9% saline vehicle or 20 mg/kg and or 80 mg/kg harmine and pargyline: with and without conjunct injection of 20 mg/kg propranolol. NAT activity, paralleled by melatonin content, was maximal 2-4 hours after pargyline injection but comparable to saline controls 6 hours after injection. Conjunct propranolol injection nullified pargyline-induced increases in NAT activity and melatonin content at all times after paryline-injection. Higher doses of harmine and pargyline produced proportionately greater increases both in NAT activity and melatonin content. These increases were negated by conjunct propranolol injection, regardless of the dose of inhibitor used. Interestingly, no significant differences between the effects of similar doses of pargyline and harmine were noted under the conditions of this study. Higher doses of MAO inhibitors produced levels of NAT activity (15.9±3.4 nmoles pineal^{-1} hour^{-1}) and melatonin content (2089.6±267.9 pg pineal^{-1}) comparable to the normal nocturnal peak of these constituents. In summary, we suggest that beta-adrenergic receptor stimulation of pineal NAT activity, regardless of changes in NAT substrate availability, regulated melatonin production under the conditions of the present experiments. (Supported by Center for Training in Reproductive Biology Postdoctoral Grant HD 07139 and NSF Grant PCM 8003441).

229.3 EFFECTS OF AFTERNOON INJECTIONS OF LARGE DOSES OF MELATONIN ON THE NEUROENDOCRINE-REPRODUCTIVE SYSTEM OF FEMALE SYRIAN HAMSTERS. B. A. Richardson*, T. S. King*, L. J. Petterborg*, M. K. Vaughan and R. J. Reiter. Dept. of Anatomy, Univ. of Texas Health Sci. Ctr., San Antonio, TX 78284.

In the hamster the pineal mediated reproductive atrophy resulting from short day exposure can be mimicked by the afternoon administration of 25 µg of melatonin (mel). The present study was designed to determine what effects large doses (2.5 mg) of mel injected in the afternoon would have on the reproductive status of adult female hamsters maintained on either stimulatory or non-stimulatory photoperiods. Female hamsters were maintained in either a long (14L:10D; lights on 0600h) or short (10L:14D; lights on 0600 h) photoperiod. Within each photoperiodic regime the animals were injected daily with either vehicle, 25 µg of mel or 2.5 mg of mel at 1600 h. Short photoperiod resulted in an inhibitory influence on vaginal cyclicity, uterine and ovarian weights, plasma FSH and pituitary PRL. One hundred percent of both 14:10 and 10:14 female hamsters receiving 25 µg injections for 11 weeks became acyclic along with significant decreases in uterine and anterior pituitary weights and increases in ovarian weight. Plasma LH, FSH and PRL titers were significantly depressed in 14:10 animals injected with 25 µg of mel while no effect on these hormones was observed in 10:14 animals. Pituitary concentrations of LH and FSH were increased in both short day and long day exposed hamsters injected with 25 µg mel; pituitary PRL was depressed in both groups. Hypothalamic LH-RH was increased in the 10:14 animals receiving 25 µg of mel. Injections of 2.5 mg of mel failed to alter any of the parameters measured in 14:10 animals with the exception of plasma FSH which was depressed. However, in 10:14 hamsters the 2.5 mg injections appeared to inhibit most of the antigonadotrophic effects of short photoperiod. These results demonstrate that a large bolus of mel given in the afternoon when smaller doses of the indole are known to have inhibitory actions on reproduction, either fails to have any effect or appears to counter the antigonadotrophic influence of a short photoperiod. These findings may be explained by the hypothesis that mel is able to regulate the number and/or sensitivity of its own receptors. Supported by F32 ND05900-01 to B.A.R. and PCM 8003441 to R.J.R.

229.4 IN VITRO ADRENERGIC STIMULATION OF PINEAL MELATONIN PRODUCTION IN THE YOUNG AND OLD SYRIAN HAMSTER. C. M. Craft* and R. J. Reiter. (SPON: J. Hansen). Dept. of Anatomy, Univ. of Texas Health Sci. Ctr., San Antonio, TX 78284.

In vivo studies have suggested that the nocturnal peak in pineal melatonin content was depressed in old Syrian hamsters in comparison to that in young Syrian hamsters (Reiter et. al., Peptides 1, Suppl. 1:69, 1980). The purpose of this study was to examine the responsiveness of the β-adrenergic receptors within cultured pineal glands after the addition of norepinephrine (NE) or isoproterenol (ISP) by measuring and comparing melatonin content in pineals from 3-month and 20-month-old hamsters. NE in post-synaptic fibers from the superior cervical ganglia stimulate melatonin production and secretion by augmenting the activity of N-acetyl transferase (NAT). During the three weeks prior to culturing these glands, all hamsters were maintained in a photoperiod of daily 14:10 LD cycle (lights on at 0600 h). Individual pineal glands were cultured in BGJ$_b$ medium for 24 hrs and tranferred to fresh medium containing tryptophan ($5x10^{-6}$M). Half of each group also contained NE or ISP ($3x10^{-4}$M). Data were statistically analyzed using an ANOVA followed by a student t-test. NE and ISP induced significantly elevated levels of melatonin content (840±112 pg/gland/hr and 1500±90 pg/gland/hr, respectively) over control levels of melatonin content (250±70 pg/gland/hr) in cultured young hamster pineal glands. A similar increase in melatonin content was observed following NE addition to cultured old hamster pineal glands. Whether young or old, the hamster pineal gland in vitro demonstrated a 16 hour latency in melatonin production in response to the addition of adrenergic stimulants when cultures were initiated during daytime. With 3-month-old vs. 20-month-old hamster pineals, no statistically significant difference was found between these two groups. However, within each NE stimulated group, earlier incubations showed no significant differences in melatonin content until 0200 ($P<0.05$), 0400 ($p<0.01$), and 0800 ($p<0.001$). At 0400 and 0800, values were significantly different ($p<0.001$) between NE old and young unstimulated glands vs. NE old and young stimulated glands. The melatonin contents of the pineals at the end of the experiment were not significantly different. This study indicates that β-adrenergic receptors do not show impared capacity to respond to NE stimulation in aged hamsters pineals in organ culture. Therefore, we suggest that the ability of the hamster pineal gland to respond to exogenous adrenergic stimulation was undiminished in culture whether young or old. (Supported by PCM 8003441.)

229.5 EFFECTS OF BLINDING OR AFTERNOON MELATONIN INJECTION ON PARAMETERS OF THYROID FUNCTION IN THE MALE GOLDEN HAMSTER (MESOCRICETUS AURATUS). L.Y. Johnson, M.K. Vaughan and R.J. Reiter. Dept. of Anatomy, The Univ. of Texas Health Science Center at San Antonio, San Antonio, TX 78284.

The pineal gland has been shown to have dramatic effects upon the reproductive axis of the golden hamster. Either blinding (and resultant pineal gland stimulation) or late afternoon injection of melatonin (a putative pineal hormone) cause total reproductive collapse in a matter of weeks. Additionally, blinding has been shown to result in pineal-mediated inhibition of the thyroid axis. To further explore possible extra-reproductive effects of the pineal gland, the effects of blinding or afternoon melatonin injections on parameters of thyroid function were determined in hamsters with regressed gonads.

Adult male hamsters housed in a 14L:10D lighting cycle (lights on at 0600h) were divided into three treatment groups: intact hamsters (INT), blinded hamsters (BL) and intact hamsters which received daily sc injections of 25 µg melatonin at 1700h (MEL). After 10 weeks, hamsters were sacrificed by decapitation between 0900 and 1100h; plasma was assayed for thyroid stimulating hormone (TSH), triiodothyronine (T_3) and thyroxine (T_4) concentrations. Additionally, T_3 uptake (a measure of the unsaturated binding capacity of serum proteins), the free triiodothyronine index (FT_3I) and the free thyroxine index (FT_4I) were determined.

Although plasma TSH levels were depressed in both BL and MEL hamsters, this decrease was significant only in the latter group (139.1±47.6 ng/ml (INT) vs. 53.1±11.9 ng/ml (MEL), $p<0.05$). However, plasma levels of T_3 were significantly depressed in both experimental groups (66.9±5.1 ng/dl (INT) vs. 34.6±4.3 ng/dl (BL) and 43.6±2.3 ng/dl (MEL), $p<0.001$) as were plasma levels of T_4 (3.88±0.31 µg/dl (INT) vs. 2.12±0.25 µg/dl (BL) and 2.44±0.13 µg/dl (MEL), $p<0.001$). Although T_3 uptake was unaltered by either treatment, the FT_3I and FT_4I were decreased by both blinding and melatonin injection. The FT_3I of INT hamsters (38.6±2.6) was significantly higher than that in BL (24.2±2.6) or MEL hamsters (29.4±1.7), $p<0.001$ and $p<0.02$, respectively. The FT_4I of INT hamsters (2.31±0.22) similarly was significantly elevated over that of BL (1.58±0.19) and MEL hamsters (1.71±0.11), $p<0.01$ and $p<0.05$, respectively.

It thus appears that both blinding and afternoon melatonin injections inhibit the thyroid axis of golden hamsters, suggesting a role for the pineal gland in the control of extra-reproductive endocrine function. The possible interaction of the effects of the pineal gland on reproductive and thyroid axes awaits further study. (Supported by NSF grant PCM 8003441.)

229.6 EFFECTS OF THYROID-INHIBITORY AND COUNTER-INHIBITORY AFTERNOON DOSES OF MELATONIN ON T_3 UPTAKE AND PLASMA LEVELS OF TSH, T_4 AND T_3 IN FEMALE HAMSTERS MAINTAINED UNDER LONG OR SHORT PHOTOPERIOD. M.K. Vaughan, B.A. Richardson*, L.Y. Johnson, T.S. King*, L.J. Petterborg* and R.J. Reiter. Dept. of Anatomy, The Univ. of Texas Health Science Center at San Antonio, San Antonio, TX 78284.

Both afternoon injections of 25 µg melatonin or short photoperiodic conditions (< 12.5 hrs light/day) leads to reproductive acylicity, ovarian enlargement and an infantile uterine appearance in female Syrian hamsters (Mesocricetus auratus). Chronic availability of melatonin either as an implant or as a large dose (2.5 mg) given in the late afternoon negates the abovementioned reproductive effects of short photoperiod or low doses of melatonin by a mechanism postulated to involve the availability and/or sensitivity of melatonin receptors. Although some of the hormones involved in this reproductive response have been well characterized (LH, FSH, prolactin), other potential hormonal imbalances in these sexually-regressed hamsters have not been fully explored. The aim of the present 2 experiments was to determine the effects of a gonad-inhibitory dose (25 µg) as well as a gonad counter-inhibitory dose (2.5 mg) of melatonin on the pituitary-thyroid axis in hamsters maintained under long or short photoperiodic conditions. Thyroid hormones (T_4, T_3), T_3 uptake and plasma TSH were evaluated in 2 experiments in which adult female Syrian hamsters were maintained under short (10:14 LD) or long (14:10 LD) photoperiods (lights on 0600) and/or injected sc with either ethanolic saline, 25 µg melatonin or 2.5 mg melatonin every afternoon. In Experiment 1, hamsters in short photoperiod for 9 weeks had significantly depressed plasma TSH, T_4, T_3 and free thyroxine index (FT_4I) as compared to animals kept in long photoperiod. In Experiment 2, sc injections of 25 µg melatonin into hamsters maintained in the long photoperiod for 11 weeks had significantly depressed plasma TSH, T_4, T_3 and FT_4I; a similar injection protocol into hamsters kept in the short photoperiod depressed plasma TSH, T_3 uptake, FT_4I and FT_3I. However, large doses (2.5 mg) of the indole given every afternoon to hamsters in long photoperiod significantly augmented the plasma levels of T_4, T_3 uptake and the FT_4I; similar treatment of animals maintained in a short photoperiod had no depressive effect on plasma thyroid hormones as did the lower 25 µg dose of melatonin. In conclusion, it appears that either short photoperiod or low doses of melatonin (25 µg/day) given in the late afternoon have a depressive effect on the pituitary-thyroid axis similar to that previously described with the pituitary-gonadal axis. Supported by F32 ND05900-01 to B.A.R. and PCM 8003441 to R.J.R.

229.7 REGULATION OF HUMAN MELATONIN SECRETION BY ENVIRONMENTAL LIGHT VIA A CENTRAL PACEMAKER AND PERIPHERAL SYMPATHETIC STIMULATION. A. J. Lewy*. (SPON: R. M. Brown). Dept. of Psychiatry, Univ. of Oregon Health Sciences Ctr., Portland, OR 97201.

Melatonin secretion occurs only at night in both diurnal and nocturnal mammalian species. Melatonin secretion is acutely suppressed by light; the light/dark cycle also entrains a central pacemaker, the suprachiasmatic nuclei (SCN) of the hypothalamus. A specific neuronal pathway, the retinohypothalamic tract, which extends from the retina to the SCN, mediates the effects of light. Melatonin secretion is the result of the circadian oscillation which originates in the SCN and which stimulates the pineal's beta-adrenergic receptors via peripheral sympathetic neurons.

Human melatonin secretion (as measured by gas chromatography-negative chemical ionization mass spectrometry) appears to be regulated in the same way as other mammalian species. Propranolol (120 - 140 mg po qhs) blocks the nighttime rise in melatonin secretion, presumably by its blockade of postganglionic beta-adrenergic receptors. Clonidine (2-3 ug/kg iv qhs) also reduces nighttime melatonin secretion (Lewy, A., Siever, L., Uhde, T., et al, in preparation), presumably by stimulating presynaptic alpha-2-auto-receptors peripherally, although a central effect cannot be excluded. These findings suggest that the human pineal is innervated by peripheral sympathetic neurons, which is true of all other mammalian species.

Light acutely suppresses human melatonin secretion; healthy subjects require much brighter light (500-1500 lux) than do other species. Patients with affective disorder appear to be supersensitive to light, even in the well state; this is consistent with, and could possibly explain, phase-advanced circadian rhythms observed in these patients. Human melatonin secretion appears to be regulated by the light/dark cycle as well, since certain blind subjects have abnormally phased or "free-running" melatonin secretory rhythms. These results also suggest that blind individuals are heterogeneous in terms of their synchronization to the day/night cycle. Future endocrine research in blind subjects, which hopefully will be stimulated by these results, should take into account the heteregeneity of this population.

Human plasma melatonin appears to be a useful "marker" for adrenergic function, the central pacemaker, and the effects of light in humans. Ocularly-mediated light appears to affect human endocrine systems. Other possible endocrine and behavioral effects of light in humans are appropriate research areas. Intensity of light should be a key methodological variable in the planning of future research in this area. Appropriately intense light may also have therapeutic uses.

229.8 DISSOCIATION OF N-ACETYLSEROTONIN AND MELATONIN LEVELS IN SERUM OF RATS, L.J. Grota, G.M. Brown, & S.F. Pang*. U. of Rochester, Rochester, N.Y. and McMaster U. Health Sciences Center, Hamilton Ontario L8S 4J9.

N-acetylserotonin is frequently considered an inactive intermediate in the synthesis of melatonin (5-methoxy, N-acetyltryptamine) from serotonin. Our laboratory has developed antisera that will specifically bind melatonin and other antisera that will specifically bind N-acetylserotonin. Using these antisera, a radioimmunoassay for serum melatonin and a radioimmunoassay for serum N-acetylserotonin have been developed and validated. These two assays were used to determine N-acetylserotonin and melatonin levels in the serum of male albino rats housed 2/cage with ad libitum feeding and with the lighting, temperature, and humidity mechanically controlled. When rats are housed under 12 hours of light and dark, serum N-acetylserotonin and melatonin have 24-hour rhythms with a characteristic crest during the latter half of the dark period. Daytime N-acetylserotonin is approximately 1.2 ng/ml with the crest level reaching 2.21 ± 0.27 ng/ml (N=9). Daytime melatonin levels are about 15 pg/ml with a crest level at 40 pg/ml occurring late in the dark period. When rats are placed in a 2 hour light 22 hour dark cycle for two months, serum melatonin has the same level and pattern as those in 12 hour light-dark cycles, i.e., crest levels occur 18 hours after light onset. In contrast to these data, serum N-acetylserotonin levels of 2L:22D animals are elevated at crest to 3.54 ± 0.61 ng/ml (N=11) and the crest occurs 8 hours after light onset. In another experiment, male rats were housed in constant light for 30 days and then placed into a 12 hour cycle of dark and light. At the end of 30 days of constant light, melatonin levels were 12 pg/ml and N-acetylserotonin levels were 0.8 ng/ml. Both of these levels are similar to trough levels observed in cycling light for the respective substances. During the initial and subsequent exposure to 12 hour dark-light cycles, melatonin showed elevations during the dark periods and supression during the light periods. In contrast to these data, N-acetylserotonin levels showed no consistent relationship with dark and light periods. These two studies demonstrate that N-acetylserotonin levels in serum are approximately 50 fold higher than melatonin levels in rat serum and that the 24-hour pattern of secretion of these two substances can be dissociated suggesting that they may be discrete hormones with separate regulatory mechanisms and target sites.

229.9 CHRONIC EXPOSURE TO 60-Hz ELECTRIC FIELDS: EFFECTS ON SEROTONIN AND ITS METABOLITES IN THE RAT PINEAL

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Serotonin, in addition to its function as an important brain neurotransmitter, serves as a precesor for the melatonin and 5-methoxytryptophol in the pineal. These indole hormones appear to be key factors in the adaptive response of the organism to external stimuli. Melatonin levels, which normally exhibit circadian rhythmicity with increased concentrations during the hours of darkness, have been measured as a general indicator of pineal function. The enzymes, which act upon serotonin in the initial step of it's conversion to melatonin and 5-methoxy tryptophol, are serotonin-N-acetyl transferase (SNAT) and monoamine oxidase respectively. Hydroxyindole-O-methyl transferase serves as the O-methylation enzyme which is common to both pathways.

We have recently reported that the normal nocturnal increase in pineal melatonin concentration is significantly depressed with a concommittant increase in 5-methoxy tryptophol concentration, by chronic (30 day) exposure to 60-Hz electric fields. Concurrent measurements in these same animals showed that overall SNAT activity was depressed in the exposed group relative to controls.

As an extension of these studies we have recently compared pineal serotonin levels for exposed and control rat populations at four different time points in a 24 hour period. High resolution glass capillary column gas chromatographic mass spectrometry was employed to monitor selected ions from butanol/ethyl acetate extracts of aqueous pineal sonicates to which internal standard had been added. The monitoring of several ions allowed simultaneous measurement of pineal serotonin and melatonin concentrations with high specificity.

Findings from these studies indicated that compared to control populations, pineal serotonin levels in exposed animals were perturbed. The observed effects on pineal serotonin concentration may be due to interference with normal SNAT activity induced by altered neuronal input to the pineal as a result of electric field exposure.

229.10 THE EFFECT OF PINEALECTOMY ON COLD-INDUCED CHANGES IN BROWN ADIPOSE TISSUE.

K. S. Kott* and B. A. Horwitz* (SPON: E. Sassenrath). Dept. of Animal Physiology, Univ. of California, Davis, CA 95616.

Previous work has suggested that the pineal gland may play a role in mammalian thermoregulation by influencing the thermogenic effector brown adipose tissue. In such experiments, hamsters receiving melatonin via subcutaneous implants of beeswax pellets had larger deposits of brown fat than did those receiving no exogenous melatonin regardless of the photoperiod imposed (Nature 247:224, 1974). In view of this finding plus the fact that cold exposure elicits increased brown fat deposition in a variety of species (Phys. Rev. 49:330, 1969), the present study was designed to investigate whether such cold-induced increases in brown fat weight are dependent on pineal secretions.

Nine Long-Evans hooded male rats were pinealectomized at 62 days of age; eight control rats were sham-operated. Throughout the experiment the animals were exposed to a 12 hr light/12 hr dark cycle, housed singly, and provided with food and water ad libitum. At 75 days of age, four pinealectomized and four sham-operated rats were transferred to 5°C, while five pinealectomized and four shams remained at 22°C. At 107 days of age, all 17 rats were sacrificed at 2330 hrs, five hours after "lights out." Brown adipose tissue from the cervical and interscapular regions was excised and weighed. A radioimmunoassay (modified from Res. Commun. Chem. Path. & Pharm. 10:693, 1975) was used to determine melatonin levels in the pineal.

After 32 days in the cold, the control (sham) rats had significantly larger amounts of brown adipose tissue (interscapular plus cervical) than did control rats maintained at 22°C, there being an increase of approximately 95% in the interscapular regions and 60% in the cervical regions of the cold-exposed controls. In contrast, pineal levels of melatonin in these cold-exposed rats (2.82 ± .75 ng/pineal, $\bar{X}$ ± S.E.) did not significantly differ from those living at 22°C (1.75 ± .38 ng/pineal). Increased brown fat weights were also observed in the cold-exposed pinealectomized rats, the magnitude of these increases being comparable to those seen in the cold-exposed controls. In rats housed at 22°C, pinealectomy did not significantly alter brown fat weights.

These results are interpreted as indicating that the pineal gland is not essential for the increased weight of brown adipose tissue induced by cold exposure. (This study was supported by NSF grant PCM 77-22706.)

229.11 KINETICS OF PHASE SHIFTS INDUCED BY PROTEIN SYNTHESIS INHIBITORS IN THE CIRCADIAN RHYTHM OF NEURONAL ACTIVITY FROM THE EYE OF APLYSIA,

D. P. Lotshaw* and J. W. Jacklet. Dept. of Biology, SUNYA, Albany, N.Y. 12222.

The isolated eye of Aplysia exhibits a circadian oscillation in the rate of spontaneous compound action potentials (CAP) recorded from the optic nerve. Phases of the oscillation are designated in H of circadian time (CT) 0 to 24. CT 0 is the phase when CAP rate has increased to 1/2 maximum. Protein synthesis inhibitors, puromycin (PURO) (Rothman and Strumwasser, J. Gen. Physiol. 68, 359) and anisomycin (ANISO) (Jacklet, Science 198, 69) induce phase dependent phase shifts of the oscillation. We re-evaluated the phase response curves (PRC) for PURO and ANISO using 6 H pulses of 265 µM PURO or 0.5 µM ANISO; lowest concentrations that induced maximal phase shifts. ANISO induced an all delay PRC with maximum 6 H delays for pulses applied between CT 18 and CT 1. PURO induced maximum phase delays of 8-9 H between CT 21 and CT 0 and phase advances of 4-5 H between CT 1 and CT 3. The ANSO and PURO PRCs differ only in the magnitude of the maximum phase delays and the PURO induced phase advances. The concentrations of ANISO and PURO used inhibit protein synthesis by 95% for ANISO and 44% for PURO, as measured by ^{3}H-leu incorporation into TCA insoluble material. Inhibition is maximal within 1 H and recovery of protein synthesis is equally rapid. To investigate the differences in phase delays produced by ANISO and PURO the effect of pulse duration was measured. For ANISO applied at CT 21, the size of the phase delay approximately equals the pulse duration up to 9 H, slope = 1, but for PURO the slope of the pulse duration vs phase delay size approximately equals 1.5. The dose of PURO needed to induce a significant phase shift during the delay or advance phases was measured. Higher doses were needed for advances (150 µM) than for delays (50 µM). However, the PURO dose needed to get maximal advances or delays is similar (265 µM). These data suggest that PURO treatments have an additional dose dependent effect that lengthens phase delays and produces phase advances. Since protein synthesis recovers equally well from ANISO or PURO after a pulse, prolonged inhibition cannot account for the difference. PURO effects may be related to accumulation of PURO containing nonsense peptides. These data also are evidence for a continual protein synthesis requirement in the circadian oscillation between CT 18 and CT 1 and suggest that the inhibition of protein synthesis phase shifts the circadian clock by stopping the oscillation during the protein synthetic phase.

Supported by NSF BNS 11154.

229.12 A GOLGI STUDY OF THE HAMSTER SUPRACHIASMATIC NUCLEUS.

Kenneth J. Mack, William T. Greenough, and Carol Sue Carter. Neural and Behavioral Biology Program, and Department of Psychology, Univ. of Illinois, Champaign, IL, 61820.

The hypothalamic suprachiasmatic nucleus (SCN) was studied using Nissl stains and Golgi-Cox impregnations in young adult male and female hamsters. Nissl studies located the nucleus immediately superior to the posterior third of the optic chiasm. An area of the SCN, 400um long, 500um wide, and 400um high was studied in Golgi-Cox coronal sections. Three types of neurons were found, classified according to the number of primary dendritic branches. These types were unipolar (25%), bipolar (50%), and multipolar (25%). The average SCN neuron studied (across classes) had two primary branches and a total dendritic length of 145um. Less than half of the neurons studied had second order branches, and uncommonly a neuron would possess up to fifth order branching. Dendritic density distribution plots, calculated as in prior work (Greenough et al, Brain Res. 126:63,1977), revealed that the highest dendritic density appeared in the central and lateral aspects of the nucleus. Statistically significant differences in dendritic density were seen between males and females, although these differences are not obvious to casual visual inspection. An analysis of dendritic fields revealed that individual SCN neurons tend more frequently to send dendrites in dorsal and lateral orientations. Females' neurons tend to send their dendritic processes out dorsally, while males' neurons send dendrites dorsomedially and ventrolaterally. Also, ventrally located cells were found to send their dendritic processes dorsally, while dorsal cells would send processes both ventrolaterally and dorsally. Finally, some SCN neurons were found to send their dendrites up to 40um across the midline into the contralateral SCN. This was primarily seen in the posterior portion of the SCN, between 60-190um above the optic chiasm. Approximately one out of every six cells within 70um of the midline would send dendritic processes into the contralateral SCN.

Supported by NS 13421.

229.13 THE SUPRACHIASMATIC NUCLEUS OF THE RAT: A MORPHOMETRIC ANALYSIS OF SYNAPTIC ORGANIZATION. M.F. Bernstein*, J.P. Card and R.Y. Moore. Department of Neurology, SUNY at Stony Brook, N.Y. 11794

Several investigations have demonstrated that the suprachiasmatic nuclei (SCN) of the rat are composed of a heterogeneous population of neurons and axon terminals which are both morphologically and chemically distinct. Afferents to the rat SCN are known to form terminal fields within clearly circumscribed subfields of the nucleus. However, with the exception of retinal afferents, little is known of the fine structural organization and termination of SCN afferents. In this study we present preliminary findings of a systematic morphometric ultrastructural analysis of the rat SCN neuropil. Five adult female Sprague-Dawley rats were perfused intracardially with buffered aldehyde solutions and brains processed for transmission electron microscopic analysis of the SCN. In order to standardize the analysis among animals and to ensure systematic sampling of all regions of the SCN, the analysis was conducted at four levels throughout the rostrocaudal axis of the SCN. These included coronal planes through the most rostral and caudal poles of the nucleus as well as two intermediate levels. Within the two intermediate levels, analysis of the neuropil was divided among four subdivisions of the nucleus; dorsomedial, dorsolateral, ventral (ventral third) spanning the mediolateral extent of the SCN/optic chiasm interface, and a small area at the ventrolateral extent of the SCN. Previous studies indicate that this method of subdivision would be effective in sampling the neuropil of the SCN in that the divisions separate areas known to be distinguishable cytoarchitectonically and immunohistochemically. In addition, this parcellation reflects divisions evident in the differential distribution of the afferent innervation of the nucleus. Axon terminals within the SCN can be separated into at least ten categories on the basis of vesicle size and shape, the prevalence of large granular vesicles, the density of the axoplasm, and in some instances, on the basis of mitochondrial fine structure. Many of the boutons establish synaptic contacts with dendrites; axosomatic synaptic contacts are rare. A number of dendro-dendritic synapses are evident within the nucleus as well as occasional synaptic contacts between vesicle-filled boutons. Preliminary evidence indicates that some of the morphologically distinct categories of axon terminals are located preferentially within specific subfields of the nucleus. Further morphometric analysis of bouton distribution within the SCN of both normal and experimental animals is currently being conducted to determine if any of the bouton categories can be correlated with any specific system of afferents. Supported by USPNS Grants NS-06247 and NS-16304.

229.14 LONG-TERM AND SHORT-TERM EFFECTS OF CLORGYLINE (A MONOAMINE OXIDASE TYPE A INHIBITOR) ON LOCOMOTOR ACTIVITY AND ON PINEAL MELATONIN IN THE HAMSTER. C. Craig*, L. Tamarkin*, N. Garrick*, and T. Wehr* (SPON: J. C. Gillin). CPB and CNB, NIMH and IRP, NICHD, NIH, Bethesda, MD 20205.

In a previous study we observed that the irreversible MAO-A inhibitor clorgyline lengthened the free-running period of the activity-rest cycle in Syrian hamsters housed in constant darkness. We now report effects of clorgyline on locomotor activity and on the pineal melatonin circadian rhythm in hamsters entrained to light:dark schedules.

Male hamsters (N=27) housed with running wheels were entrained to a light:dark schedule of LD 14:10 (lights-off 1500h to 0100h). After 4 weeks' baseline recording, time of lights-off was advanced 4 hours (LD 10:14) and 15 animals were implanted subcutaneously with osmotic mini-pumps that released clorgyline at 2 mg/kg/day. After 6 weeks of treatment in LD 10:14, all of the treated animals failed to advance the time of activity-onset to the same extent as controls. Total activity was also reduced in the treated animals. Pineal melatonin (pg/pineal) of control vs. clorgyline-treated animals was 601 ± 10 vs. 862 ± 25 at 2000h, 829 ± 14 vs. 1011 ± 6 at 2300h, 61 ± 6 vs. 496 ± 18 at 0130h, and 42 ± 4 vs. 241 ± 10 at 0300h. Mean MAO-A activity (with 5-HT as substrate) was 56 nmol/mg protein/h in control animals and 4 nmol/mg protein/h in clorgyline-treated animals. From the beginning to the end of an 8-week treatment period, clorgyline-treated male hamsters (N=5) maintained in LD 14:10 showed a 1-hour delay in the onset of activity, compared with controls. In a third study, animals in LD 14:10 were given ip injections of saline or clorgyline at 1000h on the day of sacrifice. One group was injected for 3 consecutive days. Pineal melatonin levels during exposure to light at night and total brain MAO-A activity were measured.

CLORGYLINE (mg/kg)	MELATONIN (pg/pineal)			% MAO-A
	2230h	2300h*	2400h**	Inhibition
0.0	956±15	38± 4	36± 5	0
2.0	430±17	101±11	139±14	92
4.0	854±15	545±20	500±21	91
8.0	765± 7	990±25	863±12	92
2.0 (X 3 days)	945±20	925±17	1056±19	94

*lights on 30 minutes, **lights on 90 minutes.

These data demonstrate that clorgyline treatment 1) slows re-entrainment to an advance of the lighting schedule 2) delays activity-onset relative to a fixed lighting schedule 3) reduces locomotor activity 4) inhibits MAO-A activity > 90% and 5) blocks light suppression of pineal melatonin.

229.15 POTENTIATING EFFECTS OF LESIONS OF THE INTERPEDUNCULAR NUCLEUS ON MELATONIN-INDUCED REPRODUCTIVE HYPOTROPHY IN UNDERFED MALE RATS. C. A. Stockmeier* and D. E. Blask* (SPON: J. B. Angevine). Dept. of Anatomy, Coll. of Med., Univ. of Arizona, Tucson, AZ 85724.

Melatonin (Mel) injections cause a marked decrease in weights of testes and accessory sex organs in underfed male rats. The antigonadotrophic effect of Mel, a putative pineal hormone, may be mediated by the serotonin system of the midbrain raphe. We wished to see if partial interruption of neural input to the raphe would modify this effect in underfed rats. Six groups of Sprague-Dawley male rats (40 days old) were maintained on a 14hL: 10hD light:dark cycle (lights on 0600-2000). Unoperated groups were of three kinds: fed intact (FI), underfed Mel-injected (UMel) and underfed saline-injected (US). The three operated groups were: underfed Mel-injected habenula-lesioned (UMelHb), underfed Mel-injected interpeduncular nucleus-lesioned (UMelIPN) and underfed sham interpeduncular nucleus-lesioned (UMelSham). Underfed rats received one-half the normal daily food intake of FI rats, while Mel-treated rats received daily injections (s.c.) of Mel (50 µg) between 1650-1750. Hb and IPN lesions (2mA, 30s) were placed stereotaxically in Innovar^{R}-anesthetized animals. In the sham-operated rats, the electrode was lowered to a point just above the IPN and then removed. After 35 days of Mel injections and underfeeding, the rats were weighed and decapitated; weights of testes, seminal vesicles, ventral prostates and pituitaries were recorded. The UMel group showed reduced ($p<0.05$) gonadal, accessory sex organ and pituitary weights compared to US controls. In the UMelIPN group, testicular and accessory sex organ weights were further depressed ($p<0.05$) compared to UMel controls. The ventral prostates and seminal vesicles, but not the testes, of this UMelIPN group were also lighter ($p<0.05$) than in UMelShams. Gonadal and accessory sex organ weights of UMelHb rats did not differ from UMel controls. It seems that IPN lesions potentiate Mel antigonadotrophic effects in underfed male rats. Since the reproductive organ weights of UMelSham rats fell between those of the UMel and UMelIPN animals, sham IPN lesions also appear to potentiate Mel effects, although less so than electrolytic IPN lesions. Therefore, lesions in or near the interpeduncular nucleus might interrupt inhibitory input to melatonin receptors possibly present on raphe neurons. Disinhibition of such Mel receptors might further potentiate the antigonadotrophic effect of melatonin in undernourished male rats.
(Supported by USPHS Biomedical Research Support Grant RR05675.)

229.16 PRIMATE CIRCADIAN RHYTHMS IN HYPOTHALAMIC AND BODY TEMPERATURES. Charles A. Fuller. Division of Biomedical Sciences, University of California, Riverside, CA 92521

Preoptic hypothalamic temperature in mammals has long been known to play a key role in the short-term regulation of deep body temperature. Superimposed upon this steady-state regulation of body temperature is a 24-hour circadian rhythm. We have shown in the squirrel monkey (Saimiri sciureus) that this oscillation is precisely regulated in that it is highly reproducible. However, the role of preoptic temperature in regulating this rhythm is not known. A first step in assessing this role has been to examine the relationship between preoptic and colonic temperature rhythms in the squirrel monkey.

Three chair-acclimatized squirrel monkeys, previously prepared with preoptic hypothalamic re-entrant tubes were used in this study. Chaired animals were exposed to each of three ambient temperatures (20°, 26°, 32°C) for one to three days, while measuring colonic and hypothalamic temperatures. In all cases, the animals were synchronized to a light-dark cycle, with lights on at 08:00 and lights off at 20:00 hours (LD 12:12).

The results indicate that the hypothalamic and colonic temperature rhythms are very similar in waveform, amplitude and phase at all three ambient temperatures. In all cases, the hypothalamic temperature was higher than colonic temperature. This difference was minimal (0.4°C) at the thermoneutral temperature of 26°C. At 32°C this difference was slightly higher (0.6°C) while at 20°C the temperature difference was maximal (0.9°C). The amplitude of the hypothalamic and colonic temperature rhythms was similar at all three ambient temperatures. The amplitudes, however, were maximal at the coolest temperature (2.5°C) and minimal at the thermoneutral temperature (1.9°C). This effect on amplitude was a result not only of ambient temperature influence on the steady state level of body temperature during the day, but also on the rate of fall of body temperature in the early night. The phase relationships of both rhythms at all three temperatures showed the same similarities in that the maximum body temperature was reached late during the lights on period, and the minimum temperature was reached during the latter half of the lights off period. It thus appears, that the rhythms in hypothalamic temperature and colonic temperature are very similar although the temperature difference between them is variable. This suggests other neural centers are involved in the regulation of these rhythms. (Supported by NSF Grant BNS-792441 and PHS Grant BRD-RR09070.)

230 SYMPOSIUM

NEURAL MECHANISMS OF ATTENTION. J. C. Lynch (Chairman; Univ. of Mississippi), M. I. Posner* (Univ. of Oregon), M.-M. Mesulam (Harvard Univ.), K. M. Heilman (Univ. of Florida), V. B. Mountcastle (Johns Hopkins Univ.).

Our peripheral sensory systems are continuously processing millions of bits of incoming information. However, the higher neural function of selective attention permits only a very small portion of this information to reach consciousness. Current evidence indicates that the neural systems which participate in the control of attention are distributed widely in the central nervous system, with processing nodes located in several brainstem, midbrain, thalamic, and cortical structures. The purpose of this symposium is to present recent experimental findings which relate to the anatomical organization and connectivity of these areas, to describe recent studies of the effects of damage to one or more of these processing nodes in humans and in subhuman primates, and to present the results of studies of neural processing at the single cell level related to attention at one of these nodes.

Dr. Posner will discuss three distinct components of visual attention, their separate time courses, and their dissociation in certain neurological disorders. Dr. Mesulam will describe the regions of the CNS in which lesions produce contralateral neglect or inattention, will analyze the connectivity of these regions, and will propose a model of cortical organization which could play a major role in the overall modulation of directed visual attention. Dr. Lynch will describe the effects of posterior parietal and prefrontal cortical lesions on visual attention and extinction tasks in monkeys. Dr. Heilman will describe experiments relating neglect and akinesia in brain damaged patients, and discuss the neural basis for the observation that neglect is more common after right hemisphere lesions than after left hemisphere lesions. Dr. Mountcastle will describe the influence of different states of directed visual attention upon the excitability of single light-sensitive neurons in the posterior parietal cortex of monkeys. He will relate the activation properties of these cells to their possible roles in the direction of visual attention, in spatial perception, and in the constancy of perception of the visual surround during locomotion and head movement.

231 WORKSHOP

ALTERED AXONS: IS COMPULSIVE CONDUCTION REPLACED BY SPONTANEOUS SPIKING?

W. H. Calvin (Chairman), S. G. Waxman, M. Rasminsky, J. W. Moore, J. L. Ochoa

Regenerating axons, and even "normal" axons with altered myelination or microenvironment, initiate impulses ectopicly: chemo- and mechanosensitivity, afterdischarge following a priming train, sometimes even spontaneous firing or a susceptibility to cross-talk. Alterations in conduction following demyelination will be compared to normal functional architecture, to ask: How does a trigger zone arise?

William H. Calvin, Chairman (Univ. of Washington, Neurological Surgery): "ECTOPIC SPIKING FROM REGENERATING AND DEMYELINATED AXONS."
Introduction: Neuromas and regenerating axons; focal demyelination; DRG and theory for safety factor compensation. Spontaneous spiking and afterdischarge following conduction.

Stephen G. Waxman (Stanford University, Neurology): "FUNCTIONAL ARCHITECTURE OF MYELINATED AND DEMYELINATED AXONS."
Segregation of sodium channels to nodes, potassium channels beneath myelin. Demyelination changes the channel distribition, continuous conduction properties.

Michael Rasminsky (McGill University and Montreal General Hospital, Neurology): "CONDUCTION AND ECTOPIC SPIKING IN SPINAL ROOT AXONS OF DYSTROPHIC MICE."
Saltatory and continuous conduction in dysmyelinated axons. Abnormal excitation manifested by spontaneous ectopic excitation, ephaptic excitation, autoexcitation or afterdischarge.

John W. Moore (Duke University, Physiology): "ON THE SITE OF IMPULSE INITIATION"
What makes the initial segment the normal trigger zone? Channel densities and the loading from adjacent structures. The motoneuron model.

Jose L. Ochoa (Dartmouth Medical School, Neurology): "SPONTANEOUS IMPULSES IN HUMAN SENSORY AXONS"
Single sensory unit recordings in man during both pathological and experimentally induced ectopic spiking, and their subjective sensory correlates.

232.1 NUCLEAR PROGESTIN RECEPTORS IN THE RAT BRAIN. T.C. Rainbow, M.Y. McGinnis*, L.C. Krey*, and B.S. McEwen, The Rockefeller University, New York, NY 10021.

We used an in vitro exchange assay to demonstrate that translocation of cytosol progestin receptors to nuclear fractions occurs in rat brain after administration of progesterone (P), and to relate nuclear translocation to proceptive sexual behavior produced by P. Our assay procedure was similar to that of Blaustein and Feder (Endocr. 106:1061, 1980), and utilized ^{3}H-R5020, a synthetic progestin, as a specific ligand to measure progestin receptors. As 2h after s.c. administration of 1 mg P to ovariectomized (OVX) rats primed with 20µg estradiol benzoate (EB), the amount of nuclear progestin receptor in fractions from preoptic area (POA) and medial basal hypothalamus (MBH) increased from 27 ± 8 fm/mg DNA in the absence of exogenous P to 128 ± 12 fm/mg DNA (n=10). There was also an increase in the amount of nuclear receptor in intact rats during the proestrus release of P. Nuclear receptor levels were 35 ± 5 fm/mg DNA at 1200 on proestrus before the preovulatory rise in serum P had occurred, but were 75 ± 8 fm/mg DNA at 1630 after serum P levels were elevated (n=8).

The nuclear receptors in EB-primed rats in the absence of P probably consisted mostly of unoccupied receptors because their levels were not decreased by adrenalectomy, which eliminated adrenal progesterone. Furthermore, unlike the translocated receptors, these receptors could be removed from nuclear fractions with low ionic strength buffer. We also observed that P administration resulted in low levels of nuclear translocation in MBH-POA fractions from OVX rats not given EB, and in fractions from cerebral cortex, which unlike the MBH-POA, has no estrogen-inducible progestin receptors. This indicates that both estrogen-induced and non-induced progestin receptors will translocate to cell nuclei.

In behavioral studies, the amount of receptor in MBH-POA nuclear fractions from EB-treated rats varied with the dose of P given, and was directly related to the amount of proceptive sexual behavior (quality of lordosis posture, presence or absence of solicitation), produced by 3 different doses of P (0.25, 1 and 4 mg). Our results establish that progestin receptors in rat brain will translocate to cell nuclei after administration of P, and are consistent with the notion that P influences sexual behavior by genomic activation.

Supported by NS07080, HD10168, an institutional grant RF70095 from the Rockefeller Foundation for research in reproductive biology, and postdoctoral fellowships from the NIH.

232.2 EFFECTS OF A MINIMAL ESTRADIOL STIMULUS ON SEXUAL RECEPTIVITY AND PROGESTIN RECEPTOR INDUCTION FOLLOWING OLFACTORY BULB REMOVAL. M.Y.McGinnis*, A.R.Lumia* and B.S.McEwen (SPON: B.G. Lindsey). The Rockefeller University, New York, NY 10021 and Skidmore College, Saratoga Springs, NY 12866.

In female rats primed with estrogen and progesterone (P), olfactory bulb removal potentiates feminine sexual behavior relative to sham-operated (Sham) controls. We investigated a) whether bilaterally olfactory bulbectomized (BOB) rats would show increased behavioral sensitivity to estradiol (E_2) under conditions of minimal E_2 dose and exposure times, and b) whether the facilitation of lordosis seen in BOB rats could be attributed to changes in progestin receptor (PR) induction by E_2.

In the first study, 5 mm long Silastic capsules containing 5% E_2 (diluted with cholesterol) were implanted sc for 2 days. At 24 and 48 h rats received 200 ug P in propylene glycol sc and were tested for lordosis 4-6 h later. Sham animals showed low levels of lordosis when tested at 24 h, but achieved high levels of responding by 48 h. In contrast, BOB rats displayed high levels of lordosis at both 24 and 48 h, and were significantly higher than Shams on both tests. In the second study, BOB and Sham rats received a brief, 6 h exposure to 100% E_2 in Silastic capsules (5 mm). At 24 h, 200,500,750 or 1000 ug P was administered sc and rats were tested for lordosis 4-6 h later. Mean lordosis quotients in both groups were low with 200 ug P, and rose in a dose-response manner with increasing P doses. In BOB rats, lordosis was significantly higher than in Shams after 200, 750 and 1000 ug P. Another group of rats was tested for lordosis after 4 h E_2 exposure. This dose of E_2 followed by 1 mg P was sufficient to facilitate lordosis in BOB rats, but not in Shams. Because the 4 h E_2 exposure resulted in a clear differentiation between responding (BOB) and non-responding (Sham) animals, we used this 4 h E_2 exposure to examine PR induction in pre-optic area (POA), hypothalamus (HYPO) and pituitary (PIT). Rats were sacrificed at 24 h, and PR binding measured using the synthetic progestin ^{3}HR5020 as ligand. Following this brief E_2 exposure, there was no PR induction in POA, HYPO or PIT of either BOB or Sham rats. Our behavioral results indicate that BOB rats are behaviorally more sensitive to a minimal E_2 stimulus, and display lordosis after E_2 doses and exposure times insufficient to facilitate behavior in Sham rats. The apparent dissociation between lordosis and PR induction suggests that the potentiation of lordosis following olfactory bulb removal may not be related directly to increased PR binding.

supported by PHS grant NS06156

232.3 ESTRADIOL AND PROGESTERONE CHANGE SENSITIVITY TO PROGESTERONE IN GUINEA PIGS VIA MODULATION OF THE HYPOTHALAMIC PROGESTIN RECEPTOR SYSTEM. Jeffrey D. Blaustein. Dept. of Zoology, Iowa State University, Ames, IA 50011.

In order to delineate further the role of progestin receptors in the hypothalamus-preoptic area (HP) in the regulation of lordosis in female guinea pigs, we injected estradiol benzoate (E_2B) and progesterone (P) in various regimens that result in responsiveness to, refractoriness to, or restoration of responsiveness to P-facilitation of lordosis. We attempted to correlate the effectiveness of P to facilitate lordosis with the effectiveness of P to cause accumulation of nuclear progestin receptors (NPR) in HP measured by an in vitro (^{3}H)R 5020 binding assay.

A P injection (.5 mg) 40 h after an E_2B injection (2µg) in ovariectomized guinea pigs resulted in lordosis in 83% of the guinea pigs and caused 30% depletion of cytoplasmic progestin receptors (CPR) and 200% increase in the concentration of NPR 4 h after injection compared with controls. By 24 h after injection, after heat had terminated, the concentration of NPR was no longer elevated, but the concentration of CPR remained depressed 55% lower than that of controls. This depression was seen with both untreated cytosol and with cytosol that had been pretreated by gel filtration to remove unbound steroids. These P-injected guinea pigs were refractory to P-facilitation of lordosis, and a second P injection 24 h after the first (64 h) resulted in a 70% lower concentration of NPR than in oil-injected controls. This decrease in the concentration of NPR appears to be due to the 55% lower level of CPR available at the time of the second P injection compared with controls.

E_2B (10µg) injected concurrently with the first P injection (40 h) restored behavioral sensitivity to the second P injection (64 h) and caused 160% higher concentration of NPR compared with guinea pigs that had received only P at 40 h. The increased concentration of NPR in E_2B-injected animals appears to be due to increased availability of CPR. E_2B injection at 40 h increased the concentration of CPR by 40% within 12 h, and guinea pigs that received E_2B concurrently with the P had a 50% higher concentration of CPR 24 h later compared with animals that had received only progesterone.

It seems that estradiol increases and P decreases the concentration of CPR in HP such that a subsequent P injection results in a high or low concentration of NPR, and perhaps consequently, to presence or absence of lordosis responses. The presence of sufficient NPR may be a requirement in the process by which P facilitates lordosis in estradiol-primed rodents.

Supported by BNS 8013050 from the National Science Foundation and funds from the Iowa State University Research Foundation.

232.4 IN SITU LOCALIZATION OF R5020 AND PROGESTERONE IN THE VASCULARLY SEPARATED AND ISOLATED RHESUS MONKEY HYPOTHALAMUS: LOCALIZATION OF A PROGESTERONE-CONCENTRATING LOCUS. D.R. Garris*, R.B. Billiar*, Y. Takaoka*, R.J. White* and B. Little*. (Spon: G.R. Athey) Dept. Anatomy, East Carolina Univ., Greenville, NC 27834, Dept. Reproductive Biology, Case Western Reserve Univ. and Brain Research Lab., Cleveland Metropolitan General Hospital, Cleveland, OH 44106.

Previous studies, utilizing a neurosurgical procedure for in situ hypothalamic isolation, documented that differences exist in the localization of various ovarian steroid concentrating cells in the primate hypothalamus (Neuroendocr. 32:124, 1981). The present study was designed to elucidate the location, density and pattern of progestin (R5020) and progesterone (P) concentrating neurons within the vascularly isolated and separated hypothalamus of the ovariectomized, estradiol-treated rhesus monkey. Radiolabeled steroids (80 µCi ^{3}H-R5020 or ^{3}H-P) were directly perfused in a dextran-blood solution into one-half of the isolated hypothalamus. The contralateral half of the hypothalamus was perfused in an identical manner with a mixture of radioinert and radiolabeled R5020 or P. Both sides were then perfused with radioinert microspheres for analysis of vascular patency. Brains were subsequently prepared for autoradiography. The distribution of ^{3}H-R5020 and ^{3}H-P was limited to the hypothalamus-thalamus area in all preparations and exhibited a cross-circulation of less than 10% between sides. ^{3}H-R5020 and ^{3}H-P labeling of hypothalamic neurons was markedly reduced on the side treated with the radioinert competitor. Large numbers of ^{3}H-R5020 and ^{3}H-P concentrating neurons were localized in the medial preoptic area, ventromedial (VMH), periventricular (PERI) and infundibular nuclei. Fewer labeled cells were localized in the suprachiasmatic, supraoptic and medial mammillary nuclei. Other hypothalamic areas were essentially devoid of R5020 or P concentrating cells. The localized distribution of the labeled cells was not affected by vascular perfusion since microspheres were observed in all nuclear regions regardless of whether or not labeled cells were present. A specific P-concentrating locus of cells was found in all the preparations. The locus was not confined to a specific, anatomically distinct nucleus but included the ventral PERI and medial one-third of the VMH near the mid- point of the VMH nucleus. The results of these studies demonstrate the existence and distribution of distinct R5020 and P-concentrating cells within the hypothalamus of the rhesus monkey. It is suggested that the medial-basal hypothalamic nuclei serve as a functional feedback site for P in the regulation of the hypothalamic-pituitary axis in the primate. (Supported by NICHHD Grants HD05691, HD07120 and HD07640).

232.5 SEX DIFFERENCES IN 3H-ESTRADIOL UPTAKE IN BRAIN AND PITUITARY OF THE RAT AFTER BROMOCRIPTINE TREATMENT. D. W. Gietzen,* D. E. Woolley* and M. A. Thompson* (SPON: R. Dagirmanjian). Dept. of Animal Physiology, Univ. of California, Davis, CA 95616.

Increases in 3H-estradiol ($3H-E_2$) uptake in brain and pituitary after treatment with dopaminergic agonists in the female have been reported previously by us. This study confirms these observations and extends them by measuring $3H-E_2$ uptake after bromocriptine in the male as well.

In vivo total, specific and nonspecific nuclear, supernatant and total tissue binding of $3H-E_2$ was measured 1 hr after injection (1 µg/kg $3H-E_2$ iv) in gonadectomized-adrenalectomized adult male and female rats. The dopaminergic agonist bromocriptine (5 mg/kg ip) or the vehicle was injected 1 hr prior to $3H-E_2$ administration. Specific binding was defined as diethylstilbestrol (DES) blockable radioactivity.

In the female rat bromocriptine significantly increased specific binding in the nuclear fractions of the basal hypothalamus (BH) and anterior pituitary (AP). In addition, bromocriptine increased total binding in nuclear fractions of dorsal hypothalamus (DH) and septum (SEP) and in supernatant fractions of AP, anterior hypothalamus, DH, amygdala, SEP and cortex. When values for nuclear and supernatant fractions were summed, significant increases were noted in the uptake of all tissues. Plasma levels of radioactivity were not affected by bromocriptine.

By contrast, bromocriptine pretreatment did not change $3H-E_2$ uptake in any tissue in male rats. Furthermore, $3H-E_2$ uptake did not differ in brain or AP between control male and female rats. The lack of a sex difference in the rat brain with regard to $3H-E_2$ uptake in the absence of other treatment has been noted previously in this laboratory and by others. The present report describes a sex difference in $3H-E_2$ uptake after bromocriptine treatment in AP and several brain areas of the rat known to contain E_2 receptors. Sex differences in response to apomorphine (another dopaminergic agonist), in prolactin and LH regulation, in tuberoinfundibular dopaminergic neuronal activity and in monoamine oxidase activity have been noted by others.

The fact that specific binding of $3H-E_2$ was increased in the female, but not in the male, rat in the BH and AP after treatment with a dopaminergic agonist could have important implications with regard to the sex differences in feedback effects of E_2 and in catecholamine-E_2 interactions. The observation that total tissue uptake was increased in all brain areas after dopaminergic treatment implies a general interaction between E_2 and dopamine throughout the brain in the female, but not in the male. (Supported by NIH grant HD12385.)

232.6 ESTROGEN AND PROGESTIN RECEPTOR BINDING IN BRAIN AND PITUITARY AFTER RETROCHIASMATIC KNIFE CUTS. C.P. Phelps, M.Y. McGinnis, D.M. Nance and B.S. McEwen. University of South Florida, Tampa, FL; Rockefeller University, NY, NY and Dalhousie University, Halifax, Nova Scotia, Canada.

Surgical interruption of retrochiasmatic anterior and lateral connections of the hypothalamus (HYPO) is known to interfere with LH release by the pituitary (PIT) and ovarian steroid-sensitive sexual receptivity. In this study we have examined estrogen and progestin receptor binding in the preoptic area (POA), HYPO and PIT of female rats after retrochiasmatic frontolateral cuts (FLC) or sham surgery (SHAM) made with a Halász-type knife. The behavioral effects of FLC were evaluated using pre- and post-surgical testing of lordosis quotients (LQ) after either estradiol benzoate (EB, 2µg/rat x 2d) or pure estradiol implants (E_2 in Silastic capsules x 2d), plus progesterone (P, 0.5mg/rat) injected 4-6hr before tests with experienced males. Nuclear estrogen-receptor complexes in brain and PIT were quantified using an exchange assay developed by Roy and McEwen (1977). Cytosol progestin receptor binding was determined according to the method of MacLusky and McEwen (1980) using the synthetic progestin ^{3}HR5020 as ligand. Two weeks after the final behavior test, the rats were sacrificed by decapitation 2 days after implantation of E_2 capsules and the POA and HYPO were dissected from the remainder of the brain on ice. The PIT of each animal was also removed and placed on ice. Tissue from 2 rats was pooled for each sample receptor determination. Analysis of variance revealed highly significant ($p<0.001$) effects of FLC on post- versus pre-FLC LQ's. As expected, post-hoc tests indicated that the mean LQ of the post-FLC test was significantly lower than pre-FLC LQ's and both pre- and post-SHAM LQ's. In addition, nuclear E_2-receptor binding in the HYPO of FLC rats was significantly decreased relative to corresponding SHAMS. E_2-receptor binding in POA and PIT were not affected by FLC. Cytosol progestin receptor binding values in all tissues were also not changed by FLC. This was unexpected in view of the behavioral deficit after E_2 plus P treatment. The biochemical and behavioral results of this study support the contention that there is a proportional linkage between the number of E_2-nuclear receptors in the hypothalamus and the capacity to display lordosis after E_2 plus P following surgical disconnections of anterolateral hypothalamic efferents and afferents. Supported in part by USPHS HD11345 and NS06156.

232.7 TREATMENT OF NEONATAL RATS WITH MSG ALTERS NEUROENDOCRINE FUNCTION BY DECREASING CYTOSOL ESTROGEN RECEPTORS. J.F. Rodriguez-Sierra, J.D. Blaustein, C.A. Blake, R.W. Clough and K.A. Elias*, Dept. of Anatomy, Univ. Nebraska Med. Ctr., Omaha, NE 68105 and Dept. of Zoology, Iowa State Univ., Ames, IA 50011.

We have previously reported that treatment of neonatal female rats with monosodium glutamate (MSG) interferes with normal reproductive function: delayed puberty, abnormal estrous cycles, suppression of ovulation and sexual behavior, and lack of compensatory ovarian hypertrophy (Rodriguez-Sierra et al., NEUROENDOCRINOLOGY 31:228, 1980). We ascribed neuroendocrine deficits to the neurotoxic effects of MSG on neurons in the mediobasal hypothalamus (MBH). We have now tested whether the neurotoxicity of MSG may occur in estrogen-sensitive cells to disrupt ovarian-hypothalamic-pituitary gland feedback mechanisms. Sprague-Dawley female rats were treated with MSG (4 mg/g BW, sc) on days 1,3,5,7, and 9 of age. Littermate controls were either injected with saline or remained untreated. At approximately 70 days of age, all rats were bilaterally ovariectomized. Ovaries were blotted and weighed. Two weeks after ovariectomy, rats were decapitated, trunk blood was collected, and serum was frozen until radioimmunoassays of prolactin and luteinizing hormone (LH) were performed. The pituitary gland was removed and weighed. The brain was dissected over an ice-cold glass slide, and the MBH and preoptic area (POA) were separated and weighed. The pituitary gland and both brain regions were homogenized and centrifuged, and the cytosol fractions were used for determination of estradiol receptors. The data was expressed in terms of fentomoles of ^{3}H-estradiol/mg protein. The MSG treated rats had significantly lower body weights than control rats throughout the experiment. Ovary and pituitary gland weights were 50% lower in the MSG-treated rats than in controls. Serum prolactin levels were elevated in the MSG-treated rats (255 vs. 130 ng/ml). Serum LH levels increased in both groups, but the differences were not statistically different. Nevertheless, 6 of 10 MSG-treated rats had serum LH levels below 300 ng/ml, while only 1 of 7 control rats had levels this low. The cytosol estradiol receptors were reduced significantly in the MBH and inthe POA of the MSG-treated rats. Pituitary gland cytosol estradiol receptors were virtually identical in both groups. The results support our hypothesis that neonatal MSG treatment exerts neurotoxic effects on estrogen-sensitive neurons. A reduced ability of the brain to monitor serum estradiol levels could explain some of the endocrinopathies of the MSG-treated rat. It is surprising that the pituitary gland is drastically reduced in size and yet maintains a normal complement of estradiol receptors. We are in the process of determining the specific hypothamic nuclei affected by the MSG treatment. (Supported by grants from the Univ. Nebr. Med. Center, NIH (HD11011) and BNS-8013050.

232.8 A REGIONAL ANALYSIS OF NEURAL CHANGES RELATED TO MASCULINIZATION AND DEFEMINIZATION. E. J. Nordeen* and P. Yahr. Dept. of Psychobiology, Univ. of Calif., Irvine, Irvine, CA 92717

Gonadal steroids act perinatally to masculinize and to defeminize adult sexual responsiveness. These processes can occur independently. In rats, masculinization refers to an increase in the likelihood of male sexual behavior in response to either androgenic or estrogenic stimulation in adulthood. This masculinization can be induced by exposing the developing anterior hypothalamus-preoptic area (AH-POA) to hormones such as estradiol (E_2). Defeminized animals are less likely to show either female sexual behavior (i.e., lordosis) or surges of luteinizing hormone (LH) in response to E_2 stimulation in adulthood. This defeminization occurs following hormonal stimulation of the developing medial basal hypothalamus (MBH). Sex differences in both neuroanatomy and steroid hormone binding exist in both the AH-POA and MBH. These differences develop in response to the same hormonal treatments that produce defeminization and masculinization. However, neither masculinization nor defeminization has been specifically linked to any neural change. Since masculinization and defeminization involve separate brain regions and produce opposite changes in E_2 sensitivity, sex differences in hormone binding may be regionally specific. We examined cell nuclear binding of ^{3}[H]-E_2 in the AH-POA, MBH, amygdala (AMY) and cortex (CX) of adult male and female rats. Females bound more ^{3}[H]E_2 than males in cell nuclei of both the AH-POA and MBH 60 min after an IV injection of 40 µCi [2,4,6,7-^{3}H]-E_2. Neither AMY nor CX showed a sex difference in E_2 binding. These data argue against a quantitative relationship between regional E_2 binding and E_2 sensitivity. In order to better define the relationship between steroid binding and masculinization, we are now assessing nuclear E_2 uptake in females that were masculinized by E_2 implants into the developing AH-POA.

The sexually dimorphic nucleus (SDN) of the POA provides an example of a sex difference in neuroanatomy. This nucleus is larger in males than females and its size is influenced by neonatal hormonal stimulation. However, the relationship between the size of the SDN and either masculinization or defeminization is unknown. We examined the SDN of females that were masculinized neonatally by unilateral intrahypothalamic E_2 implants. As adults these females show more mounts and intromission patterns than control females when injected daily with 2 µg estradiol benzoate + 200 µg dihydrotestosterone propionate. However, they show neither reduced female sexual behavior nor impaired LH secretion. Presently, it appears that there is no difference in the size of the SDN between these masculinized females and controls. Thus the relationship between the SDN and sexually dimorphic behavior remains unclear.

232.9 GLUCOCORTICOID BINDING IN THE BRAIN IS SEX DEPENDENT. B. B. Turner and D. A. Weaver. Depts. of Biology & Psych., Virginia Polytechnic Institute & State University, Blacksburg, VA 24061.

Sex-related neuroanatomical and neurochemical differences have been found in rodents and birds in those brain areas responsible for the control of sexual behaviors. In examining possible sex-related differences in cytosol binding of corticosterone, we chose the hippocampus (HC), which is known to have the largest number of glucocorticoid receptors, and the hypothalamic-preoptic area (HYP) which is known to be involved in the control of sexual behavior.

In a series of ten experiments, male and female Sprague-Dawley rats (70-90 days of age) were adrenalectomized 12 hrs before sacrifice. Rats were anesthetized with Metofane anesthesia and thoroughly perfused with cold dextran-saline. Tissues from 3 rats of the same sex were pooled for each experiment. Tissues were homogenized in Tris-EDTA buffer containing 10% glycerol and centrifuged at 105,000 g for 60 min. Aliquots of supernatant were then incubated with 6 concentrations of ^{3}H-corticosterone (2-30 x 10^{-9}M). Incubation in 100 fold excess unlabeled steroid was used to determine non-specific binding. The steroid bound to receptor protein was separated from free steroid by passage through LH-20 columns. Analysis of the double reciprocal plots obtained showed that ^{3}H-corticosterone binding was significantly greater in the HC in females (B_{max}=148±1 fmoles/mg protein) than in males (B_{max}=121±5 fmoles/mg protein), $p<.001$. In contrast, binding in the HYP was markedly reduced ($p<<.001$)in females (B_{max}=75±2 fmoles/mg protein) as compared to males (B_{max}=105±1 fmoles/mg protein). In HC the binding affinity was the same for males (K_d=4.16 x 10^{-9}M) and females (K_d=4.41 x 10^{-9}M). In HYP binding affinity was significantly lower ($p<<.001$) for females (K_d=7.17 x 10^{-9}M) than males (K_d=4.60 x 10^{-9}M).

This study demonstrates significant sex-dependent differences in the cytosol binding of corticosterone in HC and HYP of rats. Our results suggest that the HC of the female rat, due to its larger number of receptors, may be subject to greater modulatory control by glucocorticoids than the male. Learning, memory, and spatial orientation are functions thought to be under the control of the HC. Glucocorticoids are believed to have a modulatory role in the regulation of these functions. The sex-dependent receptor differences reported here suggest a possible explanation of the sex related differences reported to exist with respect to spatial ability. The significant decrease in receptor number and apparent affinity in the female HYP may serve a protective function with respect to reproductive capacity during episodes of stress. (Supported by an NIH BRSG grant from VPI & SU).

233.1 DIVERSE ACTIONS OF HEMICHOLINIUM-3 CONGENERS ON THE ACETYLCHOLINE ACTIVATED RECEPTOR-IONIC CHANNEL. E.G. Henderson, K.A. Alkadhi*, R.L. Volle, L.S. Reynolds*, J.J. Lambert*, and M. Ashford*. Dept. of Pharmacology, Univ. of Conn. Health Ctr., Farmington, CT 06032, and Dept. of Zool., Univ. of Nottingham, Nottingham, U.K.

The effects of hemicholinium-3 (HC-3), hemicholinium-15 (HC-15) and terphenyl-hemicholinium-3 (TPHC-3) on endplate currents (e.p.c.s) in transected frog cutaneous pectoris muscles were examined. At holding potentials (Vm) more negative than -90mV, HC-3 (50-100μM) caused a monotonic increase in the rate of e.p.c. decay (α) (Henderson et al., Fed. Proc. 1981: 40, 263). E.p.c. decay was biphasic at less negative Vm's, the initial phase being faster and the final phase slower than control. At Vm=-30mV, the relative amplitude of the terminal phase equaled that of the initial fast phase. At +30mV, a single slow exponential decay was observed (Vm=+30; α=1.75, control; α=.336 $msec^{-1}$, HC-3). Over the range Vm=-70 to -10mV, α_{slow}=.3 $msec^{-1}$ and α_{fast}=1.7 $msec^{-1}$, were independent of Vm. Peak e.p.c. amplitude (Ip) was depressed at all Vm's, but more depressed at negative Vm's. Similar results were obtained using Mg^{++} depressed intact muscles. In patch clamped chick skeletal muscles, HC-3 (50μM) decreased measured mean channel open time of ACh (5x 10^{-7}M) activated channels from 6.03±1.18 msec to 1.55±.02 msec at Em's -30 to -40mV. In the presence of HC-3, single channel events occurred in bursts. Such behavior would be expected if the blocked interval was long compared to the open time.

HC-15 (50-200μM), a mono-quaternary analog of HC-3 , caused a monotonic slowing of e.p.c. decay at all Vm's, with no effect on Ip. HC-15 (100μM) increased voltage dependence (H) of α from -144 to -118 mV. All effects of HC-3 and HC-15 were rapidly reversible. TPHC-3 (5-100μM) caused a time and concentration dependent decrease in Ip and an increase of α. While α was increased in 5μM TPHC-3, H was unchanged. TPHC-3 ≥ 10μM increased α and reduced voltage dependence. The effects of TPHC-3 on e.p.c. parameters were slowly reversible with recovery of α faster than Ip. These findings suggest that simple chemical modification of these molecules will lead to further insight into the kinetics of ACh activated ionic channels. (Supported by grants NIH NS-07540 and the U. Conn. Res. Foundation.)

233.2 MULTIPLE ACTIONS OF DMAE ON ACETYLCHOLINE-ACTIVATED IONIC CHANNELS IN THE FROG NEUROMUSCULAR JUNCTION. K.A. Alkadhi*, L.S. Reynolds*, E.G. Henderson and R.L. Volle. Dept. of Pharmacology, Univ. of Connecticut Health Center, Farmington, CT 06032.

The effect of α,α'bis(dimethylammonium acetylaldehyde diethylacetal)-p-p'-diacetylbiphenyl dibromide (DMAE), an analogue of hemicholinium-3, was investigated on neurally evoked endplate currents (e.p.c.s) in the transected cutaneous pectoris muscle of the frog. Within one minute of perfusion of DMAE (50 or 100μM), a marked initial prolongation of e.p.c. decay was observed at all holding potentials. At -90mV holding potential the prolongation of e.p.c. decay was immediately followed by marked shortening. However, at +30mV the initial prolongation of the decay persisted and no shortening was seen. Similar results were obtained in intact Mg^{++}-depressed muscles. The normal relationship between decay and holding potential was reversed by DMAE (H: control=-145mV; DMAE=+344.8mV) but the single exponential nature of the decay was unchanged at all holding potentials. DMAE depressed the peak e.p.c. and the current-voltage relationship became non-linear at hyperpolarized potentials with no change in the reversal potential. Pretreatment of the muscle with neostigmine (5μM) to inactivate acetylcholinesterase partially antagonized the initial prolongation of the e.p.c. decay but had no effect on the subsequent shortening of the decay phase or the depression of the peak e.p.c. amplitude. Washout of DMAE resulted in marked prolongation of the decay phase of e.p.c.s. This prolongation of the decay as well as the depression of the e.p.c. peak amplitude were not readily reversed by continued perfusion of drug-free Ringer solution (α=rate constant of decay, Ip=e.p.c. peak current at -90mV: control, α=0.752, Ip=319nA; 100μM DMAE, α=1.492, Ip=96nA; 25 min washout, α=0.495, Ip=110nA). Smaller concentrations (5μM) of DMAE produced only long-lasting prolongation of the decay phase and a small increase of peak e.p.c. amplitude. These results suggest that DMAE action involves more than one site at the acetylcholine-activated ionic channel. (Supported by NIH NS07540 and the Univ. of Conn. Res. Found.)

233.3 TWO PHASE DECAY OF MPSPS AT HATCHETFISH GIANT SYNAPSE. Day,J.W., Huse,W.D., Bennett,M.V.L. Dept. of Neurosci., Albert Einstein College of Medicine, Bronx, New York 10461.

In the medulla of the South American hatchetfish, Gasteropelecus, the Mauthner fibers are presynaptic to 10-14 myelinated giant fibers. The axo-axonic synapses formed by these fibers appear to be nicotinic cholinergic (see Hall,D.H. et al., this meeting). The input resistance of the giant fiber is 1 MΩ, its time constant is less than 100 μs, and it has a space constant of 3 mm.

Under voltage clamp the postsynaptic current (PSC) reverses at -15 mv, has a rise time of 80 μs, and decays in two phases (Huse, W.D., Bennett, M.V.L., J. Biophys. 33:14a, 1981). The early, fast falling phase shows little voltage dependence and has a decay constant of 300 μs. The late, slowly falling phase is voltage dependent and is slower at hyperpolarized membrane potentials, similar to the PSC of the frog neuromuscular junction. Its decay constant is 750 μs at the resting potential of -90 mv. Miniature postsynaptic currents (MPSCs) have a rise time of 50-100 μs when clamped at the resting potential, but falling phases were difficult to characterize because of noise.

Because of the fast time constant of the membrane the decay phase could be studied in unclamped voltage records. Miniature postsynaptic potentials (MPSPs), which averaged 1 mv in amplitude, were recorded on an FM tape recorder. A computer was used to digitize records and select MPSPs on the basis of rapid rise, slow fall, and amplitude within a given range. 300-500 MPSPs were averaged together, aligning the points of greatest rate of rise. The rise time of the averaged MPSP was 75-80 μs. The similarity of the rise time for the MPSP and MPSC shows that the high frequency components of the current are reflected in the voltage records, and are not appreciably filtered by the membrane or recording apparatus. The decay of the averaged MPSP is similar to that of the PSC and has two phases. The decay constant of the faster phase is 350 μs, and that of the slower phase is 700-800 μs. These data complement the voltage clamp findings and show that the two decay phases of the PSC reflect mechanisms operating at the level of the quantal event. The presence of a rapid phase followed by a slower phase in the decay of the PSC cannot be described, with any combination of rate constants, by the three-state model that Katz and Miledi used for the frog neuromuscular junction. More complicated schemes with two open states, multiple transmitter binding, or a blocking action of acetylcholine like that seen with some local anesthetics are possible explanations that require further study.

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233.4 CALMODULIN STIMULATES PHOSPHORYLATION OF THE ACETYLCHOLINE RECEPTOR, H. Smilowitz, R.A. Hadjian*, J. Dwyer* & M.B. Feinstein* Dept. Pharmacol., U. Conn. Hlth. Ctr., Farmington, CT 06032

Acetylcholine receptor (AChR) enriched membranes were prepared from frozen electric organ of Torpedo, C. by differential and density gradient centrifugation. Membranes from various fractions along the gradient were used to assay endogenous phosphorylation in the absence and presence of Ca^{++} and calmodulin (CDR). Each of the AChR enriched membrane fractions exhibited a 3-5 fold stimulation of endog. phosphorylation by Ca^{++} and CDR. Both Ca^{++} and CDR are needed for maximal stimulation although Ca^{++} alone affords a small but reproducible stimulation of endog. phosphorylation. The stimulation of endog. phosphorylation of the Torpedo membranes by Ca^{++} and CDR is completely inhibited by 25μM trifluoperazine (TFP). TFP at 50μM inhibits basal phosphorylation by 60% suggesting that most or all of the basal phosphorylation may be due to endogenous CDR. In fact, heat resistant material which stimulates CDR dependent phosphodiesterase activity is present in our membrane preparation.

SDS polyacrylamide gel electrophoresis shows that the major phosphorylated species (both in the presence and absence of Ca^{++} and CDR) are Mr 65K, 58K, 41K, and 39K polypeptides. A Mr 50K polypeptide is also phosphorylated to a lesser extent. The Mr 65K, 58K and 50K phosphorylated polypeptides correspond to components of the purified AChR from Torpedo, C. The identity of the Mr 41K polypeptide is still unresolved. The 39K polypeptide is not a component of the AChR. Polypeptides of 43K and high molecular weight are not appreciably phosphorylated.

Fluoride (5mM), Vanadate (100μM) and ouabain (1 mM) inhibit ATPase activity present in our membranes and further stimulate AChR phosphorylation. Ouabain stimulates less well than Vanadate or fluoride which inhibit all ATPase activity in our membranes. The anti-ATPase activity of fluoride can, therefore, account for most but not all of the stimulatory effect of fluoride in the phosphorylation assay; fluoride stimulation is 1.5 fold greater than vanadate in the presence of high levels of CDR. The mechanism is not yet known.

This phosphorylation system has been further characterized. The concentration of CDR which affects half maximal stimulation is 55nM. The apparent Km of the kinase for ATP is about 80μM. The phosphorylation reaction stimulated by potassium and inhibited by sodium. Under our optimal assay conditions including 100μM ATP, 100μM Vanadate, 0.4μM CDR, 50μM free Ca^{++} and 100 mM KCl, maximal phosphorylation is achieved in 30-45 min at 20°C and represents about 1 nM phosphate per mg of membrane protein of which, 10-15% is the AChR. (Supported by the Muscular Dystrophy Association.)

233.5 AUTAPTIC EXCITATION IN TURTLE OLFACTORY BULB IN VITRO. R.A. Nicoll and C.E. Jahr, Depts. of Pharmacology and Physiology, UCSF, San Francisco, CA 94143.

Excitation of olfactory bulb mitral cells is followed by an inhibitory postsynaptic potential (IPSP) which is mediated predominantly by activation of dendrodendritic reciprocal synapses located between the mitral (excitatory) and granule (inhibitory) cells (C.E. Jahr and R.A. Nicoll, Science 207:1473, 1980). When the inhibitory half of the reciprocal synapse is blocked by the GABA antagonists, bicuculline or picrotoxin, mitral cell activation is followed by an excitatory depolarizing potential instead of an IPSP. This depolarizing after-potential (DAP) can be sufficiently intense to elicit prolonged repetitive firing.

We first considered the possibility that the DAP was produced by a slowly inactivating, tetrodotoxin (TTX) sensitive, sodium conductance as is seen in cerebellar Purkinje cells (R. Llinás and M. Sugimori, J. Physiol. 305:171, 1980). TTX blocks directly evoked fast spikes in mitral cells revealing calcium spikes. However, TTX did not block the DAP.

The DAP is calcium-dependent since it was blocked by the calcium antagonists, cadmium and cobalt. This finding suggested that the DAP might represent a slow voltage-dependent calcium conductance or, alternatively, a feedback of excitatory transmitter released from the same mitral cell. If the DAP were due to a voltage-dependent calcium conductance, it should be blocked by injecting a hyperpolarizing current pulse, as has been shown in other cells. Such a pulse did not abort the mitral cell DAP. Furthermore, barium, which readily passes through calcium channels but does not support synaptic transmission, blocked the DAP while actually increasing the size of the TTX-resistant spike. This suggests that the DAP is transmitter mediated. Iontophoresis of either glutamate or aspartate (putative transmitters for the mitral cell) in the presence of bicuculline or picrotoxin, depolarized mitral cells. Addition of α-amino adipate, which antagonizes aspartate responses in other systems, reversibly blocked the DAP with no effect on the calcium spike. Since TTX did not block the DAP, a pathway dependent on propagated action potentials is excluded. We suggest that mitral cell activation causes the dendritic release of an excitatory transmitter, possibly aspartate, which feeds back directly onto the same mitral cell.

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233.6 DL-MUSCARINE DECREASES A POTASSIUM CONDUCTANCE TO DEPOLARIZE MAMMALIAN SPINAL CORD NEURONS IN CELL CULTURE. L.M. Nowak and R.L. Macdonald. Dept. of Neurology, The University of Michigan, Ann Arbor, MI 48109.

We have investigated the postsynaptic actions of dl-muscarine (MUS) on multipolar spinal cord neurons in primary dissociated cell culture and report that MUS evoked atropine-sensitive, slow membrane depolarizations associated with a decrease in a membrane potassium conductance (g_K).

Spinal cords and attached dorsal root ganglia were dissected from 12-13.5 day old fetal mice, and grown in culture at 35° for 4-10 weeks prior to electrophysiological investigations. Intracellular recordings of voltage and current responses were made from neurons bathed in buffered saline on the modified, heated (30°-35°), stage of an inverted phase-contrast microscope. High impedance (30-45 MΩ) 4M potassium acetate-(KAc) or 3M potassium chloride-filled (KCl) micropipettes were used for conventional intracellular recording with the bridge technique. Low impedance (10-20 MΩ) KCl-filled micropipettes, shielded with conductive paint (within 1-2 mm of tips), were used for single electrode voltage-clamp recordings (3KHz switching frequency; a 50% duty cycle). MUS and 10 μM GABA were applied to the neuronal surface by pressure pulses (1-2 sec at .25-3.0 psi) from blunt (2-10 μm tips) glass micropipettes.

MUS evoked dose-dependent (1-10 μM), slow (4-6 sec to peak at 30°C), reversible depolarizations accompanied by decreased membrane conductance during recordings with KAc-filled and KCl-filled micropipettes. Responses to 10 μM MUS were eliminated by <2 μM atropine. GABA evoked rapid (2 sec to peak) depolarizing responses and increased membrane conductance during intracellular recording with KCl-filled micropipettes. Both MUS and GABA evoked inward currents from the same neuron at resting membrane potential (RMP). GABA current responses decreased as membrane potential was voltage-clamped at increasingly depolarized potentials and response polarity reversed between -20 and -10mV. MUS-evoked inward currents increased with depolarization, decreased with hyperpolarization and had extrapolated reversal potentials (RP_es) 15-30 mV below RMP, suggesting that MUS decreased a g_K. Potassium-dependence of MUS-responses was confirmed by a shift of RP_es and RMPs to more depolarized potentials when bath potassium concentration was changed from 5 to 25mM.

MUS-currents, in contrast to GABA-currents, did not vary linearly with membrane potential at hyperpolarized potentials suggesting that MUS may decrease a voltage-dependent g_K in mammalian CNS neurons as has been described in frog sympathetic ganglion neurons (Brown & Adams, 1980).

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233.7 NOREPINEPHRINE (NE) AND ISOPROTERENOL (IP) DO NOT CONSISTENTLY HYPERPOLARIZE CA1 PYRAMIDAL NEURONS (PNs) OF THE RAT HIPPOCAMPUS SLICE IN VITRO. G.R. Siggins and D.L. Gruol. A.V. Davis Center, The Salk Institute, P.O. Box 85800, San Diego, CA 92138.

Many past extracellular studies show that iontophoretically applied NE inhibits hippocampal PNs in vivo. Although two recent studies on the hippocampal slice preparation (Langmoen et al., Brain Res. 208: 349, 1981; Segal, Brain Res. 206: 107, 1981) have described weak hyperpolarizing actions of NE applied to CA1 PNs by a micro-drop method, the precise mechanism of this inhibition is still equivocal. We have tested on PNs of the rat hippocampal slice (120-160 gm rats, 350-400 μm slices) the effects of NE or IP applied by superfusion or by pressure from pipettes (10 μm tips) containing 10 mM IP or NE in HEPES buffered CSF (pH 7.4). In both cases perfusion of the slice with artificial CSF (pH 7.4; temperature held at 33,34, or 35°) was continuously maintained at 1-2 ml/min over all surfaces of the slice, to prevent artifactual environmental changes with drug application. In the 12 PN's chosen for study, resting membrane potentials were steady for 1-5 hours at -55 to -75 mV, spikes were 80-100 mV and input resistance was 20-40 Mohms. NE was applied to 6 cells, in 8 complete tests by perfusion (20 μM to 1 mM) and in 10 tests by pressure (40 psi); IP was tested on 6 PNs, 15 times by perfusion (50 μM to 2 mM) and 3 times by pressure (40 psi). NE or IP slowed spontaneous activity in 44% of tests, speeded in 33% and had no effect in 23%. However, membrane potentials generally were not effected (NE, 48% of tests; IP, 61%) or slightly depolarized (NE, 29%; IP, 28%), rather than hyperpolarized (NE, 23%; IP, 11%). Potential changes for any one cell were inconsistent and small: 0.5-4 mV (1.36 ± 0.91 mV, mean ± S.D.). These changes in firing rate and membrane potential usually occurred without change in input resistance. Occasionally, paroxysmal depolarization shifts developed with NE or IP, or on washout. Lack of hyperpolarization was seen whether KCl or K acetate recording electrodes were used. Perfusion of NE in CSF with 10 mM $MgCl_2$ (to block synaptic activity) did not reveal hyperpolarizations. NE or IP were ineffective even in cells which displayed hyperpolarizations to adenosine and somatostatin and depolarizations to glutamate. We conclude either that NE and IP, even in high concentrations, inhibit PN's in CA1 by some mechanism other than hyperpolarization of the PN soma, or that the present hippocampal slice preparation is not adequate for analysis of mechanisms of adrenergic responses seen in vivo.

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233.8 PATCH CLAMP ANALYSIS OF GLUTAMATE RECEPTORS. K.A.F. Gration*, J.J. Lambert*, R.R. Ramsey* and P.N.R. Usherwood* (SPON: S.J. Potashner). Dept. of Zool., Univ. Nottingham, Nottingham NG7 2RD, U.K.

The extracellular patch clamp technique has been used to record currents passing through single glutamate D-receptor channels in the extrajunctional membrane of locust muscle. This preparation is particularly well suited to the patch clamp technique for the following reasons:-

1) The conductance of the glutamate channels is sufficiently large (~120 pS) to enable information to be gained on channel life-times <1 ms.

2) Pretreatment with Concanavalin A inhibits receptor desensitization enabling the effects of relatively high concentrations of glutamate (10^{-4}M - 10^{-2}M) on channel activity to be studied. For relatively low concentrations of L-glutamate (10^{-6}M - 5 x 10^{-5}M) mean channel life-time does not change significantly with glutamate concentration. Within this concentration range frequency distribution of channel life-times constructed with 50 μs bin widths are not exponential but exhibit a peak. The distributions can be described by two exponentials as follows $A\,(e^{-t/\tau_2} - e^{-t/\tau_1})$ where for glutamate $\tau_1 < \tau_2$. This suggests that the life-time of the glutamate channel involves two time-dependent processes. The process characterized by τ_1 has the effect of delaying the observed event of channel closure which is characterized by τ_2. At glutamate concentrations > 5 x 10^{-5}M the mean life-time of the channel appears to increase with increasing glutamate concentration. With 10^{-3}M glutamate, the channel remains mainly in the open state with only transient closings. Relatively long open times have been observed in the absence of Con A suggesting that this phenomenon is a natural property of the glutamate channel.

We have extended these studies to other glutamate agonists (quisqualate a more potent agonist than glutamate and cysteine sulphinate a weaker agonist than glutamate) in an attempt to discover the nature of the different potencies of agonists. Our results suggest that the agonists gate channels of similar conductance and that the relative potency of an agonist is determined mainly by channel life-time and the probability of receptor activation. (Supported by a grant from the British Science Research Council to P.N.R. Usherwood.)

233.9 Low agonist concentrations reveal complex kinetic behavior of GABA-induced membrane channels in tissue cultured mouse spinal neurons. David A Mathers* and Jeffery L. Barker. Laboratory of Neurophysiology, NINCDS, NIH, Bethesda, Md. 20205

Standard noise analysis procedures were used to study the influence of agonist concentration on the power spectral density of membrane current fluctuations induced by γ-aminobutyric acid (GABA) in tissue cultured mouse spinal cord (SC) neurons.

When GABA concentrations above 25μM were used, we obtained power spectra which were well described by a single time constant (Lorentzian) spectrum of the form $S(f) = S(0)/[1 + (f/f_c)^2]$, where fc is the half-power frequency of the spectrum. This observation is consistant with the view that, at high agonist concentrations, GABA spectra largely reflect the activity of a single population of two-state ionic channels. The lifetimes of these channels are then inferred to be exponentially distributed with mean value $\tau_{GABA} = 1/(2\pi f_c)$. At 23°C, τ_{GABA} = 30 ± 2.2 msec (mean ± S.D.) was calculated. Muscimol, an isoxazole believed to interact with vertebrate GABA receptors, also produced single time constant spectra when applied at concentrations above 25μM. In this case, however τ_{musc} = 76 ± 14.9 msec at 23°C was found.

When low agonist concentrations (1-10μM) were used, GABA spectra frequently deviated from the single Lorentzian form. These spectra were adequately described by the sum of two Lorentzian terms $S(f) = S(0)_s/[1 + (f/f_{cs})^2] + S(0)_f/[1 + (f/f_{cf})^2]$. The corresponding time constants calculated from such two component GABA spectra were $\tau_s = 1/(2\pi f_{cs})$ = 46 ± 8.9 msec and $\tau_f = 1/(2\pi f_{cf})$ = 9 ± 1.6 msec at 20°C (n = 5 cells). Qualitatively similar double Lorentzian spectra were also obtained using muscimol as agonist at concentrations in the range 1-10μM. In this case τ_s = 85 ± 19.6 msec and τ_f = 13 ± 6.4 msec at 20°C were calculated. In the case of both agonists, τ_s and τ_f were not appreciably influenced by membrane potential or by the mean amplitude of the agonist induced current, μ_I. For both agonists, however, the spectral ratio $S(0)_s/S(0)_f$ was found to increase at more positive membrane potentials and with increasing values of μ_I.

The results show that, at low agonist concentration and at negative membrane potentials, an additional fast kinetic process can be detected in the response of the SC cell membrane to GABA and muscimol. It is not yet clear whether this additional process, which is also seen in extracellular patch clamp records (Biophys. J., 33: 14a, 1981), reflects the presence of more than one type of GABA-operated ion channel in the SC cell membrane.

233.10 AN INCREASE IN K^+ CONDUCTANCE PRODUCES THE SLOW IPSP IN BULLFROG SYMPATHETIC GANGLIA. J.P. Horn and J. Dodd*. Dept. of Neurobiology, Harvard Medical School, Boston, MA. 02115.

In the 9th and 10th paravertebral sympathetic ganglia of the bullfrog, the slow IPSP is produced by cholinergic activation of muscarinic receptors on C-type neurons(Dodd & Horn, this volume). We have investigated the ionic mechanism of this synaptic potential by determining the IPSP's dependence upon membrane potential and ion concentration gradients. Conventional balanced bridge recordings were made with a single intracellular microelectrode.

At membrane potentials greater than -65mV, IPSP amplitude varies as a linear function of membrane potential, with a reversal potential near -100mV. However, the IPSP diminishes in amplitude as membrane potential is depolarized from -65mV. This complex behavior appears to be produced by the non-linear I-V relation of C cells. The decrease in IPSP amplitude with depolarization below -65mV always corresponds to a sharp drop in cell input resistance. This suggests that the IPSP is shunted by the non-synaptic membrane when the cell is depolarized beyond -65mV. The synaptic conductance increase implicit in these results was also investigated by passing 0.5 second constant current pulses before and during the IPSP. As expected, there was always a decrease in membrane resistance during the IPSP.

The IPSP reversal potential implies an increase in membrane conductance to K^+ and/or Cl^-. Since use of KCl filled microelectrodes and substitution of extracellular Cl^- by methylsulfate had no detectable effect on the IPSP reversal potential, Cl^- appears not to be involved. When extracellular K^+ was varied, the IPSP reversal potential changed in close agreement with values predicted by the Nernst equation. In summary, our results indicate that the muscarinic IPSP in bullfrog sympathetic neurons is produced by an increase in membrane K^+ conductance. (Supported by NIH grants NS13288 and NS07112 and a Harkness Fellowship to J.D.).

233.11 MONOSYNAPTIC MEDIATION OF THE MUSCARINIC SLOW IPSP IN SYMPATHETIC GANGLIA OF THE BULLFROG: INTRACELLULAR EVIDENCE. J. Dodd* and J.P. Horn (SPON: E. Furshpan). Dept. of Neurobiology, Harvard Medical School, Boston, MA. 02115.

In sympathetic ganglia of many species preganglionic nerve stimulation produces a fast suprathreshold nicotinic EPSP followed by a slow muscarinic IPSP. In the bullfrog there are conflicting hypotheses, based on extracellular recordings, as to the cellular location of the muscarinic receptors responsible for the IPSP. Libet and his coworkers[1] have proposed that the IPSP is produced by muscarinic excitation of catecholamine-releasing interneurons. They suggest that chromaffin-like SIF (small intensely fluorescent) cells are the interneurons. On the other hand, Weight and his colleagues[2] have made observations to the contrary and proposed that acetylcholine activates inhibitory muscarinic receptors located directly on sympathetic neurons. We sought to resolve this issue with intracellular recording.

In the 9th and 10th paravertebral ganglia of the bullfrog there are two types of principle neurons which are identifiable based on the latency and origin of their fast nicotinic inputs. The IPSP occurs only in long latency C-type neurons which are innervated by spinal nerves 7 and 8. Our experiments were done on C cells visualized with Nomarski optics. In confirmation of intracellular studies by others[3], we found that the IPSP was clearly present in normal drug-free preparations. In curare, totally atropine-sensitive IPSPs, of up to 35mV amplitude, were obtained and could be closely mimicked by iontophoresis of acetylcholine onto C cells. The best mimicry was achieved when the iontophoretic pipette was very close to the impaled cell. More important, when synaptic transmission was blocked in low Ca^{++}/high Mg^{++}, the acetylcholine response remained undiminished. These results demonstrate the presence of inhibitory muscarinic receptors on C neurons and indicate that the slow IPSP is monosynaptic. In light of these findings, it would be interesting to do similar experiments on mammalian sympathetic ganglia where the situation remains unclear. (Supported by NIH grants NS13288 and NS07112 and a Harkness Fellowship to J.D.).

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233.12 INTEGRATION OF PEPTIDERGIC AND MUSCARINIC SYNAPTIC TRANSMISSION IN AMPHIBIAN SYMPATHETIC GANGLION NEURONS. T. J. Sejnowski and S. W. Kuffler†. Dept. of Neurobiology, Harvard Medical School, Boston, MA 02115.

Intracellular recording from paravertebral sympathetic ganglia of the bullfrog has established that in the large B neurons two slow excitatory postsynaptic potentials are produced by stimulating two different nerves: a muscarinic response lasting up to 1 min, which is mediated by acetylcholine, and a noncholinergic response lasting up to 10 min, which is mediated by a peptide resembling luteinizing hormone-releasing hormone (LHRH) (Jan, Jan & Kuffler, Proc. Nat. Acad. Sci. 76: 1501, 1979; 77: 5008, 1980).

Using a single-electrode voltage clamp, we have confirmed previous reports that a conductance decrease occurs in most ganglion cells near the resting potential during the peptidergic response to nerve stimulation or external application of LHRH. However, when a cell was clamped at hyperpolarized potentials, conductance increases often occurred during the response; in these ganglion cells the peak amplitude of the peptidergic current increased with hyperpolarization. Among the cells in which only a decrease in conductance occurred, it was possible in a few cases to reverse the polarity of the peptidergic current at a hyperpolarized potential. More than one mechanism may contribute to the generation of the peptidergic response.

Conductance decreases and conductance increases also occurred during the slow muscarinic responses. In the same cell the muscarinic and peptidergic responses had the same voltage dependence and were accompanied by the same conductance changes.

The similarity between the muscarinic and peptidergic responses, despite their different time courses, suggests that they are produced by similar mechanisms. Their interaction was examined to test whether the mechanisms are shared. When evoked together, their combined currents were generally less than the sum of the individual currents. Furthermore, during a prolonged saturating response to bethanechol, a muscarinic agonist, the response of a cell to LHRH was completely blocked, and conversely, a saturating response to LHRH completely blocked the effect of bethanechol. Since the muscarinic and peptidergic receptors are independently blocked by pharmacological agents, the interaction between the two responses occurs at a stage beyond activation of the receptors.

†deceased (Supported by NIH grant NS 13288)

233.13 VERAPAMIL, A CARDIAC "CALCIUM CHANNEL BLOCKER" PROMOTES DESENSITIZATION OF ACETYLCHOLINE RECEPTORS. ANGELES B. RIBERA* AND WILLIAM L. NASTUK, DEPT. OF PHYSIOL., COLUMBIA U., N.Y.C., 10032

Calcium ion (Ca^{++}) influx increases across the agonist-depolarized post-junctional membrane (PJM). Nastuk and Parsons (J Gen Physiol 56:218, 1970) postulated that desensitization (DS) of the acetylcholine receptor (AChR) at the frog neuromuscular junction involves the intracellular accumulation of Ca^{++}. Thus, blockage of Ca^{++} influx would be expected to inhibit DS. On this basis, we examined the effects of Verapamil (Ver), an antagonist of Ca^{++} channels in cardiac muscle, on DS of the AChR.

DS was assayed by measuring the decline in depolarization of the PJM produced during the repetitive ionophoretic application of carbachol (carb). The standard test was a 1 Hz 45 sec train of 10 msec duration carb pulses. In preliminary investigations with 10 mM Ca^{++} Hepes-Ringer bathing a frog sartorius muscle preparation a 25% DS level was achieved. Contrary to expectations, the addition of 10 µM Ver to the bathing medium increased the DS level substantially. With the Ca^{++} concentration reduced to 1.8 mM the carb train did not produce measurable DS. Under these conditions, following the addition of 10 µM Ver the DS amounted to 40-50% with a $t_{1/2}$ of 2-3 sec. Recovery from DS was 90% completed in 5 sec. 10^{-4} µM Ver did not decrease the mepp amplitude, block nerve evoked neuromuscular transmission or decrease PJM sensitivity as measured by single iontophoretic carb pulses. Apparently AChR activation is necessary for 10 µM Ver to manifest its effects. The $t_{1/2}$ and DS level were not altered by an increase in the iontophoretic current amplitude, but an increase in the current duration to 50 msec led to a decrease in the $t_{1/2}$ to less than 1 sec and an increase in the DS level to 80%. In the range of 1-50 µM Ver these effects were concentration dependent.

Our paradoxical results with Ver can be understood if one assumes that Ver binds to sites exposed during AChR activation and thereby decreases single channel ionic conductances.

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234.1 LIGHT-INDUCED VOLTAGE FLUCTUATIONS IN BARNACLE PHOTORECEPTORS, Ehud Kaplan*, A. Mauro* and S. Poitry* (SPON: Frank Brink). The Rockefeller University, N.Y., N.Y. 10021.

In dim light, the membrane potential of many photoreceptors exhibit discrete waves (quantal bumps). These waves are believed to result from single photon absorptions, and summation of them produces the receptor potential. In bright light the mean frequency of the discrete waves increases, but the mean amplitude decreases, thus providing a mechanism for light adaptation.

We studied the lateral eye photoreceptors of the barnacle, Balanus Eburneus, to find out whether they, too, show discrete waves. They did not. Even when the eye was fully dark adapted, we never saw discrete waves. This result confirms the reports by previous investigators (S.R. Shaw, J. Physiol. (1972), 220, 145; A.J. Hudspeth and A.E. Stuart, J. Physiol. (1977), 272, 1; A. Fein, personal communication). However, dim light induced small (<1 mV) fluctuations, which increased with light intensity up to moderate levels. Voltage clamp studies are under way to obtain light-induced current fluctuations, which should yield a spectral analysis uncompromised by the appreciable membrane time constant (≈250 ms.). Meanwhile a spectral analysis of the light-induced voltage fluctuations was made as a first approximation and showed that the frequency components were predominately below 10 Hz. The power spectrum of the fluctuations did not match the frequency response deduced by a Fourier transform analysis of the response to dim flashes, namely, the responses were too slow to account for the spectral density of the voltage noise. This suggests that more than one process is involved in generating the light-induced voltage fluctuations.

234.2 VOLTAGE-ACTIVATED AND CALCIUM-ACTIVATED CURRENTS STUDIED IN SOLITARY ROD INNER-SEGMENTS FROM THE SALAMANDER RETINA. C. R. Bader, D. Bertrand* and E. A. Schwartz*. Dept. de Physiologie, Ecole de Médecine, 1211 Geneve 4, Switzerland and Dept. of Pharmacological and Physiological Sciences, University of Chicago, Chicago, IL 60637, U.S.A.

Solitary inner-segments of rod-photoreceptors were obtained by the enzymatic dissociation of tiger salamander (Ambystoma tigrinum) retina. Their voltage-dependent currents were studied using the voltage-clamp technique (Bader, MacLeish and Schwartz, 1979, J. Physiol. 296:1-26). These solitary inner-segments were continuously superfused with a medium to which combinations of drugs could be added to selectively isolate individual currents. Five currents were identified: 1) a voltage-dependent K+ current, blocked by tetraethylammonium, with a reversal potential at -72 mV; 2) a current responsible for inward rectification, blocked by extracellular caesium and carried by Na+ and K+, with a reversal potential at -32 mV; 3) a regenerative calcium current, blocked by cobalt, with a null potential at +45 mV; 4) a calcium-activated potassium current, blocked by intracellular EGTA or intracellular caesium; 5) a calcium-activated anionic current, carried in part by chloride, blocked by intracellular EGTA, with a reversal potential at -17 mV. These currents can all be activated in the physiological range of voltage in which vertebrate photoreceptors function.

234.3 VOLTAGE- AND TIME-DEPENDENT SUPPRESSION OF THE LIGHT RESPONSE IN VERTEBRATE RODS. Stuart A. Lipton* (SPON: D.D. Potter). Dept. of Neurobiology, Harvard Medical School, Boston, MA 02115.

Solitary rods from the tiger salamander were dissociated with papain, maintained in culture for several days, and retained their light sensitive properties. Responses in voltage and voltage-clamp were studied prior to and following a hyperpolarizing step that ranged from 50 to 95 mV from the resting potential. Immediately after the step, the light response to a saturating flash was decreased in amplitude but recovered with time. The extent of the decrease seemed to depend on the size of the hyperpolarization and fit the Boltzmann relation; for the largest step, there was no detectable response to light for 0-2 secs. Generally, 30-40 secs were needed for complete recovery of the original response. No decrement of the light response was observed with hyperpolarizations of less than 50 mV from rest or with depolarizing pulses. Concurrent with the depression of the light response after a hyperpolarizing step, most voltage records showed a depolarizing overshoot. In voltage-clamp this was manifest as a depolarizing tail current. This tail current was of similar duration to the decrease in light response and was eliminated by a bright flash.

To decide whether the tail current and the decrease in response amplitude were related to a voltage-sensitive current involved in the light response or to another conductance which was sequentially triggered after photostimulation, TEA, Co, and Cs were used to suppress the voltage-dependent currents of the inner segment (Bader, Bertrand & Schwartz, 1981, Neurosci. absts). Under these conditions and in low Ca^{2+} (MacLeish, Schwartz & Tachibana, 1981, Neurosci. absts), the tail current was blocked by illumination and had a reversal potential between 0 and 10 mV. If the only effect of a hyperpolarizing step were the increased conductance and the tail current, one might see no change or even an increase in the amplitude of the light response during this period (since more channels may be available for blockade). Instead, as described above, smaller light responses were observed. The mechanism for this is unclear; there may be a voltage-dependent desensitization of the channels to blocking particles or a decrease in availability of blocking particles. Because Ca^{2+} has been proposed as the blocking particle, it was of interest to examine its role in these phenomena. When impaled with EGTA-filled electrodes, rods in both normal Ringer's and low Ca^{2+}, TEA, Co, Cs cocktail exhibit little or no tail current or inhibition of the light response following hyperpolarization. Since voltage-dependent suppression does not occur with EGTA, and analysis by the Boltzmann relation predicts that a valency of 2 is associated with the decrement in the light response after hyperpolarization, the data might suggest that Ca^{2+} interacts with the channels responsible for the light response.

234.4 VOLTAGE AND TIME DEPENDENCE OF THE GENERATOR CURRENT IN SOLITARY RODS FROM THE SALAMANDER RETINA. P. R. MacLeish, E. A. Schwartz* and M. Tachibana. Dept. of Neurobiology, Harvard Medical School, Boston MA 02115 and Dept. of Pharmacological and Physiological Sciences, University of Chicago, Chicago IL 60637.

Solitary rod photoreceptors were obtained by enzymatic dissociation of the tiger salamander (Ambystoma tigrinum) retina. Voltage and time dependent currents that normally flow through the inner segment were suppressed by a mixture of pharmacological agents (Bader, Bertrand and Schwartz, 1981, Neurosci. Abstr.). The current entering the outer segment and controlled by light, the generator current, was studied with the voltage-clamp technique (Bader, MacLeish and Schwartz, 1979, J. Physio. 296, 1-26). When the concentration of extracellular calcium was greater than 2 mM, the generator current was voltage dependent but showed little time dependence as previously reported (Bader, MacLeish and Schwartz, 1979, J. Physiol. 296, 1-26). When, however, the concentration of extracellular calcium was less than 0.1 mM, the generator current was both voltage- and time-dependent. The magnitude of the time-dependent part depended on the holding potential and was greater at -70 mV than at -30 mV for small voltage steps. The current-voltage curve, measured 100 msec after the voltage was stepped from -70 mV to a new value within the physiological range, had a shallow negative resistance region. The entire current reversed polarity between 0 and +5 mV.

In the absence of the above pharmacological agents and in the presence of a concentration of 3 mM external calcium, the time course of the voltage response to a flash differed from that of the current observed in voltage clamp. The difference was attributed to voltage- and time-dependent currents that were not controlled by light (Bader, MacLeish and Schwartz, 1979, J. Physiol. 296:1-26). In the presence of the pharmacological agents that block these voltage- and time-dependent currents and with a concentration of external calcium of 0.1 mM, the time course of the voltage response to a flash also differed from that of the current observed in voltage clamp. In this case, the difference is attributed to the voltage- and time-dependent properties of the generator current that are increased in size when external calcium is lowered in concentration.

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234.5 EFFECTS OF D.C. MAGNETIC FIELDS ON VERTEBRATE PHOTORECEPTION
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Recent work has shown that rhodopsin has a finite diamagnetic anisotropy that results in physical orientation of isolated rod outer segments by external d.c. magnetic fields of 1-2 Kgauss. Since bleaching reduces this effect, the summed anisotropy of oriented rhodopsin molecules is a postulated mechanism. The physiological significance of these effects is unknown.

We are using d.c. electromagnetic fields (up to 10K gauss) to study this question electrophysiologically. Extracellular recordings from the in vitro turtle eyecup during exposure to magnetic fields are being used to quantitate any effects. In brief, we are substituting d.c. magnetic fields for background illumination and monitoring the changes in retinal responses and sensitivity in the ERG a-and b-wave intensity-response functions. Both rod-cone (Chelydra) and all-cone (Pseudemys)retinas are being used in order to study any photoreceptor cell differences in magnetic field susceptibility.

Our results so far indicate:(1) external d.c. magnetic fields have a large suppressive effect (25-75%) on the response amplitude of both photoreceptor types to light flashes during exposure; (2) this response compression is not manifest in any subsequent reduction of retinal sensitivity;(3) the phase relationship between the time of exposure and the diurnal light:dark(L:D) cycle is the most important, and surprising, factor in these results; and (4) while our earlier data showed clear response compression at 2.5K gauss and higher, more recent data suffest that this effect is seen as low as 25-50 gauss and saturates around 100 gauss.

These results indicate that external d.c. magnetic fields have a significant effect on vertebrate phototransduction and that very low field strength exposures act, in some unknown way, as if they are shunting the transmembrane current fluxes due to photoisomerization. The central importance of the diurnal L:D cycle implicates a metabolic aspect hitherto unsuspected. Coupled with the low field strength effects and the fact that both rod and cone photoreceptors are affected, this suggest that the diamagnetic anisotropy of rhodopsin is not crucial for these effects. (Supported by the Department of Energy).

234.6 TRACKING VARIABLE WAVEFORM AND AMPLITUDE ACTION POTENTIALS WITH A MICROCOMPUTER SYSTEM BASED UPON A NOVEL ARCHITECTURE.
Robert C. Gesteland and Dennis L. Director*. Northwestern University Evanston, IL 60201.

Extracellularly recorded action potentials from single neurons often have large variations in amplitude and waveform. These variations are prominent in cells with small axon diameters such as olfactory receptors. In such cells the locus of spike origin varies with firing rate and the stimulus often drives the cell to depolarization block. Activity of one cell cannot be separated from that of neighbors with the usual techniques of amplitude selection and derivative analysis (counting phases of the field potential). However, the waveform of the field potential from any particular cell does change in a regular way for successive spikes. Activity of the cell can be tracked if a faithful representation of the waveform is recorded. Film is adequate to the task. However the quantity required and the analysis effort are prodigious. Digital recording of the waveforms of the delayed signal initiated by triggers from an amplitude selector operating on the real time signal is much more efficient. Attempts to use commonly available laboratory computers for this recording task are frustrated by inability to acquire data rapidly enough or by failure to store the data as rapidly as it is acquired. Even a fast machine dedicated to data acquisition may not suffice because transferring acquired data to disc cannot occur while the bus is dedicated to acquisition. Specialized machines can be constructed using dual-ported memory as the acquisition buffer. Not only is this memory expensive, it also must be rigidly tied to the acquisition device. The solution to this problem lies in an innovative architecture. An inexpensive laboratory computer with a dual bus can be used to acquire and store real time data from one or more sources at high sampling rates. We use the DEC LSI-11 microcomputer. The Q bus used for intersystem communication is split into a processor bus and an acquisition bus. An arbiter connected to both decides when to allow information transfer between the two. The A/D converter runs at full speed, the processor runs independently of data acquisition, and the system disc can store the data from the acquisition buffer as quickly as if it were in the system main memory. All commercially available LSI-11 peripherals can be used without modification. The computer configured in this way remains as a general purpose machine in the laboratory and can be used for data analysis, document preparation and other standard computing tasks. This architecture improves throughput by about a decimal order of magnitude.

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234.7 SURFACES OF ROD PHOTORECEPTOR DISC MEMBRANES.
Dorothy Roof, Juan Korenbrot, and John Heuser*. Dept. of Biochemistry, University of California, San Francisco, and Dept. of Physiology, Washington University, St. Louis, MO.

The surfaces of disc membranes in rod photoreceptor cells from the toad, Bufo marinus, were exposed by quick freezing, followed by freeze fracture and deep etching. The luminal surface of the disc membrane exhibits a rough texture which appears to be composed of 6 nm. particles tightly packed at a density of about 30,000/um^2. These dimensions suggest that the texture arises from protrusions of the integral membrane protein, rhodopsin, into the intradisc space. The cytoplasmic surface of the disc membrane exhibits a collection of much larger bumps (8-12 nm.) present at about 1/15 the concentration of rhodopsin. These large particles can be removed by washing the membranes in the dark with 0.1 iso-osmotic medium plus EDTA; biochemically this treatment depletes the membranes of GTP binding protein (GBP) leaving cGMP phosphodiesterase (PDE) intact. The large particles can be partially returned to stripped membranes after reconstitution with a GBP-enriched supernatant. In contrast, the density of large particles does not correlate with the presence or absence of PDE. Thus, the large particles probably represent GTP binding protein, one of the major peripheral proteins known to associate with the cytoplasmic surface of discs. In addition, the large particles are missing within a narrow 30 nm. band around the disc rim, and the rims are connected by wisp-like filaments stretching from disc to disc, across disc incisures, and from discs to plasma membrane.

235.1 MOLECULAR MECHANISM OF SWEET TASTE: EVIDENCE FOR THE IMPORTANCE OF HYDROGEN BONDING. S.S. Schiffman. Dept. of Psychiatry, Duke University Medical Center. Durham, N.C. 27710, U.S.A.

Two lines of evidence point to the importance of units capable of intermolecular hydrogen bonding (i.e. AH-B systems) for the sweet taste. First, application of the psychophysical method of cross adaptation revealed that more than one type of receptor site mediates the perception of sweetness. Fourteen stimuli, seven artificial sweeteners varying widely in chemical structure as well as seven sugars, were cross adapted with one another. The best mutual cross-adaptation occurred for stimuli with identical AH-B site types, i.e. acetosulfam and sodium saccharin. In addition, taste detection thresholds for 11 sweeteners varying widely in chemical structure were determined for young and elderly subjects. For both groups, sweeteners with the lowest detection thresholds tended to have the greatest number of units (AH-B systems) capable of intermolecular hydrogen bonding. Use of the method of magnitude estimation with 10 sweeteners revealed that the elderly perceived less growth in intensity with increasing concentration than young subjects. The slopes of the psychophysical functions relating concentration and perceived intensity were flatter in all cases for elderly subjects; the mean ratio, slope(young)/slope(elderly), was 2.06. The largest relative decline in slope was found for those stimuli with the greatest number of possible AH-B types, suggesting that the possibilities for concerted intermolecular hydrogen bonding may decline with age.

235.2 ELECTRO-CHEMICAL STIMULATION OF TASTE. T.C. Pritchard and C. Pfaffmann. Rockefeller Univ., New York, NY 10021

Electrical polarization of the tongue elicits gustatory activity, but its mechanism has been in dispute. On the basis of multi and single unit experiments, Pfaffmann and Pritchard (Olfaction and Taste VII, 1980, 175-178) have proposed that electric taste is the product of an iontophoretic process. Thus, electric taste would be expected to have many properties in common with fluid stimulation of the tongue. The present investigation compares chorda tympani (CT) single unit responses evoked by chemical and electrical stimulation of the tongue.

Single units of the CT were isolated by microdissection in nembutalized golden hamsters. The anterior half of the tongue was drawn into a special flow chamber which permitted either chemical (i.e. fluid) or electrical stimulation. The electrical polarizing current was passed through a .001 M Na saccharin solution which bathed the tongue thereby allowing either Na^+ cations or the saccharin anion to be iontophoresed depending upon the polarity of the current.

The results support the hypothesis that electric taste is a special form of chemical stimulation of the tongue at the current levels employed. Single units which were sensitive only to electrolytes (NaCl or HCl) responded to stimulation by the anode (passing Na^+) but not to that of the cathode (passing saccharin). Other single units exclusively sensitive to sucrose responded to cathodal but not anodal stimulation. Units which responded to both electrolytes and sucrose responded to anode and cathode. Somatosensory units isolated from the lingual nerve unresponsive to gustatory stimulation could not be driven with this electric taste paradigm.

The threshold for cathodal stimulation (.25 $\mu A/cm^2$) is about ten times higher than that observed for the anode. Response latencies for anodal and NaCl stimulation approach 30 ms whereas for cathodal and sucrose stimulation the latencies consistently exceed 300 ms. In general single units which responded to low intensity electrical polarization also responded to fluid stimulation at very weak concentrations. The converse was true for higher threshold fibers.

The striking similarity of electrically and chemically induced gustatory neural activity has already enabled its use as a probe during CNS exploration. Further research will assess the potential of electric taste for studies of neural coding, receptor biophysics and clinical diagnostics.

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235.3 THE DISTRIBUTION OF GUSTATORY NERVES WITHIN THE NUCLEUS OF THE SOLITARY TRACT IN RAT. R.B. Hamilton and R. Norgren. The Rockefeller University, 1230 York Avenue, New York, NY 10021.

Gustatory receptors occur in five concentrations in the oral cavity. Fungiform papillae on the anterior tongue are innervated by the chorda tympani nerve (CT). Foliate and circumvallate papillae on the posterior tongue receive gustatory afferent axons via the lingual branch of the glossopharyngeal nerve (LIX). The taste buds on the palate and larynx are innervated by the greater superficial petrosal (GSP) and superior laryngeal nerves (SLX), respectively. These nerves primarily terminate in the nucleus of the solitary tract (NST), however, the relationship of the terminal distributions to one another is not well described. We applied crystals of horseradish peroxidase (HRP) to the cut central end of CT, GSP, LIX, and SLX, as well as to the otic ganglion (OG), the lingual nerve (LV) and the cervical vagus (X). After 60-72 hours survival, the tissue was processed using modified tetramethyl benzidine procedures for visualizing transganglionic anterograde transport of HRP (Kalia & Mesulam, 1980). The distribution of anterior HRP reaction product was as follows. CT terminals first appear just dorsal to the spinal trigeminal nucleus oralis at the level of the genu of the facial nerve, become heaviest in lateral NST 400u caudally, and finally end 1400u after their first appearance. GSP label begins at the level of heaviest CT labeling, becomes most extensive in NST 800u further caudally and finally ends in dorsolateral NST 1600u after it begins. Afferent axons that travel through OG overlap the densest areas of both CT and GSP termination. LIX terminals first appear in lateral NST just rostral to the densest CT label, but fill lateral NST approximately 1000u further caudally. Caudal to this level LIX terminals in lateral NST diminish rapidly, but small neurons clustered within the tract and in the dorsal third of medial NST remain heavily labeled. SLX terminates sparsely in lateral NST at levels where LIX label is heaviest. SLX label only becomes heavy, however, over the densely packed neurons situated within the caudal solitary tract. Label from X axons appears very sparsely in the caudal half of lateral NST, but becomes very dense in the medial division just rostral to the obex, filling the commissural nucleus on both sides. Axons from LV terminate in nucleus oralis just lateral to the CT distribution in NST and hugs its lateral edge until the roots of X reach the nucleus. At this level many LV axons invade lateral NST and terminate extensively just rostral to obex. A few terminals reach the dorsal third of medial NST in the same area innervated by LIX axons. Axons innervating gustatory receptors in the anterior tongue (CT) and palate (GSP) overlap considerably in rostral NST. Those innervating the posterior tongue (LIX) and larynx (SLX) terminate caudally and do not overlap substantially.Supported by NSF BNS80-06444/NIH NS01050

235.4 EARLY EVENTS IN THE FORMATION OF OLFACTORY AXONS. Albert I. Farbman, Richard E. Heller and Diane Scholz.* Department of Anatomy, Northwestern University, Chicago, Illinois 60611.

During differentiation of olfactory receptor cells, the proximal cellular processes which become the axons must penetrate the barrier of the epithelial basement lamina as they grow toward the presumptive olfactory bulb. This morphological study, using Golgi impregnation and transmission electron microscopy, considered the following questions: 1) which cells are involved in enabling the growing receptor cell axons to penetrate the basement lamina? 2) what is the origin of olfactory nerve Schwann cells? Olfactory mucosa from fetal rats on days E15, E16 and E17 (E1 = day when mother is sperm positive) were studied. Golgi material reveals that before the receptor cell's proximal process leaves the epithelial compartment, it forms a triangular, foot-like extension, the base of which lies on the basement lamina and the apex is directed toward the perikaryon. Electron micrographs of these foot processes show that in their early stage they contain abundant free ribosomes and a moderate amount of dense filamentous substance. Later, the number of ribosomes is significantly diminished and an increased amount of filamentous material imparts an electron density to the cytoplasm. Those proximal processes which have penetrated the basement lamina are uniformly narrow (0.1 - 0.3 μm in diameter), contain axially oriented microtubules and significantly less filamentous substance than the foot processes. Golgi preparations show these axons to be narrow with few varicosities. Axons first reach the ventral aspect of the presumptive bulb on day E15. Islands of cartilage, the precursor of the cribriform plate, first appear between bundles of axons on day E16. Schwann cell processes accompanying olfactory axons have been seen as continuous cytoplasmic strands crossing from the epithelial compartment into the lamina propria. Pericytes and endothelial cells of blood vessels in the region of the basement lamina send cytoplasmic processes into the epithelial compartment. It is tentatively concluded that 1) both epithelial and connective tissue elements contribute to the alteration of the basement lamina, thus enabling the olfactory axon to exit from the epithelial compartment; 2) Schwann cells originate in the olfactory epithelium and accompany the growing axons as they leave the epithelium.

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235.5 CONNECTIONS OF THE MAIN OLFACTORY BULB (MOB) IN MICE. G.D. Adamek & M.T. Shipley. Graduate Program in Neuroscience, Northwestern University, Evanston, IL 60201 and Department of Cell Biology & Anatomy, Northwestern Univ. Medical School, Chicago, IL 60611.

The odor of a strange conspecific male causes pregnant mice to abort. Although this effect depends upon the olfactory system, the central connections of the mouse olfactory bulb have never been systematically studied. Thus it is unclear whether this salient, odor-dependent reaction is reflected in this species' neural circuitry. We have determined the connections of the MOB using antero- and retrograde labeling with 1-2% wheat germ agglutinin-HRP injections confined to the MOB in BALB-C mice.

Efferents: The MOB projects ipsilaterally to the accessory olfactory nucleus (AON), the ventral and dorsal portion of the hippocampal rudiment (V and DHR), the entire extent of the piriform cortex (PC) and olfactory tubercle (OT), the anterior cortical (ACo) and posterolateral cortical (PLCo) amygdaloid nuclei, parts of the lateral entorhinal cortex (LEC) and the nucleus of the lateral olfactory tract (nLOT). The projection to the PC extends dorsally into the insular cortex. A projection to the contralateral AON was observed. Afferents: Neurons projecting to the MOB were found ipsilaterally in all divisions of the AON except the external, the VHR, all the PC, part of LEC, nLOT, vertical and horizontal limbs of the diagonal band, preoptic area, several sub- and hypothalamic areas, 3 raphe nuclei and the locus coeruleus (LC). A few labeled cells were seen in the hippocampal formation. All divisions of the contralateral AON project to the MOB.

The pattern of MOB connections in the mouse is similar to that of several other species but the hypothalamic, LC and raphe inputs to the mouse MOB appear to be more prominent than in the rat (de Olmos et al, J.C.N. 1978) and hamster (Davis et al, Br. Res. Bull. 1979); subthalamic inputs to the bulb have not been described in any species. The connections of MOB in the rat and mouse are more extensive than those reported for the hamster (Davis et al, op cit). These may represent species differences or the fact that relatively large MOB injections are needed to demonstrate certain circuits. Injections in AON rule out large MOB injections that may lead to ambiguity in the MOB connections reported here.

Studies of the accessory olfactory bulb and the AON are underway to determine if the mouse's central olfactory pathways differ from those species that do not exhibit odor-dependent abortion. Potential differences between male and female mice will also be examined to see if the pregnancy blocking effect has a demonstrable anatomical basis.

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235.6 ARE THE OLFACTORY BULB EFFERENT AXONS TOPOGRAPHICALLY ORGANIZED? William S. Seibly*, George Maeda*, and T. J. Willey. Dept. of Physiology, Loma Linda Univ., Loma Linda, CA 92350.

Olfactory bulb efferents in the cat pass caudally over the prepyriform cortex in a broad fiber sheet called the Lateral Olfactory Tract (LOT). The LOT can be visually traced to the periamygdaloid cortex where the bundle of fibers becomes narrow and compact. Near the bulb the LOT circumferentially bounds the olfactory peduncle. A multielectrode monitoring array with equi-spaced probes at one mm intervals was constructed. The array was built to conform to the curved surface and geometry of the prepyriform cortex including the surface projecting pathway of the LOT. Stimulating electrodes were positioned in the olfactory bulb to straddle the mitral cell layer and to activate dorsal, lateral, ventral and medial bulbar aspects. Action potentials were recorded from the array as they propagated past each electrode and computer processed by averaging. Surface contour maps and potential profiles were developed to test the hypothesis for topographical representation of mitral cell projections in the LOT. The potentials were spatially represented for each sampling interval (35 μ sec) for the entire conducted action potentials. Antidromic conduction conditions were achieved by stimulating on the multi-electrode matrix and recording from a fixed electrode across the mitral cell dipole in the olfactory bulb. Conduction velocities and spatial and temporal dispersions were also computed from these data.

The velocity of nerve impulses decreased by 50% between the rostral to caudal aspects of the LOT. Velocities ranged from 20 m/sec at the peduncle to about 10 m/sec at the posterior cortex. Computed "hot-line" trajectories for the axis of bulbar projection reveal orderly topographic relationships at the peduncle. By mid cortex or about 5 mm posteriorly this projection is diffuse. These findings support the anatomical data of Price and Sprich (1975) and Devor (1976) showing the LOT as a flattened representation of the bulb with considerable intermingling of the axons originating in the dorsal and ventral. Sensory template analysis disputes the need for neural images from bulb to cortex to be precisely topographically organized in contrast to other sensory systems.

235.7 GUSTATORY SPECIFICITY OF PREABSORPTIVE INSULIN SECRETION. Kent Berridge* and Harvey J. Grill (SPON: L. Hurvich). Dept. Psychol. and Inst. Neurol. Sci., U. Pennsylvania, Phila., Pa. 19104.

Preabsorptive insulin release (PIR) occurs prior to enteric absorption when palatable nutrients are taken or infused into the mouth. While both the palatability and the caloric significance of tastes have been shown to be important in the elicitation of this neuroendocrine reflex, the characteristics of the adequate stimulus for the PIR remains unknown. This study examined the ability of a wide variety of novel palatable nutrients and nonnutritive substances to elicit a PIR. Nine sugars and sugar alcohols (glucose, fructose, sucrose, maltose, galactose, mannose, sorbitol and glycerol: all .8 M; lactose: .5 M), 6 amino acids (L-phenylalanine, L-arginine, L-leucine, L-lysine, L-proline, L-tyrosine: .05 M), two oils (sunflower and mineral), a complex nutrient (fresh rat milk), and Na saccharin (.007 M) were tested. In addition, the sensitivity of the PIR to taste concentration was examined in a study of 4 concentrations of glucose (.17, .125, .55, 2.76 M). Each experiment tested the PIRs of 5 rats to 4 novel tastes. One taste was always .8 M glucose, which served as a comparison standard. Each rat received one taste presentation per day, and received all 4 tastes in counterbalanced order. No rat was used in more than 1 experiment, and each experiment was replicated 1-3 times. Procedure: 5 male rats were implanted with oral and jugular cannulae 1 day prior to testing. Test: 4 h food-deprived rats were habituated to the test chamber and a 200 ul jugular blood sample was taken. One ml of a novel taste solution was infused into the mouth over 1 min, and consummatory responses and quantity ingested were recorded. Blood samples were taken each min for the first 5 min, and every other min for the next 4 (Berridge, Grill, & Norgren, 1981). Blood samples were centrifuged, and the plasma radioimmunoassayed for insulin and assayed for glucose. We found that: a) sugars and sugar alcohols were far stronger PIR elicitors than the other tested substances, b) the ability of sugars and sugar alcohols to elicit a PIR was unequal (equimolar glucose, maltose, & glycerol elicited high amplitude PIRs (.7 - 1 ng/ml), sucrose & sorbitol elicited intermediate responses (.4 - .6 ng/ml), and fructose, mannose, lactose, & galactose produced little or no PIR), and c) the PIR magnitude was correlated with stimulus concentration. These differences occurred in spite of the very similar consummatory responses evoked by the different tastes. These and earlier data suggest that neither the palatability nor actual nutrient value of a taste stimulus predicts PIR magnitude. Rather, some complex and as yet unspecified property of gustatory afferent stimulation determines the amplitude of the PIR. (Supported by NIH grant AM-20397 and by the Diabetes Center of the University of Pennsylvania.)

236.1 VISUAL ACUITY DEFICITS IN GOLDFISH WITH COMPRESSED RETINOTECTAL PROJECTION: COMPARISON OF HORIZONTAL AND VERTICAL GRATINGS. Dean Yager and Martha Romeskie. State University of New York, College of Optometry, New York, New York 10010.

Visual acuity for vertical and horizontal square-wave gratings was measured by a technique of conditioned suppression of respiration. Normal baselines were established for the two eyes separately; acuity was better for vertical gratings than for horizontal gratings. Next, one optic nerve was crushed in the orbit, and the contralateral caudal half-tectum was ablated. Sensitivity to light in the operated eye-tectum pair returned within 3-4 weeks following surgery. Acuity was less than 0.5 cycle per degree until about three months later, at which time there was a rapid increase in acuity.

Acuity for vertical gratings did not return to normal values; there was a permanent deficit, confirming results which have been reported earlier. For horizontal gratings, acuity for the operated eye-tectum pair also failed to return to normal. These results are consistent with the notion that compression of the retinotectal projection in the rostro-caudal tectal axis produces receptive fields of post-sysnaptic tectal cells that are expanded in both the temporal-nasal and superior-inferior directions of the visual field. In addition, post-synaptic tectal recording should reveal a greater sensitivity to vertically-oriented stimuli than to horizontally-oriented stimuli in normal as well as in compressed-projection fish.

236.2 GRADIENTS OF SYNAPTOGENESIS AND CELLULAR MATURATION IN THE PRIMATE SUPERIOR COLLICULUS DO NOT CORRESPOND TO GRADIENTS OF NEURONAL GENERATION. M.L. Cooper and P. Rakic, Sect. Neuroanatomy, Yale University School of Medicine, New Haven, Ct. 06510

The timing of cell origin and order of neuronal settling have been frequently suggested as determinants of the sequence of cell maturation and of neuronal connectivity. To test this hypothesis in the rhesus monkey, we analyzed spatial gradients of cellular maturation, including synaptogenesis, in the superficial grey layer (SGS) of the superior colliculus (SC) at various embryonic (E) ages. At E47, most neurons destined for the developing superficial layers have been generated and have attained their final relative positions (Cooper & Rakic, Neurosci. Abstr., '80). These cells are contained in a uniform band of small, darkly Nissl-stained neurons situated at the outer edge of the SC. By E54, the superficial band is no longer uniform in appearance across its mediolateral (ML) or anteroposterior extents.Rather, superficial cells in the middle of the rostral pole of the SC are considerably larger and stain paler than the more medially or laterally located cells.This sharp middle-peripheral difference in cell staining becomes progressively less pronounced at more caudal levels of the SC. Such dissimilarities in Nissl-staining characteristics are also evident at E61, but have largely disappeared by midgestation (E83). The presence of regional differences in the tempo of SGS maturation was confirmed and amplified by quantitative EM analysis in E54 and E61 specimens. We made probes consisting of overlapping electron micrographs through the outer 200μ (the approximate thickness of the SGS at these ages) of the medial, middle, and lateral regions of the SC at each of three coronal levels. Point-counting of EM montages through the medial and lateral portions of the anterior SC revealed consistently greater amounts of extracellular space than were present in the middle region. The amount of neuropil (the cross-sectional area excluding extracellular space, somata, radial glia and blood vessels) was smaller medially and laterally than in the middle, as were the densities of synapses, both in terms of synapses/μ^2 of total cross-sectional area and synapses/μ^2 of neuropil alone. (The synaptic density is fairly constant across the ML extent of the adult anterior SGS.) These differences in synaptic density and neuropil development were no longer evident at the most posterior coronal level. Thus, although our previous ^{3}H-thymidine studies (Cooper & Rakic, '80) showed no significant ML variation in the time of neuron origin in the superficial SC, the middle region begins to mature much earlier than the more medial or lateral areas. This finding demonstrates that in the primate, SC gradients of neuronal maturation (as defined by synaptogenesis,development of neuropil and light microscopic appearance) do not correspond to those of cellular proliferation. (Supported by EY-02593).

236.3 ALTERED INDIVIDUAL TERMINAL ARBORS IN COMPRESSED PROJECTIONS TO THE SUPERIOR COLLICULUS. George M. Sachs* and Gerald E. Schneider (SPON: R. Held), Dept. Psychology, M.I.T., Cambridge, MA 02139.

After ablation of a caudal portion of the superior colliculus (SC) in newborn hamsters, projections from the contralateral retina compress onto the remaining portion of the SC (Jhaveri and Schneider,'74, Anat. Rec., 178,383; Finlay,et al.,'79,Nature, 280,153). By visualizing single axons in these compressed projections, we investigated alterations in their terminal arborizations.

Small amounts of HRP were injected into the optic tract of normal adult hamsters and hamsters that had undergone caudal tectum ablations at birth. Parasagittal sections were reacted with a DAB procedure. Individual HRP-filled axons were traced into the SC and their arborizations were charted with the aid of a drawing tube. We focused our analysis on a type of axon which forms dense arbors in the upper portion of the stratum griseum superficiale. Previous work has indicated that such axons originate in the contralateral retina. In normal animals, fibers of this type show terminal arbors of consistent dimensions, extending 90-150 μm in the rostrocaudal axis. After the early caudal tectum lesions, arbors of such axons showed significant reduction in their rostrocaudal extent. Occasionally, axons in early lesioned animals showed bizarre trajectories. Some fibers extended caudally into the SC only to loop back in an abrupt 180°turn and course rostrally, leaving the SC through the optic tract. Their ultimate termination could not be traced because of dark reaction product from the injection site. In the SC, these looping fibers sent out a thin collateral which distributed a small number of terminals in either stratum griseum superficiale or stratum opticum.

Udin and Schneider (Exp. Br. Res., in press) have found a reduction in the number of retinal ganglion cells participating in compressed retinotectal projections. The results of the present study show that compression also involves a reduction in the extent of individual terminal arbors, for at least some retinotectal axons. It is likely that removal of tectal tissue increases competition for terminal space, and that this heightened interaxonal competition serves to decrease the size of arborizations. Fibers with drastically reduced terminal arbors may be sustained by abnormal terminations in the diencephalon.

(Supported by NIH grant EY 00126)

236.4 WIDESPREAD BRANCHING OF RETINOTECTAL AXONS: TRANSIENT IN NORMAL DEVELOPMENT AND ANOMALOUS IN ADULTS WITH NEONATAL LESIONS. G.E. Schneider, L. Rava*, G.M. Sachs*, and S. Jhaveri*[1], Dept. of Psychology, M.I.T., Cambridge, MA 02139.

Brains of Syrian hamsters, aged 0-21 days postnatal, were prepared with Rapid Golgi methods and cut sagittally. Immature axons of the optic fiber layer of the superior colliculus (SC) were found to pass through a stage of multiple, widespread branching before the several types of single, dense terminal arbors characteristic of the adult (Sachs and Schneider, Soc. Neurosci. abstracts, 1980) became recognizable. Axons with widespread branching patterns first became prominent in 3-day-old animals.

Single axons in adult animals which had been subjected to partial tectal lesions on the first or second postnatal day were visualized by an HRP-filling technique (Ibid.). In cases of rostral- or mid-tectal damage, examples of axons with two distinct, widely separated end-arbors were found. Each of these arbors was smaller than most arbors found in similar studies of normal adult hamsters. In cases of early unilateral lesions of superficial tectal layers with simultaneous early removal of the ipsilateral eye, individual axons entering the damaged SC were followed across the midline to the intact superficial gray layer. A series of examples of such axons revealed multiple branching patterns with distinct, abnormally small end-arbors.

The single-axon descriptions fit studies of retinotectal topography in similar cases with early tectal lesions. In these studies, small retinal lesions were made in the adult to elicit anterograde degeneration, which was traced with the Fink-Heimer technique. Results showed that axons from single regions of the retina, if deflected from their normal trajectories, often terminated in one or more small patches in addition to a larger patch in the tectal region expected from electrophysiological mapping studies (Finlay et al.'79: JCN 183,721; Nature 280,153).

We conclude that during normal development, multiple widespread branches of single optic-tract axons within the superficial tectal layers progressively lose all branches except those forming a single terminal cluster. This process can be disrupted by neonatal lesions so that more than one cluster of terminals can persist into adulthood. The normal narrowing of the end-arbor may be influenced by competitive interactions which are altered after the early lesions. However, when multiple end-arbors form, the total number of terminals does not appear to be appreciably expanded; thus, each end-arbor is abnormally small.

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236.5 RELATIONSHIP BETWEEN DEVELOPING OPTIC AXONS AND THEIR TARGET CELLS IN THE SUPERFICIAL SUPERIOR COLLICULUS OF THE MOUSE. M.A. Edwards,G.E.Schneider, and V.S. Caviness, Jr. MIT, Cambridge, MA and E.K. Shriver Center, Waltham, MA (Sponsor:Roger Williams)

The earliest afferent axons from the retina to the superior colliculus of the rodent are largely concentrated at the surface (rat: Lund and Bunt,1976; hamster:Frost,So and Schneider,1978). In the adult animal optic fibers travel primarily within the stratum opticum (SO),deep to the surface,and arborize among their target cells in the overlying suficial gray stratum (SGS). We examined this developmental change in the mouse by determining:(1) the time of origin and migration of superficial collicular neurons with H^3-thymidine autoradiography,(2) their state of differentiation with Golgi impregnations, and (3) the time of arrival and distribution of retinal afferents with anterograde HRP labelling after eye injection in utero and silver impregnation of normal fibers.

Most cells of the colliculus become postmitotic on embryonic days E11 through E13. Although the cells generated on these days overlap in their final distribution within the upper collicular layers, cells labelled on E11 predominate near the surface of SGS.Relative positions of these two cohorts become set by E15, suggesting that the majority of the E11 and E13 cohorts have completed their migrations by this time.Golgi impregnations of neurons within the outer 100 μm of the colliculus at E15-17 reveal a rapid growth and differentiation of dendrites and axons which is consistent with the autoradiographic findings.Within the same period the early retinal projection is composed of axon fascicles concentrated at the collicular surface, although more widespread fiber bundles course through the subjacent cell populations to a depth of 80-100μm.From E17 to birth(P-0=E19) the rostrocaudally coursing bundles of optic fibers become more evenly distributed through a depth of 120 um,without a surface concentration.Thereafter the superficial gray layer appears as a fiber-sparse zone above the longitudinal fiber fascicles, progressively widening to a nearly adult thickness by the time of eye opening(P-13).

The observed sequence of events suggests that the earliest optic fiber fascicles concentrated at the collicular surface are lost by degeneration and/or dispersion in the course of neuropil development in the perinatal period.The lower level of the band of optic fiber fascicles present from E15 to PO presumably marks the level at which the definitive stratum opticum develops by continued ingress of optic fibers, while cells above this level,although largely fixed in their post-migratory positions,form the SGS by a protracted period of expansion by differentiation. *NEI Postdoctoral Fellowship

236.6 OPTOKINETIC NYSTAGMUS IN LONG-TERM MONOCULARLY DEPRIVED CATS. R. Malach*, N.P. Strong* and R.C. Van Sluyters. School of Optometry, University of California, Berkeley, CA 94720.

Prolonged monocular deprivation (MD) is known to cause substantial functional disconnection of cortical visual pathways, but whether it also exerts a direct effect on subcortical visual function remains unclear. We feel the optokinetic nystagmus (OKN) system of the cat can serve as a behavioral model for studying the relative effects of MD on cortical and subcortical pathways, since both are involved in mediating this reflex in the cat.

Cats were monocularly deprived from eye opening to about one year of age and then the deprived eye was opened for OKN testing. OKN was elicited by rotating a large patterned drum around the cat at various velocities while monitoring eye movements with the search coil technique.

The gain of the monocular OKN reflex was severely reduced when stimuli were presented through the deprived eye. This deficit was most pronounced at higher drum velocities. While some OKN response remained when the stimulus to the deprived eye moved in a nasalward direction (N-OKN), the reflex was almost totally abolished for stimuli moved in a temporalward direction (T-OKN), where occasionally even a reverse-OKN was elicited. OKN through the non-deprived eye was strikingly more vigorous (with gains close to one at low velocities), although here too the gain of the T-OKN response was usually lower than that of the N-OKN.

The results of this study, and others in normal and lesioned cats, will be discussed in terms of the effects of MD on the subcortical pathways mediating OKN in the cat.

237.1 AUTORADIOGRAPHIC, HRP AND BEHAVIORAL DATA SUGGEST THAT N.R. MAGNOCELLULARIS MODULATES PAIN WHILE N.R. GIGANTOCELLULARIS CONTROLS POSTURE AND LOCOMOTION. Lee-Ming Kow, Donald W. Pfaff and Frank P. Zemlan. The Rockefeller University, N.Y., N.Y. 10021 and Department of Psychiatry, University of Cincinnati College of Medicine, Cincinnati, OH 45267.

HRP application to unilaterally transected lateral or ventral column fibers at C_2 or T_{10} in the rat indicated that an extensive group of N.r. magnocellularis (nmc) neurons projected primarily through the lateral columns to caudal spinal levels. After microiortophoretic deposit of ^{3}H-amino acids (aa's) to nmc, labeled fibers were observed to project to the lateral reticular nucleus, hypoglosal nucleus, dorsal motor nucleus of the vagus, and raphe pallidus. The majority of labeled fibers coursed caudally through the lateral columns to the dorsal aspect of the dorsal horn, the lateral aspect of laminae V, VI and VII, and X through lumbar levels.

A large bilateral group of HRP labeled cells was observed in n.r. gigantocellularis (ngc) after transection and enzyme application to the ventral columns at C_2. After microiontophoretic deposit of ^{3}H-aa's in the rostral aspect of ngc, labeled fibers were observed to project laterally to the lrn, the facial nucleus, and contralateral ngc. Caudally, labeled fibers formed facicles in the MLF, ultimately joined the spinal ventral columns to project to laminae VII, VIII and X, through cervical levels.

Bilateral electrolytic lesions were placed at the caudal extent of nmc. Significant analgesia was observed during the first 2 postoperative weeks as indicated by increased response latencies to thermal stimulation and increased pressure thresholds for eliciting vocalization. Bilateral electrolytic lesions were also placed in ngc + nmc. In addition to the analgesic effect observed after nmc only lesions, a time dependent motor syndrome was observed postoperatively. Initially, posture and locomotion were characterized by limb hyperextension followed by a second phase where locomation was characterized by alternate hyperextension and hyperflexion of the limbs. The rapid balistic movements indicated that the effect of the lesion may have been due to altered gamma-loop activiy in both extensor and flexor muscle groups.

The anatomical and functional data taken together suggest that spinal lateral column fibers (nmc) are concerned with the modulation of sensory activity, particularly nociception; while ventral column projections (ngc) are more concerned with motor function.

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237.2 THE EXTENT AND NUMBER OF MEDIAL MEDULLARY NEURONS SUFFICIENT TO INHIBIT TAIL-FLICK IN RATS. I.D. Hentall, G. Zorman and H.L. Fields. Depts. Neurol., Neurosurg., Univ. Calif., San Francisco, San Francisco, CA 94143

We showed previously that 8 μA, 200 μsec, 50 Hz, monopolar stimuli in the rat's nucleus raphe magnus (NRM) will inhibit tail flick to noxious heat. The average radius of suprathreshold current spread and the number of excited neurons is estimated here.

Microelectrodes placed in the medial medulla for concurrent extracellular stimulation and recording of single cells consisted of Pt-plated stainless steel. Their impedances were about 500 kΩ at 1KHz. With the amplifier input AC-coupled, artefact from the 0-10 μA, 200 μsec pulses subsided in 1.5 msec. Cell excitation was detected by collision with an antidromic spike, which was activated by electrodes on the dorsolateral funiculus; thus all tested neurons were medullospinal in their projections. A prolonged ability to "follow" at 100 Hz was used to indicate direct excitation; indirect excitation rarely appeared. For each cell, thresholds (I_t) and peak-to-peak spike heights (V) were found at different depths. Ferric ions were deposited by current for anatomical localization.

All 29 cells fitted $I_t=K_2r^2$ ($P<0.05$), with the radial distance to the initiation zone (r) and constant K_2 determined by 2nd order regression. The equation $I_t=K_1r$ fitted a few cells better, but most far worse. The analogous constant of spike spread ($1/V=C_2r^2$) increased with K_2, suggesting extracellular conductance affects these constants. Also, conduction velocity and K_2 appeared inversely related, implying a role for cell size. The successful square law must approximate a more complicated equation; the soma's extracellular current flow vector being constant at threshold under this law is probably irrelevant. A more complicated relationship, involving integration of voltage in the somato-dendritic region, was explored with some success; lack of space here prevents its description.

NRM cell density found by standard nuclei-counting was 4560 per mm^3. Applying the square law, for the average cell the suprathreshold current spread with an 8 μA stimulus is 126 microns. This is well within the NRM, whose smallest dimension is 1 mm. Assuming like-distributed sample and population K_2 values, the number of stimulated cells is 61. Available anatomical evidence suggests only 5% of these are the behaviorally relevant ones which contain serotonin or enkephalin and project to lumbar cord. Hence only 3 medullospinal neurons at most are required to prime analgesia circuitry. Neuronal redundancy or, more likely, multifunctional overlap, must be high, since all NRM sites suppress tail-flick equally well.

237.3 CROSS TOLERANCE BETWEEN TWO BRAINSTEM SITES SUPPORTING, STIMULATION-PRODUCED ANALGESIA. B.E. Thorn-Gray, R.M. Ashbrook* and M.H. Johnson* Dept. of Psychology, The Ohio State University, Columbus, Ohio 43210.

Electrical stimulation of the periaqueductal gray (PAG) produces inhibition of pain response to a number of noxious stimuli with a variety of species. Research investigating the neural/chemical substrate of stimulation-produced analgesia (SPA) has raised questions as to whether SPA is mediated by the endogenous opiate system (at least part of which purported to the pain inhibitory in function).

As a preliminary step in investigating the neural substrate of SPA, our lab has been interested in whether cross-tolerance can be demonstrated between neural areas supporting SPA. If the effect is more electrode-locus-specific, rather than anatomical-system-specific, we would not expect cross-tolerance between areas.

Thirty rats were implanted with two bipolar stimulating electrodes (either two PAG loci or one PAG and one dorsal raphe magnus-DRM). Stimulation consisted of biphasic rectangular wave pulse pairs of 1 msec. with 100 μsec. pauses in between pairs. Stimulation intensity ranged between 40 μA - 250 μA depending upon site and variability within the animal. Sites of initial stimulation were counterbalanced. Tail flick latencies were taken both during and immediately after stimulation. After one week recovery, stimulation was given in the second area (method identical to the first). After an additional week recovery, animals were given repeated stimulations (between 10-20 series) until tolerance to the analgesia had occurred. Immediately following demonstration of SPA tolerance in the area given repeated stimulation, animals were stimulated in the second site and observed for TF latencies to determine whether tolerance spreads across areas or remains localized in the area of stimulation.

When compared to implanted sham-stimulated controls, and when compared to their own pre-stimulation baseline (baseline $\bar{x}$ = 3.1 sec.) animals with two PAG implants showed significant analgesia in both sites, both during and immediately after stimulation (Site 1 $\bar{x}$ on = 5.7 sec; $\bar{x}$ off = 6.0 sec; Site 2 $\bar{x}$ on = 6.8 sec; $\bar{x}$ off = 4.7 sec). On test day three, significant cross tolerance was achieved between sites 1 and 2 ($\bar{x}$ baseline = 3.3; pre-tolerance analgesia: $\bar{x}$ on = 7.9, $\bar{x}$ off = 5.4; tolerance (site 1): $\bar{x}$ on = 2.7, $\bar{x}$ off = 1.8; $\bar{x}$ tolerance (site 2): $\bar{x}$ on = 4.4, $\bar{x}$ off = 3.4). Preliminary evidence indicates that cross tolerance is also demonstrable between PAG and DRM sites. The above evidence suggests a neuroanatomical system mediation of stimulation produced analgesia.

237.4 MEDIAL THALAMIC LESIONS REDUCE THE AVERSION-GATING ACTION BUT NOT THE REWARD OF LATERAL HYPOTHALAMIC STIMULATION. K. D. CARR, K. A. BONNET* and E. J. SIMON. Depts. of Psychiatry and Pharmacology, New York Univ. Med. Ctr., New York 10016.

As reported previously (Carr and Coons, Brain Research, in press) lateral hypothalamic stimulation has an aversion-gating action which is correlated with, but dissociable from, self-stimulation reward elicited through the same electrodes. Rats that lever-press to escape electrical stimulation of medullary nucleus reticularis gigantocellularis (NGC) respond less when sub-reward-threshold pulses to the lateral hypothalamus (LH) are paired with those to NGC. If NGC stimulation is made inescapable, these rats lever-press at high rates to obtain trains of LH pulses of the same amplitude as had inhibited escape. This lever-pressing behavior (for low intensity LH trains during aversive NGC stimulation) is not inhibited by gastric loading although classical self-stimulation (for higher intensity LH trains in the absence of NGC stimulation) is inhibited.

To investigate further the aversion-gating phenomenon and its relationship to self-stimulation reward, rats were implanted with monopolar stimulating electrodes in both the LH and NGC. In addition, electrodes to be used for making electrolytic lesions were implanted bilaterally in the medial/intralaminar thalamus. During each daily test session, threshold currents were determined for: (i) elicitation of a criterion rate of 12 (or more) lever-presses per minute to obtain 2-sec 25 pps trains of LH stimulation, (ii) elicitation of a criterion rate of 12 (or more) lever-presses per minute to obtain 2-sec escapes from 25 pps stimulation of NGC, and (iii) experimenter-delivered LH pulses which when paired with pulses in the NGC train would reduce escape lever-pressing from the former criterion rate of 12 (or more) to 8 (or less). Following a period of training and threshold stabilization, data were collected over four consecutive daily sessions. All animals were then anesthetized but only some received bilateral thalamic lesions. The next four consecutive days of testing revealed that the ability of LH stimulation to inhibit NGC-escape is impaired (i.e. thresholds in test (iii) increase) by medial/intralaminar thalamic lesions although self-stimulation reward (through the same LH electrodes) is unaltered. These results indicate that the aversion-gating triggered by stimulation of an appetitive behavioral system in the LH is indeed a separate function from appetitive reward and is mediated, at least in part, by a medial/intralaminar thalamic integrator.

237.5 ROLE OF THE ADRENAL MEDULLA IN OPIOID STRESS ANALGESIA. J. W. Lewis, M. G. Tordoff, J. E. Sherman* and J. C. Liebeskind. Dept. of Psychology and Brain Research Institute, University of California, Los Angeles, CA 90024.

The analgesic response to prolonged footshock stress appears to be mediated by opioid peptides, whereas that following brief footshock stress is nonopioid (Lewis et al., 1980). Hypophysectomy attenuates the opioid but not the nonopioid form of stress analgesia. While suggesting a role for pituitary opioids, these results may also be due to secondary changes in hormone deprived target glands. Therefore, we investigated the role of the adrenal in both forms of stress analgesia.

Two weeks after either adrenalectomy (ADX), adrenal demedullation (MDX), adrenal medullary denervation by celiac ganglionectomy (CGX), or sham surgery, rats were tested for baseline pain sensitivity and for analgesia to either prolonged (2.5 mA, 1 sec shock/5 sec, for 20 min) or brief (2.5 mA, continuously for 3 min) stress using the tail-flick test. All rats received both stresses in counterbalanced fashion.

ADX, MDX, and CGX antagonized opioid ($p < .01$, compared to shams), but not nonopioid, stress analgesia. MDX and CGX were as effective as ADX. None of these manipulations affected baseline response latencies. RIA indicated that ADX, but not MDX or sham surgery, reduced serum corticosterone. Thus, MDX appeared not to impair adrenocortical function. These results suggest an important role for the adrenal medulla, but not adrenal cortex, in opioid stress analgesia.

Stimulation of adrenal medulla releases both catecholamines and enkephalin-like peptides. To determine which of these may be critical to stress analgesia, rats were tested for analgesia to prolonged stress 24 hr after the last of two daily injections of reserpine (2 mg/kg). This drug regime is known to deplete catecholamine and increase enkephalin-like peptide concentrations in the adrenal (Viveros et al., 1980). Reserpine treated animals displayed significantly enhanced analgesia ($p < .01$, compared to saline controls). This enhancement appears to be mediated by opioids since naltrexone (3 mg/kg) blocked opioid stress analgesia as profoundly in reserpine treated as in saline treated animals.

While not precluding the participation of opioids of brain and pituitary origin, these findings strongly suggest that enkephalin-like peptides, released from the adrenal medulla, are critically involved in certain forms of stress analgesia. (Supported by NIH grant #NS07628 and MHTP grant #MH15345.)

237.6 NOCICEPTIVE RESPONSES OF SPONTANEOUSLY HYPERTENSIVE AND WISTAR-KYOTO NORMOTENSIVE RATS. A. Randich* (SPON: L. Mitchell) Dept. of Psychology, The Univ. of Iowa, Iowa City, IA 52242

Nociceptive responses of spontaneously hypertensive rats (SHRs) and age-matched Wistar-Kyoto normotensive rats (WKYs) were assessed with the hot-plate assay, flinch-jump assay, and conditioned suppression paradigm. SHRs showed significantly longer latencies to either paw-lick or jump than WKYs in the hot-plate assay ($55^{o}C \pm 0.05^{o}C$). In contrast, SHRs showed significantly lower response thresholds to both flinch and jump than WKYs to an electric grid shock stimulus in the flinch-jump assay. In accord with the latter findings, SHRs acquired conditioned suppression of instrumental responding at a significantly faster rate than WKYs to a 3-min white noise stimulus repeatedly paired with a 1.0-mA, 0.8-sec electric grid shock stimulus.

These findings demonstrate a fundamental dissociation of nociceptive responses to different aversive stimuli in SHRs, and support the view that blood pressure and pain regulatory systems are physiologically linked. It is suggested that hypoalgetic behavior in the hot-plate assay may involve compensation for cardiopulmonary baroreceptor resetting and enhanced activation of endogenous opioid pain-inhibition systems, whereas hyperalgetic behaviors in the flinch-jump assay and conditioned suppression paradigm may involve sino-aortic baroreceptor resetting and reduced activation of endogenous pain-inhibition systems.

238.1 A BEHAVIORAL ANALYSIS OF CHRONIC PHENYLETHYLAMINE ADMINISTRATION IN RATS. K. Mueller. Dept. of Pediatrics, Univ. of Calif. - San Diego, La Jolla, CA 92093*

Altered phenylethylamine (PEA) levels have been reported in metabolic (PKU) and behavioral disorders (depression, aggression, schizophrenia) in humans. Exogenous PEA has been reported to produce abnormal behavior in rats which under certain conditions is similar to that produced by amphetamine. In fact, endogenous PEA has been suggested to mediate the central effects of amphetamine. The "PEA hypothesis" of behavioral disorders invites a more careful examination of the behavioral effects of chronic PEA.

Long Evans hooded rats (n=24) were administered 60 or 100 mg/kg PEA intraperitoneally (IP) or subcutaneously (SC) for 21 days (SC injections were terminated after 13 days due to multiple cutaneous lesions). The animals were observed in their home cages every 4th day for 40 (60 mg/kg) or 70 (100 mg/kg) min. An additional 6 chronic saline injected animals were also observed.

After a few days of PEA the animals exhibited amphetamine-like behavior but with important differences. In general, IP animals exhibited increasingly long periods of immobility which were not necessarily accompanied by the stereotyped sniffing characteristic of amphetamine. On the other hand, SC animals exhibited increasing locomotion, which by day 13 assumed the 3 phase pattern characteristic of amphetamine. Paradoxically, however, obviously stereotyped head movements (SHM) were often accompanied by locomotion equal to or greater than that exhibited by saline controls. Thus, like amphetamine, the route of administration markedly influenced the behavioral response. There was a significant change in SHM over time only in the 60 mg/kg SC group. These animals exhibited an increase in SHM from days 1 to 9 but a decrease from days 9 to 13. In the saline controls, rearing and locomotion were highly correlated with rears generally slightly exceeding locomotions. In SC PEA aniamals rearing and locomotion were poorly correlated with locomotions generally exceeding rears. Piloerection and copious salivation were common in all PEA rats. Other behaviors such as Straub tail, hindlimb abduction, tremor, and treading were occasionally observed. Thus although chronic PEA produced stereotyped behavior similar to that produced by amphetamine it also produced a dissociation of the various components of the amphetamine response, supporting the hypothesis that these components may be subserved by different mechanisms.

*This research was performed in the laboratory of W. L. Nyhan.

238.2 BARREL ROTATION IN RATS INDUCED BY INTRAVENTRICULAR CHLORPROMAZINE METHIODIDE: AN ANTIMUSCARINIC EFFECT. R.E. Burke*, H. R. Wagner, and S. Fahn. (SPON: T. Pedley), Dept. Neurology, Columbia Univ. College of P & S, New York, NY 10032.

Barrel rotation is a unique motor phenomenon in rats in which sustained twisting develops along the animal's long axis, resulting in repetitive lateral rolling. It was first reported with intraventricular injection of somatostatin (SRIF) (Cohn & Cohn, Brain Res. 96: 138, 1975), and subsequently with intraventricular injection of substance P and vasopressin. Barrel rotation has recently been reported folowing intraventricular injection of chlorpromazine methiodide (CPZMI), a quaternary ammonium derivative, and suggested as an animal model of torsion dystonia (Rotrosin et al., Neurology 30: 878, 1980). Unlike chlorpromazine, CPZMI is not a dopmaine antagonist, because it has no effect on CNS dopamine metabolism even when injected intraventricularly.

We have studied the specificity and pharmacologic basis of CPZMI barrel rotation. In a series of 31 control injections, including CNS depressants, excitants, and agonists and antagonists of putative neurotransmitters, (dopamine, ACh, GABA, NE, serotonin, glutamate, glycine), only antimuscarinic agents induced barrel rotation. Six antimuscarinics, representing different chemical classes, induced the effect. Simultaneous intraventricular injection of the muscarinic agonist carbachol inhibited CPZMI barrel rotation; whereas atropine, a muscarinic antagonist, enhanced it.

CPZMI is a weak inhibitor for the binding of ^{3}H-spiroperidol, a dopamine receptor ligand (K_i=6 μM). In contrast, CPZMI is a potent inhibitor for the binding of ^{3}H-quinuclidynl benzyilate, a muscarinic receptor ligand (K_i=11 nM).

We conclude that CPZMI-induced barrel rotation is an antimuscarinic effect. The similarity of CPZMI barrel rotation to that induced by SRIF suggests a role for SRIF in modulating cholinergic function. The relevance of barrel rotation to clinical movement disorders remains to be determined.

238.3 PREVENTION OF THE SEROTONIN SYNDROME IN RATS BY REPEATED ADMINISTRATION OF MONOAMINE OXIDASE INHIBITORS. I. Lucki and A. Frazer. Depts. of Psychiat. and Pharmacol., Univ., of Penna. and Vet. Adm. Hospital, Phila., Penna. 19104.

The binding of ^{3}H-serotonin in brain is decreased by repeated treatment of rats with monoamine oxidase inhibitors (MAOIs) but not by treatment with tricyclic antidepressants (TCAs). In the present study, we examined whether drug-induced decreases in serotonin receptors would inhibit a behavioral response due to the activation of such receptors. The response studied was the "serotonin syndrome", which is characterized by repetitive forepaw treading, hindlimb abduction, body rigidity, tremor, lateral head weaving, and Straub tail. In one experiment, repeated (40mg/kg twice daily for 7 days) but not acute treatment of rats with nialamide blocked completely the development of the serotonin syndrome induced by either 5-methoxy-N,N-dimethyltryptamine (5MeDMT; 9mg/kg) or d-lysergic acid diethylamide (LSD; 4mg/kg). In rats treated repeatedly with nialamide, even a supramaximal dose of 5MeDMT (27mg/kg) failed to produce the serotonin syndrome. By contrast, neither acute nor repeated treatment of rats with amitriptyline altered the appearance of the behavioral syndorme caused by either serotonin agonist.

In addition, treatment of rats for 7 days with other MAOIs like pargyline (25mg/kg) or phenelzine (10mg/kg) reduced significantly the ability of 5MeDMT (3mg/kg) to cause the serotonin syndrome. 5MeDMT did produce a robust syndrome in rats treated repeatedly with either desmethylimipramine, chorimipramine, or iprindole. If rats were pretreated with parachlorophenylalanine, then repeated administration of nialamide no longer blocked the serotonin syndrome induced by 5MeDMT.

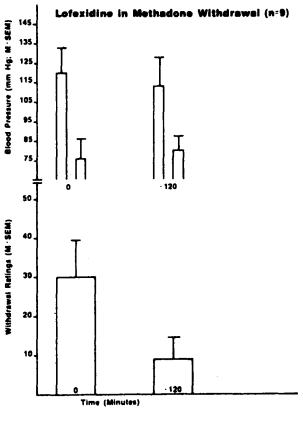
The relationship between the ability of MAOIs to reduce ^{3}H-serotonin binding sites and to block the ability of serotonin agonists to cause the serotonin syndrome indicate that these neurochemical and behavioral effects are related. Furthermore, our results suggest that the serotonin syndrome is produced by activation of serotonin$_1$ rather than the recently described serotonin$_2$ receptors, as it has been shown by others (Science, 210:88-90, 1980) that repeated treatment of rats with either MAOIs, TCAs, or iprindole decrease serotonin$_2$ binding sites in the central nervous system. (Supported the Vet. Admin., MH 29094 and MH 14654).

238.4 LOFEXIDINE BLOCKS ACUTE OPIATE WITHDRAWAL. M.S.Gold, A.L.C. Pottash, W.J. Annitto,* I.Extein, H.D.Kleber. Fair Oaks Hospital and Psychiatric Diagnostic Laboratories of America, Summit, N.J. 07901, and Yale Univ. School of Medicine, New Haven, CT 06510.

We have described potent antiwithdrawal effects for clonidine the alpha-2 adrenergic agonist (1,2) which reduces brain noradrenergic activity. On the basis of clonidine's efficacy in human opiate withdrawal(1,2) and preclinical rodent and primate studies (3) we have postulated noradrenergic hyperactivity in opiate withdrawal and suggested that new nonopiate medications with central anti-norepinephrine (NE) activity might be found which are equipotent to clonidine in antiwithdrawal efficacy but without clonidine's marked sedative and hypotensive effects. More recently, we have suggested that lofexidine, an imidazoline derivative which is a structurally related analogue of clonidine, may be the ideal non-opiate antiwithdrawal agent.

We have recent data from 9 male chronic methadone addicts which demonstrates potent antiwithdrawal activity for lofexidine and offers additional support for the NE hyperactivity hypothesis of opiate withdrawal.

Following procedures reported previously (1,2) lofexidine (3 ug/kg) was administered, the withdrawal score fell rapidly and significantly ($p < 0.01$) from 30.3.3 ± 8.6 to 8.2 ± 5.5 at 120 minutes after lofexidine. Systolic and distolic blood pressure were not significantly decreased and remained in the normal range. All patients were successfully detoxified from chronic methadone addiction and switched to long acting opiate antagonist naltrexone in this inpatient study. Our data with lofexidine suggest that antiwithdrawal effects can be separated from antihypertensive and sedating effects.



1. MS Gold, et al. JAMA 243: 343, 1980.
2. MS Gold, et al. N.Engl.J Med. 302:1421, 1980.
3. MS Gold, et al. Biomedicine 30:1, 1979.

238.5 ASPECTS OF THE PHARMACOLOGY OF LIMB FLICKING AND YAWNING IN CATS. J.L. Marini* & M.H. Sheard. Dept. of Psychiatry, Yale Univ. Sch. of Med., 34 Park Street, New Haven, CT 06508

Relative to their occurrence after saline, high frequencies of limb flicking (LF) and yawning (YA) are seen in cats after i.p. doses of d-lysergic acid diethylamide (LSD) and other hallucinogens with serotonergic properties, and LF plays a central role in a behavior model for such drugs (Jacobs et al., Commun. Psychopharm. 1:243, 1977). Although it has recently been shown that LF is not specific for hallucinogens (e.g., Marini et al., Neurosci. Abst. 6:700, 1980), the elicitation of LF and YA by such drugs, and their susceptibility to tolerance after single doses, makes them useful in the behavioral pharmacology of hallucinogens and related substances.

Adult, home-cage-acclimated male and female mongrel cats in good health were used in these studies. Results are rates of occurrence for the 90 min following i.p. doses of drugs or saline (control).

The muscarinic agonist pilocarpine (PILO, 0.125-1.0 mg/kg as HCl salt, n=4-6) did not elicit YA, but gave a dose-dependent increase in LF that was maximal at 0.5 mg/kg (26 $\pm$ 5.8; control, 0.3 $\pm$ 0.2, $P < .01$). This confirms that LF is elicitable by non-hallucinogens. The effects of PILO (0.5 mg/kg, n=4) were antagonized by pretreatment (15 min) with 0.5 mg/kg of the peripherally-acting antimuscarinic agent, N-methylscopolamine bromide (MESCO), showing that a peripheral mechanism can elicit LF.

LSD (0.01, .025 & .05 mg/kg as bitartrate, n=6-9) produced increased rates of LF (40-50 per 90 min, $P < .03$) and YA (6-8 per 90 min, $P < .03$). The related ergolene, lisuride (LIS, as hydrogen maleate, .025 & .05 mg/kg, n=8-9), elicited LF (19-33 per 90 min, $P < .05$) but not YA. Pretreatment with MESCO (0.5 mg/kg) 15 min before LSD (.01 mg/kg, n=6) or LIS (.05 mg/kg, n=6) had no effect on LF or YA. Pretreatment with methysergide was reported to antagonize LSD-elicited LF and YA and LIS-elicited LF, results consistent with a serotonergic mechanism for LSD- and LIS-elicited LF and YA (Marini & Sheard, Europ. J. Pharmac., in press, 1981).

Combining LSD and LIS (.025 mg/kg each, n=4) resulted in an additive effect on LF compared to either drug alone, but in a reduced rate of YA. Apomorphine (APO, .05 or 1.0 mg/kg as HCl salt) given 15 min before either .01 or .025 mg/kg of LSD did not affect LF relative to LSD alone, but the higher APO dose antagonized YA (n=5-6, $P < .05$). These results suggest that a dopamine (DA) mechanism inhibits serotonin (5HT)-mediated YA in the cat. Drug-induced YA may be useful in studying concomitant DA and 5HT effects of drugs (e.g., LIS).

238.6 BEHAVIOURAL EVIDENCE FOR NEUROCHEMICAL PATHOLOGY AFTER TREATMENT OF NEWLY HATCHED CHICKS WITH CYCLOHEXIMIDE OR GLUTAMATE. Paul R. Sanberg and Richard F. Mark*. Department of Behavioural Biology, Research School of Biological Sciences, Australian National University, Canberra, A.C.T., 2600, Australia.

Previous studies have demonstrated profound behavioural alterations in older chickens which received forebrain injections of cycloheximide (CXM) or glutamate (GLU) when newly hatched. The acquisition, attentional and emotional deficits found in these birds have not been related to any brain pathology[1]. CXM has been shown to increase the concentration in the brain of GLU in neonatal chicks. It has been suggested that an accumulation of this amino acid may underlie the behavioural deficits induced by CXM[2]. In order to assess possible pathology in these birds, we have studied the integrity of the dopaminergic and cholinergic neurotransmitter systems in the paleostriatum using drug-induced motor activity.

Compared to saline-injected controls, bilateral forebrain injections of CXM (20 µg in 25 µl saline) or GLU (210 µg in 25 µl buffered saline) in 2-day-old chicks resulted in significantly less activity in response to intraperitoneal injections of the dopaminergic agonist, apomorphine (2 mg/kg) or the cholinergic antagonist, scopolamine (2 mg/kg) when tested in BRS activity platforms four weeks later (Fig. 1). In addition, these birds were found to be much more susceptable than controls to convulsions induced by pentylenetetrazol (50, 100 and 200 mg/kg).

The results suggest that CXM or GLU injections in neonatal chicks cause damage to neurotransmitter mechanisms localized on intrinsic and/or efferent neurons in the paleostriatal complex (basal ganglia), the nuclei responsible for motor behaviour in birds.

[1]Sanberg, P.R., Faulks, I.J., Anson, J.M. & Mark, R.F., *Soc. Neurosci. Abst.* 6: 1980, 111.

[2]Hambley, J.W. & Rogers, L.J., *Neurosci.* 4: 1979, 677-684.

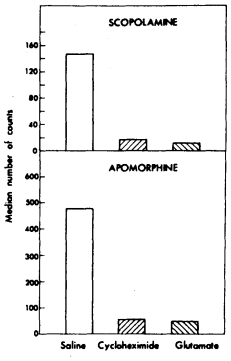


Fig. 1. Median increase in activity over baseline levels following scopolamine (upper) or apomorphine (lower) during 20 min test.

238.7 DOPAMINE-NOREPINEPHRINE ANTAGONISM IN ATTENTION DEFICIT DISORDER WITH HYPERACTIVITY IN CHILDREN. W. O. Shekim, J. Javaid, H. Dekirmenjian, D. B. Bylund, J. M. Davis. Children and Youth Center and the Clinical Research Center, University of Missouri Health Sciences Center, Columbia, MO 65201.

None of the biochemical studies in attention disorder with hyperactivity in children consider the possibility that hyperactivity may result from an imbalance or abnormal interaction between dopamine (DA) and noradrenergic (NE) systems. To test the hypothesis of possible abnormal interaction between DA and NE, the authors examined the relationship between the excretion of 3-methoxy-4-hydroxy-phenylglycol (MHPG), the main metabolite of central nervous system NE, and homovanillic acid (HVA), the main metabolite of DA in 16 hyperactive boys and 12 controls. They further examined the effect of d-amphetamine (0.5 mg/kg body weight daily for two weeks) has on that relationship. The hyperactive children were admitted to a Clinical Research Center after meeting certain exclusionary and inclusionary criteria. Twenty-four hour urine samples were collected on ice in bottles containing 0.5 gm sodium meta bisulfite/liter of urine. MHPG was analyzed according to the method of Dekirmenjian and Maas (1970) and HVA according to a modification by Dekirmenjian and Dziedzic et al, 1972.

The correlation co-efficients r between MHPG and HVA excretion were significantly negative in hyperactive boys when the rational effects of age, body surface, and 24-hour urinary creatinine were removed. (Partial r with age removed: - 0.542, df = 14, $p < 0.05$, partial r with body surface removed - 0.559, df = 14, $p < 0.05$, partial r with 24-hour urinary creatinine removed - 0.544, df = 14, p = ns). Furthermore, the correlation co-efficient r on the Pearson Product Correlation Co-efficient Test in hyperactive boys and in responders at baseline differed significantly from the correlation co-efficients in post-treatment and in controls. The correlation co-efficient post-treatment did not differ from controls. The administration of d-amphetamine to hyperactive boys changed the negative correlation that existed in the excretion of MHPG and HVA to a positive one similar to that in controls, i.e., pre-treatment r - 0.537 vs post-treatment 0.544 $p < 0.01$, controls r: 0.328.

The evidence presented in this paper indicates the involvement of an altered relationship between NE and DA in the etiology of hyperactivity and d-amphetamine may correct such a relationship.

239.1 SPECIFIC HIGH AFFINITY ^{3}H-Ro5-4864 BENZODIAZEPINE BINDING SITES IN RAT BRAIN AND PERIPHERAL TISSUES. Hans Schoemaker*, Marla Bliss*, Susan H. Yamamura* and Henry I. Yamamura (SPON: Paul F. Consroe). Dept. Pharmacology, Univ. Arizona Hlth. Sci. Ctr., Tucson, Arizona 85724.

Benzodiazepines bind with high affinity to specific receptors in the mammalian brain and various peripheral tissues, e.g. the kidney. The benzodiazepine receptors present in the brain and peripheral tissues can be distinguished by both clonazepam and the chloro-diazepam analog Ro5-4864. Thus, whereas clonazepam has high affinity for the central benzodiazepine receptor, it has only very low affinity for the peripheral receptor. The reverse is true for Ro5-4864. Recently, ^{3}H-Ro5-4864 has become available and the specific binding of this new ligand to rat brain and peripheral tissues was studied. After decapitation, the brain and peripheral tissues were dissected and homogenized in 50 mM Na/K phosphate buffered saline (pH 7.4). Aliquots were incubated with ^{3}H-Ro5-4864 (NEN) in the presence or absence of various drugs. After 120 min incubation at 0-4^{0}C, membranes were harvested by filtration over Whatman GF/B filters and washed with 3x5 ml cold buffer. Binding in the presence of 1μM unlabelled Ro5-4864 was defined as nonspecific. Equilibrium binding experiments indicate that ^{3}H-Ro5-4864 binds with high affinity (Kd= 1.3nM) to rat kidney membranes. The pharmacological profile of this receptor is similar to that already described for the peripheral receptor labelled by ^{3}H-flunitrazepam. Diazepam, flunitrazepam and Ro5-4864 potently displace ^{3}H-Ro5-4864 binding in low nanomolar concentrations whereas clonazepam shows only a weak inhibition at 1μM. A high affinity ^{3}H-Ro5-4864 binding site could also be demonstrated in several brain areas. ^{3}H-Ro5-4864 binds with high affinity (Kd=1.2nM; Bmax=220fmol/mg protein) to membranes of the cerebral cortex, the pharmacological profile of this site being comparable to that of the peripheral ^{3}H-Ro5-4864 receptor. In addition a second ^{3}H-Ro5-4864 binding site may be present in this tissue. Binding is not or only slightly affected by several non-benzodiazepine anxiolytics, sedatives/anticonvulsants, stimulants /convulsants, or alkyl-β-carboline-3-carboxylate derivatives. The pyrazolopyridine derivative tracazolate, which enhances ^{3}H-flunitrazepam binding to cerebral cortical membranes, inhibits ^{3}H-Ro5-4864 binding in this tissue. ^{3}H-Ro5-4864 binding shows a regional distribution, binding being highest in the brainstem and cerebellum, and lowest in the striatum. The cellular localization in the brain, i.e. neuronal or non-neuronal (e.g. glial) is currently being investigated as well as the subcellular localization. This study was supported in part by USPHS grants and a RSDA to H.I.Y.

239.2 γ-AMINOBUTYRIC ACID AND BENZODIAZEPINE RECEPTORS: COPURIFICATION AND CHARACTERIZATION. Moshe Gavish* and Solomon H. Snyder+. *Israel Institute of Technology, Dept. of Pharmacology, Haifa, Israel and +Johns Hopkins University, School of Medicine, Depts. of Neuroscience, Pharmacology and Psychiatry, Baltimore, Maryland 21205

γ-Aminobutyric acid (GABA) and benzodiazepine receptors have been solubilized and purified by procedures such as gel filtration, ion-exchange, lectin, and affinity chromatographies. All of these procedures enhance the specific activity of each receptor to a similar extent. The drug specificities of [^{3}H]muscimol and [^{3}H]flunitrazepam binding sites are the same after extensive purification by affinity chromatography compared to the membrane bound and initially solubilized receptors. GABA and chloride stimulation of benzodiazepine binding is retained in pure receptors. Two bands are covalently labeled with [^{3}H]flunitrazepam after ultraviolet irradiation of the purified receptor. The persistent association of GABA, benzodiazepine, and chloride recognition sites after extensive purification suggests that they may be part of a single macromolecular complex. Further purification in attempts to isolate homogeneous receptors has clarified the benzodiazepine-GABA linkage.

239.3 BRAIN REGIONAL VARIATION IN BENZODIAZEPINE AND GABA RECEPTOR ASSOCIATION. Fredrik Leeb-Lundberg* and Richard W. Olsen (SPON. M.A. Baker). Division of Biomedical Sciences, University of California, Riverside, CA 92521.

Anesthetic barbiturates (pentobarb.) enhanced the binding of both [^{3}H]benzodiazepines (BZ) (Leeb-Lundberg, Snowman and Olsen, PNAS 77, 7468-7472 (1980) and [^{3}H] γ-aminobutyric acid (GABA) (Olsen, Snowman, and Leeb-Lundberg, Fed. Proc. 40, 309 (1981)) to receptor sites in rat brain membranes. Only part of these GABA and benzodiazepine receptor sites seem to be coupled as indicated by interactions with barbiturates and the GABA receptor antagonist bicuculline. BZ receptor binding in fresh, well-disrupted and washed rat brain membranes was assayed by filtration, using 0.5 nM [^{3}H]diazepam in 20 mM KPO_4, 0.2 M KCl, pH 7.5 at 0^{0}C. GABA receptor binding in frozen-thawed and well washed rat brain membranes was assayed by centrifugation, using 2 nM [^{3}H]GABA in the same buffer at 0^{0}C. Pentobarb enhancement of BZ binding was due to a change in K_D, while enhancement of GABA binding was due to a change in B_{max}, apparently by converting GABA receptor sites of immeasurably low affinity to higher affinity. Both effects, mediated by barbiturate/picrotoxinin receptors, were saturable (EC_{50} 100-200 μM), competitively blocked by picrotoxinin (μM), dependent on Cl^- or other anions able to penetrate GABA ionophores, and correlated very well with anesthetic potency and enhancement of GABAergic postsynaptic responses by a series of barbiturates. Bicuculline only blocked a fraction of the pentobarb-enhanced BZ binding; this fraction varied with brain region, from 10-70%. The maximal percent enhancement of BZ and GABA binding by pentobarb also varied with brain region, from 20-180%. While pentobarb enhancement of GABA binding in various brain regions did not correlate with total pentobarb-enhanced BZ binding, it agreed very well with regional variation in bicuculline-sensitive pentobarb-enhanced BZ binding, with cortex > hippocampus ≥ thalamus ≥ striatum ≥ pons-medulla = cerebellum. The bicuculline-insensitive fraction of pentobarb-enhanced BZ binding sites appears to be associated with higher affinity β-carboline binding sites. These results suggest that barbiturates affect multiple BZ receptor sites, only part of them being coupled to GABA/bicuculline receptors.

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239.4 BENZODIAZEPINE "TYPE 1" RECEPTORS: A BIOCHEMICAL STUDY. D.L. Niehoff, J.M. Palacios, M.J. Kuhar, †R. O'Brien and †D. Horst. Dept. of Neuroscience, Johns Hopkins Univ., Sch. of Med., Balto., MD 21205; †Hoffman-La Roche, Nutley, NJ.

A variety of pharmacological, biochemical, behavioral and anatomical methods have confirmed the existence of a heterogeneous population of benzodiazepine (BZ) receptors in rat brain. The triazolopyridizine (TPZ) drugs, exemplified by CL218,872 (CL) are a class of compounds which can discriminate between two subpopulations of BZ receptors: Type 1 has a high affinity for TPZ's and Type II has a low affinity. We have used ^{3}H-CL to label and characterize BZ receptors in the rat cerebellum, which contains primarily Type 1 receptors (Young et al., J. Pharmacol. Exp. Ther., 216:825, 1981).

Synaptosomal membrane preparations (P_2) of rat cerebellum, suspended to 10 mg/ml in 50 mM Tris citrate (pH 7.1) were used in most of these experiments. When investigating the effects of GABA, chloride ion, and pyrazolopyridines on ^{3}H-CL binding, the pellet was resuspended and washed in 50 mM Tris citrate 3 times to remove endogenous GABA. ^{3}H-CL (2.5 nM), displacing drugs, and membranes (1 ml. final volume) were incubated on ice for 60 min. prior to termination by centrifugation. Blank values were determined in the presence of 1 uM clonazepam.

^{3}H-CL binding was saturable and had highest densities in the cerebellum, followed by cerebral cortex and hippocampus. Scatchard analysis revealed a K_D = 23 nM and B_{max} = 32.9 fmol/mg tissue. The use of higher concentrations of ^{3}H-CL suggested the presence of an additional lower affinity site. The binding of ^{3}H-CL was inhibited more potently by (+)-B10 than by (-)-B10 (IC_{50} values = 1 nM and 50 nM respectively), and by clonazepam and methyl-beta-carboline carboxylate in the 1-10 nM range. In multiply-washed membranes, 1-100 uM GABA potentiated ^{3}H-CL binding. However, 25-500 mM NaCl failed to potentiate ^{3}H-CL binding, while enhancing ^{3}H-flunitrazepam binding in the same membranes. Similarly, cartzolate (1 μM) (SQ 65396), potentiated ^{3}H-flunitrazepam binding in the presence of 150 mM NaCl, while failing to potentiate ^{3}H-CL binding in the same membrane preparation.

In summary, ^{3}H-CL binding has many, but not all, of the same characteristics as ^{3}H-BZ binding. ^{3}H-CL binding is not potentiated by chloride ion, even in the presence of cartazolate. Thus, the Type 1 site appears coupled to the GABA site, but may not be closely associated with the chloride conductance channel.

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239.5 ADDITIONAL EVIDENCE FOR SEVERAL BENZODIAZEPINE/ANION/GABA RECEPTOR COMPLEXES IN RAT CEREBELLUM AND FOREBRAIN. R. F. Squires. Rockland Research Institute, Orangeburg, NY 10962.

NaCl protects dialysed benzodiazepine (BZ) receptors (from rat forebrain or cerebellum) against heat inactivation (30 min, 60°C) in a concentration dependent way with complete protection above 500 mM. GABA and several GABA mimetics are without protective effect alone but reduce the concentration of NaCl required for 50% protection (from 280 mM without GABA to 30 mM in the presence of 1 mM GABA). LiCl, KCl, CsCl and NaCl yield similar protection patterns while sodium or potassium phosphate yield a different pattern. In the presence of NaCl (20-100 mM) GABA enhances protection in a saturable, concentration dependent way, with Hill numbers (α) near 0.7 and "Kd" values ranging from 10 µM (at 100 mM NaCl to 75 µM (at 20 mM). Picrotoxin potentiates the protective effect of high ($\geq$ 200 mM), but not low, NaCl concentration. Other GABA mimetics, including imidazoleacetate (ImAA), piperidine-4-sulfonate (P4S) and isoguvacine (IGV) also protect in saturable, concentration dependent ways, but provide less maximum protection than GABA. Time courses of heat inactivation at 60°C in 50 mM NaCl using saturating concentrations of several GABA mimetics show that P4S or IGV selectively protect a small (30-40%) sub-population of BZ receptor complexes in both cerebellum and forebrain while ImAA protects a larger subpopulation (44 and 56%, respectively, in cerebellum and forebrain). GABA, 3-amino propane sulfonate (APSA) and, beta-guanidinopropionate provide the greatest protection (62-69% in cerebellum, 70-80% in forebrain). These results indicate the existence of at least four receptor complexes in both cerebellum and forebrain which differ with respect to their constituent GABA and anion recognition sites. All GABA mimetics tested, protected less in cerebellum than in forebrain. APSA and ImAA showed the greatest differences in their effects on cerebellum and forebrain, respectively. Evidence from other laboratories suggest that brain BZ receptors exist at tetramers of MW near 200,000 containing (at least) two subunits with MW's near 50,000. The lactate dehydrogenase model for BZ receptor multiplicity is one interesting possibility which may account for several of the above findings (α_4, $\beta_1\alpha_3$,$\beta_2\alpha_2$, $\beta_3\alpha_1$,β_4). Several substances partially inhibit ^{3}H-FLU binding ("plateau" inhibition). These include strychnine (Imax 64%, "Kd" 135 µM, α = 0.66), cyproheptadine (63%, 62 µM, 0.92), 4-guanidinobenzoate (27%, 56 µM, 0.87) and tabernanthine (82%, 38 µM, 1.1). These substances appear to uniformly affect all BZ receptors by allosterically decreasing Bmax and/or increasing the Kd for flunitrazepam.

239.6 PHYSICOCHEMICAL PROPERTIES OF THE GABA AND BENZODIAZEPINE RECEPTOR PROTEINS FROM MAMMALIAN BRAIN. F.A. Stephenson,* A.E. Watkins,* R. King,* D. Gusman,* D.V. Greenlee* and R.W. Olsen. University of California, Riverside, CA 92521

Detergent-solubilized GABA receptor (GABA-R) and benzodiazepine receptor (BZ-R) were both found to co-migrate on gel filtration chromatography and sucrose gradient centrifugation with a molecular weight of 355,000. Soluble binding of [^{3}H]muscimol (GABA-R) and [^{3}H]flunitrazepam (BZ-R) was assayed by polyethylene-glycol precipitation and centrifugation, determining nondisplaceable background with 10 µ M muscimol or flunitrazepam respectively. Triton X-100 (0.5%) solubilized 55% (5.3/10.3 pmol/g bovine cortex tissue) of BZ-R but only 5% (2.5/64 pmol/g tissue) of GABA-R. However, deoxycholate (0.5%) solubilized 63% of BZ-R and 31% of GABA-R and both these values could be further improved by including protease inhibitors. Deoxycholate extracts were unstable at 0°, so were exchanged by gel filtration column chromatography into 0.5% Triton X-100, in which both binding activities were more stable at -20° and 4°C. Scatchard plots of soluble GABA-R gave K_D values for [^{3}H] muscimol and [^{3}H] GABA of 12 nM and 50 nM respectively; both gave a B_{max} of 1.55 pmol/mg protein; soluble [^{3}H] flunitrazepam binding gave a K_D of 8 nM and B_{max} of 0.8 pmol/mg (4 expts.). Solubilized BZ-R binding was enhanced $\geq$ 20% by both GABA (10 µ M) and pentobarbital (0.5 mM). Both GABA-R and BZ-R comigrated on Sepharose 6B columns, with a Stokes radius of 6.81 nm. Fractionated BZ-R binding was still enhanced by GABA but not pentobarbital. GABA-R and BZ-R also co-migrated on centrifugation in sucrose gradients in H_2O and D_2O (+0.5% Triton X-100), with $s^0_{20,w}$ = 12.5 and $\bar{v}$ = 0.73 ml/g, indicating very little bound detergent. The frictional coefficient was 1.46 and the calculated molecular weight of both proteins was 355,000. Thus at least a portion of GABA-R and BZ-R in brain membranes seem to be associated with a large multisubunit protein complex which can be solubilized under certain conditions and is consistent with both in vivo and in vitro interactions between benzodiazepines and GABA receptors. This complex may also contain the GABA-regulated chloride ion channels and the modulatory sites for barbiturates and related depressant drugs and picrotoxinin-like convulsants.

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240.1 REFLEXIVE AND ACQUIRED CONTROL OF A HUMAN LIMB MUSCLE. M. C. Wetzel and J. W. Davis*. Psych. Dept., Univ. of Arizona, Tucson, AZ 85721.

Classical conditioning procedures extended work to distinguish reflexive from conditioned movements. Previous findings showed that the pretouchdown EMG burst in quadriceps (biceps or rectus) femoris could be produced or eliminated by discriminative stimulus (operant conditioning) control during both cat (Wetzel, M. C., 1981a, b, Am. J. Phys. Med., in press) and human (Wetzel, M. C., 1981c, J. Human Movement Stud., in press) treadmill locomotion. The present experiments examined some conditions that influenced the effectiveness of 1) an eliciting stimulus, tap to the patellar tendon, over the subsequent reflexive extensor EMG and 2) several events that preceded the tap and therefore might acquire conditioned reflex or discriminative stimulus control over the same quadriceps muscle.

Results for 6 Ss accorded with previous findings in several respects. The reflexive EMGs were larger when the fists were clenched in response to a click discriminative stimulus (heard when the tape recorder switched on) than when there was no clench; most responses were ipsilateral; and EMG amplitude varied appreciably within daily sessions of 60 - 200 trials, during which reinforcement contingencies were invariant.

Conditioned responses were highly dependent upon conditions and Ss. When present, however, differential control was seen by the recorder click that occurred about 500 msec before the tap, the clench discriminated by that click, a tone that began 300 msec before the tap, and/or a touch to the skin covering the patellar tendon. The touch was not established as an effective conditioned stimulus when its onset coincided closely with the tap.

Bilateral acquired responses were rare, but the ipsilateral EMGs comprised several bursts distinguished by amplitudes and latencies that varied with the controlling stimuli. There was little "temporal" conditioning.

It was concluded that early reports of knee jerk conditioning must be repeated and reinterpreted (e.g., Twitmyer, 1902, reprinted in 1974, J. Exp. Psychol., 103, 1047-1066). In most previous studies of motor control in intact animals or humans, multiple sources of stimulation have been present but confounded. Differential reinforcement procedures are recommended to identify specific eliciting (reflexive), conditioned, and discriminative stimuli. In this way the relative strength of the three control sources can be disclosed.

240.2 CHARACTERISTICS OF HUMAN ARM TRAJECTORIES. W. K. Abend*, E. Bizzi and P. Morasso*. Dept. of Psychology, M.I.T., Cambridge, MA 02139.

In order to investigate the strategies used to plan and control arm trajectories, we have recorded two-joint arm movements performed by normal adult humans. Subjects grasped the vertical handle of a lightweight hand-position transducer and briskly moved the handle to each of a series of visual targets. The arm was restricted to move in a horizontal plane and the wrist was braced, so that only the shoulder and elbow joints were active. Signals from the transducer allowed the computation of the position and speed of the hand and joints during the movement.

When subjects were asked simply to move the handle from one target to another, they generated a roughly straight trajectory. The speed profile of these straight trajectories was bell-shaped. When the subjects were asked to produce curved hand trajectories, three unexpected results were obtained. First, the trajectory curvature profile usually displayed one or more peaks, so that the trajectory had a segmented appearance, as if the subjects were trying to approximate the curve with low curvature segments. Occasionally, curved movements without any clear discontinuity were produced. Second, at the time of peaks in the curvature of the trajectory, the hand slowed briefly. Therefore, while the speed profile for a straight hand movement was bell-shaped, the profile for curved movements was multipeaked. Third, the average duration of the curved trajectories was significantly greater than that of straight movements having the same path length. These three findings were obtained in all parts of the work space and even when the subject traced constant curvature guide paths without visual feedback.

The results are surprising because there is no a priori reason why the curved trajectory should so frequently appear segmented, or why the velocity and duration characteristics of curved and straight hand trajectories should differ. These characteristics may reflect central mechanisms involved in trajectory planning and control, but other explanations can be advanced. The Coriolis and reaction forces may be more significant during curved movements and affect the trajectory and velocity. Another possibility is that muscles which span two joints (e.g., biceps) may allow the implementation of straight hand paths without the need for central trajectory planning (Hogan, N., Proc. Joint Automatic Controls Conf., 1:TA10-B, 1980). If these muscles actually impose a tendency toward straight trajectories, this could result in the segmented appearance of curved movements and the associated velocity irregularities. (Research supported by NIH grants NS09343, NS06416 and AM26710.)

240.3 SEPARATE CORTICAL CELL SYSTEMS FOR THE CONTROL OF JOINT MOVEMENT AND OF JOINT STIFFNESS. D.R. Humphrey and D.J. Reed*, Lab. of Neurophysiol., Emory Univ. Sch. Med., Atlanta, GA 30322.

With simple, voluntary movements about a joint, agonist and antagonist muscles are activated reciprocally. Such movements are accompanied often, however, by a co-contraction of antagonistic muscles at joints which must be stiffened, in order to provide postural stabilization for the moving part. To date, little is known about the central systems which control such co-contraction, though there is ample evidence to suggest that such systems may malfunction in various motor disorders.

To study further this basic form of motor control, we have recorded from wrist flexor-extensor (F-E) motor units (N=128) and from motor cortex cells (N=108) in monkeys that have been trained to control the position of the wrist in the presence of imposed forces which attempt alternately to flex and then to extend the wrist. As the frequency of such perturbation increases, the animal shifts from a mode of joint position control by graded, reciprocal activation of wrist F-E muscles toward one in which the wrist is stiffened tonically by a sustained co-contraction of these muscles. At the level of the spinal cord, the convergence of these two, apparently distinct control signals is detectable in the discharge patterns of single motor units. At the level of the motor cortex, however, the two signals appear to originate in part from separate neuronal populations, both of which contain pyramidal tract and other, identified output cells. Cells which lie within the center of the low-threshold, microstimulation-defined, wrist F or E zones tend to fire in a graded, reciprocal manner; these cells are also highly responsive to cutaneous inputs from the palm, or from joint receptors in the fingers and/or wrist. In contrast, units which lie in an adjacent, slightly anterior zone tend to fire in a manner which correlates highly with the degree of agonist-antagonist co-contraction. These cells are essentially unresponsive to somatosensory input from the arm and hand. Moreover, microstimulation within this zone tends to evoke F-E co-contraction.

We propose, therefore, that, at the level of the motor cortex, these two fundamentally different modes of muscle control are mediated in part by separate neuronal systems. By virtue of these separate systems, the motor cortex appears to be capable of controlling independently (1) reciprocal activation of antagonistic muscles, and hence joint movement, and (2) co-activation of these muscles, and hence the stability or mechanical stiffness of the joint. (Supported by NIH Grant NS 10183).

240.4 MOTOR UNIT CONTROL IN SKILLED PERFORMERS. A. P. Xenakis*, R. S. LeFever*, C. J. De Luca. (SPON: T.F. Weiss) NeuroMuscular Research Lab., Children's Hospital Medical Center, Harvard Medical School, Boston, MA 02115.

Multiple channel myoelectric (ME) signals were recorded from an indwelling bipolar needle electrode during both constant-force and force-varying isometric contractions of the First Dorsal Interosseous (FDI) and the deltoid at levels up to 80% of maximal voluntary contraction (MVC). The data was obtained from three groups of highly skilled individuals: three 1500 meter freestyle swimmers, three powerlifters and three concert pianists. These subjects ranked among the best in the world (including Olympic and World Champions). A computer assisted visual procedure was used on the ME signal to reliably detect the firings of concurrently active motor units (up to 8) throughout the contraction [R.S. LeFever and C.J. De Luca, Abstracts of the Soc. for Neuroscience, 1978].

Utilization of this methodology on 209 motor units demonstrated that the firing rate behaviour was different for the deltoid and the FDI. This difference in behaviour was noted in: the firing rate at recruitment(8.41 $\pm$ 1.99 pps for the FDI and 12.84 $\pm$ 2.55 pps for the deltoid), the near maximal firing rate (43.0 $\pm$ 5.19 pps for the FDI and 33.0 $\pm$ 3.54 pps for the deltoid), the firing rate at decruitment(7.86 $\pm$ 2.33 pps for the FDI and 8.61 $\pm$ 2.57 pps for the deltoid). Also, the firing rate of the FDI was linearly related (r=0.65) to the force, whereas the deltoid motor units demonstrated a preferred level for the firing rates. This behaviour was significantly (typically $p<0.23$) invariant with subject groups (training) and force rate of the contraction. Furthermore, these findings are in direct support of data reported on unskilled subjects by previous authors [R.S. LeFever and C.J. De Luca, Abstracts of the Soc. for Neuroscience, 1979].

The results show that the effects of training appear to have no demonstrable affect on the CNS control of the firing rate properties of motor units. According to other investigators, the training effects primarily impose themselves on the properties of the end organ itself ("use hypertrophy of the muscle). (Supported in part by NIAMDD Grant AM 19665, the Insurance Institute for Highway Safety and Liberty Mutual Insurance Company).

240.5 CHANGES IN FIRING OF PALLIDAL NEURONS DURING ARM REACHING MOVEMENTS IN A REACTION TIME TASK. M.E. Anderson and F.B. Horak. Depts of Physiology and Biophysics and Rehabilitation Medicine and Regional Primate Research Center, University of Wash., Seattle WA 98195.

The firing of many neurons in the globus pallidus changes in a manner that is temporally correlated with the repetitive trained movements used in most testing situations. The timing of these changes has been reported by some to precede the movement, and the suggestion has been made that their activity might be involved in the initiation of or preparation for the movement.

We have reported earlier (Horak and Anderson, 1980) that stimulation in the globus pallidus of monkeys making rapid arm reaching movements in a forced reaction time task changes the movement time but does not change the reaction time, which is terminated by the onset of the movement. The time at which stimuli must be applied is critical. For example, stimuli that cause the movement to be slowed must be applied within a period about 50 to 150 msec preceding movement initiation. Earlier stimulation does not change either movement time or reaction time.

We now report the firing patterns of pallidal neurons studied in the same tracks in which stimuli subsequently were applied. Changes in activity of neurons with movement-correlated discharge usually were initiated in a period of time 50 to 150 msec prior to the onset of the movement, the same time period during which stimulation caused a change in movement time. EMG activity was recorded from wrist, elbow, shoulder, and thoracic back muscles during the same trials, and in most cases, changes in unit activity occurred following, instead of preceding the onset of EMG activity. Current data seem to indicate that exceptions are units in most rostral portions of GP,

We would hypothesize that pallidal neurons associated with movement, at least those in posterior portions of GP, play a role in scaling the buildup of EMG activity during a movement, and, thus, in determining the speed of movement initiated or gated by other mechanisms.

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240.6 FEEDBACK IN FOREARM MOVEMENTS AND ABSOLUTE POSITION SENSE: AN INFORMATION THEORY ANALYSIS, Barbara Sakitt, Massachusetts Institute of Technology, E10, Cambridge, Mass. 02139, U.S.A.

Experiments were conducted to study the role of feedback in achieving precision in human visually triggered forearm movements. The conditions used were: (1) Deliberate smooth movements without corrections: These are characterized by a single peak in the velocity trace, intermediate values of velocity, and attainment of the final average EMG activities of biceps and triceps before the movement is completed. (2) Correcting movements: The subject keeps moving until he thinks that his arm is pointed at the target. These trials are characterized by a series of step-like movements, with the EMGs changing at each step. The velocity trace has many peaks and varies in an irregular fashion. (3) Passive movement-passive posture: The subject keeps his arm limp while it is passively moved in a zigzag trajectory, and then maintained at final position by external forces. The subject then estimates the elbow joint angle. (4) Passive movement-active posture: After the arm is moved passively, the subject tenses his arm so as to actively maintain final posture. He then estimates his joint angle. For all conditions, the subject does not see his arm during the movement, but after each trial, receives visual feedback.

The data were examined by both Information Theory and a conventional error analysis. The results were: (1) Absolute position sense for passive movements followed by either active or passive posture was considerably worse than the precision attained by both the deliberate smooth and the correcting movements; i.e., the subject could move more precisely to a target than he could estimate by absolute position sense. (2) Correcting movements were slightly more precise than the deliberate smooth ones. The small amount of improvement of the correcting versus smooth deliberate movements is quantitatively accounted for by the conscious position sense results. This suggests that the difference in precision between movements and position sense is not due to the former having access to better position feedback than that available to consciousness.

How can one move consciously to a target more precisely than one knows where one's arm is? Corollary discharge and proprioceptive feedback by themselves are not precise enough to explain the precision of voluntary movements. This paradox might be explained by the hypothesis that the system has a more precise way to compare "motor" information, i.e. corollary discharge and proprioceptive feedback, with visual feedback after each trial than it can judge "motor" information on an absolute basis. This more precise comparative information is then used to increase the accuracy of future movements.

240.7 DYNAMIC INTERACTIONS BETWEEN LIMB SEGMENTS DURING PLANAR ARM MOVEMENT. T. Flash* and J. M. Hollerbach* (SPON: T. Zeffiro). Dept. of Psychology, M.I.T., Cambridge, MA 02139.

Movement of multiple segment limbs requires generation of appropriate joint torques which include contributing terms arising from dynamic interactions among the moving segments. These interactions, arising from reaction, centripetal, and Coriolis forces, are not present for single joint movements. This work is an assessment of the significance of the interaction forces relative to single joint movements for planar two degree of freedom arm movement.

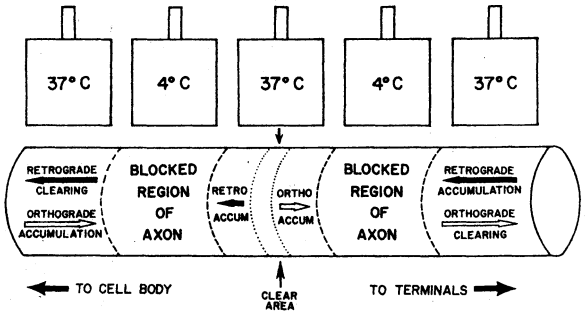
Measurements of human arm movement between two targets, involving the shoulder and elbow joints, were made to yield a time sequence of joint angles and, after differentiation, the joint velocities and accelerations. A general purpose simulation program for arbitrary open loop kinematic chains was developed and used to solve for the interaction forces given the experimental kinematic data. Segmental parameters such as principal inertias, lengths, masses, and internal axes were obtained from a computational model by Hatze (C.S.I.R. Tech. Report, TWISK 79, Pretoria, 1979).

The results indicate that the dynamic interaction terms are significant, even for slow movements. Consequently, models of movement which simplify the motor control dynamics by exclusing some interaction terms, usually the velocity terms, can lead to major errors in the computed magnitudes of the joint torques. This study stresses the fact that motor control programs must devise means for compensating for joint interactions during multiple joint movement. These interactions cannot be overlooked when physiological studies of trajectory formation by multiple segment limbs are conducted. (Research supported by NIH grant AM26710.)

241.1 THE MEMBRANOUS RETICULUM WITHIN MYELINATED AXONS IS NOT RAPIDLY TRANSPORTED. Mark H. Ellisman and James D. Lindsey, Department of Neurosciences, University of California, San Diego, School of Medicine, La Jolla, CA 92093.

The axoplasmic reticulum has been described for myelinated axons in many reports as an anastomosing network of tubules. We have used a focal chilling method to block axonal transport in two places (see diagram below). After 4 hrs. all axoplasmic materials transported faster than 25 mm/day should clear from this central warm area. Whatever formed elements that remain in this central region would not have been rapidly transported. These double blockade experiments were conducted on mouse saphenous nerve. In these studies we have found that: 1) the axonal reticulum does not accumulate against a block designed to trap orthogradely moving vectors; 2) there is no evidence of continuity between the axonal reticulum and the orthogradely moving vectors; 3) the axonal reticulum remains in the region between two cold blocks were only non-motile elements should be found; 4) the axonal reticulum does not accumulate against a dam designed to trap retrogradely moving elements; 5) although retrogradely moving elements appear to associate with the axonal reticulum they do not fuse with it and are not contained in it.

We conclude that the axoplasmic reticulum represents a separate membrane system from either orthogradely or retrogradely moving rapid transport vectors and that this cisternal system is not rapidly transported. Supported by grants to MHE from MDAA, MS, and NIH NS14718.



241.2 AXONAL TRANSPORT OF NA-K-ATPASE IN HAMSTER OPTIC NERVE. Susan C. Specht, Teresa Candelas* and Roberto Ocasio-Rivera*. Dept. of Pharmacol., Univ. P. R. Sch. Med., San Juan, P. R. 00936.

The NaK-ATPase, a major membrane-bound enzyme of neural tissue, is responsible for maintaining transmembrane NaK-gradients. Nerve endings are particularly enriched in this enzymatic activity. The mammalian enzyme consists of two subunits, a catalytic subunit and a smaller glycoprotein of unknown function. Enzymatic activity in rodent brain increases markedly during the first two weeks of life. To obtain a better understanding of factors involved in the development of nerve ending membrane, we have examined the axonal transport and enzymatic activity of NaK-ATPase of adult and immature (12 days-old) hamster brain.

The NaK-ATPase of hamster brain was partially purified by NaI and glycerol treatments. The catalytic subunit was identified by phosphorylation with ^{32}P-ATP in the presence of 100 mM NaCl or KCl. Its approximate molecular weight on SDS-PAGE was 96,000. Associated proteins found in most preparations had molecular weights of approximately 47,000 and 51,000. Staining by the periodic acid-Schiff's reaction indicated that both are glycoproteins.

To examine the axonal transport of the enzyme and associated glycoproteins, hamsters were injected intraocularly with ^{35}S-methionine or ^{3}H-fucose (250 μCi per preparation) and sacrificed 24 h later. The enzyme was isolated, run on SDS-PAGE and counted. The data demonstrate that both the catalytic subunit (labeled with ^{35}S-methionine) and the associated glycoproteins (labeled with ^{3}H-fucose) are transported rapidly to the optic nerve ending.

Kinetic parameters were determined for enzyme from both adult and immature hamsters. The pH optimum for both was pH 7.6. The optimal Na/K ratio was 90/60 mM for adult and 140/10 mM for enzyme from 12 day-old hamsters. Activity of the adult enzyme was 96-100% sodium dependent, whereas that from the 12 day-olds was only 88% sodium dependent.

These preliminary studies suggest that investigation into the axonal transport of NaK-ATPase will provide insight into the maturation of nerve ending membrane.

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241.3 AXONAL TRANSPORT OF $NA^{+}K^{+}$ATPASE AND PROTEIN I IN RETINAL GANGLION CELLS. C. Baitinger* and M. Willard* (SPON: D. Gottlieb). Dept. Anat. and Neurobiol., Wash. Univ. Sch. Med., St. Louis, MO 63110.

In order to compare the axonal transport of proteins destined for two different membranous organelles, we identified among the radiolabeled axonally transported proteins NaKATPase (the sodium pump, specifically associated with the axolemma) and Protein I, a synaptic vesicle- and postsynaptic density-associated substrate for cAMP-dependent protein kinase (Bloom, F.E. et al., PNAS 76: 5982,1979). Proteins synthesized in rabbit retinal ganglion cells were labeled by an intravitreal injection of ^{35}S-methionine, and the radioactive axonally transported proteins were recovered in the optic tract and superior colliculus. ATPase and Protein I were separated from other labeled proteins by two-dimensional gel electrophoresis, and identified by comparison with standards identified by their characteristic phosphorylations with γ-^{32}P-ATP (K^{+}-sensitive in the case of the two catalytic subunits of ATPase (α, MW=100K and α+, MW=105K); cAMP-dependent in the case of the two components of Protein I (Ia, MW=85K, Ib, MW=80K). The contribution of local protein synthesis to labeled ATPase and Protein I present in the tract and colliculus was assessed by direct injection of ^{35}S-methionine into these structures; labeled ATPase and Protein I were at most minor components of proteins labeled in this manner.

Both of the catalytic subunits of ATPase were labeled in the optic tract and superior colliculus by 3 hours, indicating that they are transported down the axons at a velocity of at least 240 mm/day, the velocity of the most rapidly transported proteins (Group I). ATPase remained labeled in both the optic tract and superior colliculus for at least 16 days, consistent with the interpretation that it turns over slowly at its destination in the axolemma. Labeling of Protein I was first detected in the optic tract at 6 hr, increased until 4 days, and was barely detectable at 8 days; on the other hand, Protein I remained heavily labeled in the colliculus until at least 16 days. This behavior is consistent with the interpretation that Protein I passes through the axons and accumulates in the synaptic terminals, as expected for a synaptic vesicle-associated protein.

These results provide the first assignment of a function to an electrophoretically identified Group I protein, the NaKATPase, supporting the hypothesis (Lorenz & Willard, PNAS 75:505, 1978) that Group I serves in whole or in part to supply proteins to the plasma membrane. The delayed labeling of Protein I is a characteristic of Group II proteins; the reported association of Protein I with synaptic vesicles is consistent with the possibility that Group II (which includes mitochondria) serves to supply cytoplasmic membrane-bounded organelles.

241.4 VELOCITY AND METABOLISM OF AXONALLY TRANSPORTED LABELED CATECHOLAMINES IN BULLFROG SYMPATHETIC NERVE. W. J. Litchy*, S. Brimijoin, and C. Reiter*. Depts. Neurology and Pharmacology, Mayo Fdn., Rochester, MN 55905.

Values for transport velocity of endogenous norepinephrine have previously been reported in mammals, amphibians, and teleosts. However, direct, full scale studies of the transport of exogenous, radiolabelled catecholamines have never been carried out. We now report on the metabolism and axonal transport of ^{3}H-labeled catecholamines in adrenergic axons of the sciatic nerve of the bullfrog (Rana catesbeiana). After injection of ^{3}H-norepinephrine (sp. act. 46.5 Ci/mmole) into the capsule surrounding a sympathetic ganglion (S_G) the sciatic nerve was incubated for 4, 8, and 12 hours at 20°C in a frog Ringer's solution. After incubation the nerve was cut into 3-mm sections, which were homogenized in 0.4 N $HClO_4$ with 10 mM EDTA, and centrifuged at 1000 g for 10 minutes. Radioactivity in portions of the supernatant fractions was determined by liquid scintillation spectroscopy. A profile of labeled material was observed that migrated rapidly in a distal direction. From the displacement of the front of this profile, a maximum transport velocity of 5.3 mm/hour was calculated. This is similar to the velocity determined by stop-flow techniques for endogenous catecholamines, when corrected for temperature. Regression analysis of this data indicated that the lag time for transport was less than 20 minutes as compared to 90 minutes for proteins labeled with ^{35}S-methionine.

To determine the nature of the catecholamines that were transported, the material that accumulated at a ligature was analyzed by reverse-phase high performance liquid chromatography (HPLC) using electrochemical detection. It was found that 8 hours after injection greater than 95% of the radioactivity that accumulated at the ligature had an elution time similar to that of an epinephrine standard. On the other hand, only about 50% of the radioactivity remaining in the ganglia had an elution time similar to epinephrine. The remainder eluted with the norepinephrine standard. We conclude that the synthesis and processing of transported catecholamines in this species is largely completed in the nerve cell body. (Supported by Mayo Foundation and NIH Grant NS 11855.)

241.5 BLOCK OF FAST AXONAL TRANSPORT CAUSED BY MICRO-INJECTION OF THE ACTIN DEPOLYMERIZER, DNase I, INTO THE AXON OF AN IDENTIFIED NEURON. Daniel J. Goldberg, Dept. Pharmacol. and Center for Neurobiol. & Behav., Columbia U. Coll. P&S, New York, N.Y. 10032

It has often been suggested that actin participates in generating the force for fast axonal transport. Until recently there has been scant experimental support for this idea, one difficulty having been the lack of specific actin inhibitors that could easily permeate the axolemma to gain access to the transport apparatus. Within the past year, this difficulty has been surmounted by micro-injecting inhibitors directly into the cell bodies of giant identified invertebrate neurons. Deoxyribonuclease I (DNase I), which, in addition to its enzymatic activity, specifically depolymerizes actin filaments, decreased the transport of serotonin in the giant cerebral neuron of Aplysia (Goldberg et al., Proc. Nat. Acad. Sci., U.S.A., 77:7448, 1980) and of protein in the leech Retzius cell (Isenberg et al., Brain Res., 194:588, 1980). Because the inhibitors were injected into the cell bodies, however, it could not be concluded that they blocked the actual movement of material along the axon rather than the assembly of organelles in the cell body or the export of the organelles into the axon.

In the experiments reported here, DNase I has been unequivocally shown to block fast axonal transport by injecting it directly into the axon. In initial experiments, DNase I was injected into the cell body of the Aplysia cell R2 prior to intrasomatic injection of ^{3}H-fucose to label glycoprotein for transport. During a 4 hour period after ^{3}H-fucose injection, much less ^{3}H-glycoprotein exited the ganglion in the axon of R2 (measured as a percentage of total ^{3}H-glycoprotein in the cell) than in the absence of DNase I. The amount of ^{3}H-fucose incorporated into glycoprotein in the cell body was also greatly reduced, indicating that DNase I injected into the cell body can inhibit stages in the processing of material for transport.

In subsequent experiments, DNase I was injected into the axon of R2 rather than the cell body. It was found that essentially no ^{3}H-glycoprotein progressed past the site of DNase I injection and, in fact, a very large accumulation of radioactivity was observed just proximal to that site.

In addition to demonstrating that an actin depolymerizer blocks fast transport of materials along the axon, these experiments establish a protocol to assess unambiguously the effects of large, hitherto unusable, probes, such as enzymes and antibodies, on transport within the axon.

241.6 CHANGES IN THE AXONAL TRANSPORT OF THE CYTOSKELETON DURING DEVELOPMENT, AGING, AND REGENERATION. P.N. Hoffman, J.W. Griffin* and D.L. Price*. Depts. of Ophthalmology, Neurology, and Pathology, The Johns Hopkins Univ. Sch. of Med., Baltimore, MD 21205.

In order to test the hypothesis that the pattern of slow axonal transport in regenerating neurons resembles the developing state, we have examined the effects of development/aging on the axonal transport of the principal cytoskeletal proteins (actin, tubulin, and the neurofilament triplet polypeptides) and compared these results with studies of the transport of these proteins in regenerating neurons.

Axonal transport of the cytoskeletal proteins was examined in the motor neurons of male Sprague-Dawley rats at 3 and 8 weeks and one year of age. The transport velocities of each of the principal cytoskeletal proteins declined with age. At 3 and 8 weeks and one year of age, the leading edge of the actin wave moved at average rates of 5.2, 3.9, and 2.1 mm/d, respectively, while tubulin was transported at average rates of 3.8, 2.8, and 1.8 mm/d, respectively. The average velocity of the neurofilament triplet proteins also declined.

Hoffman and Lasek [Brain Res. 202:317-333, 1980] have shown that the transport of actin and tubulin is increased during axon regeneration. The present study indicates that this increase is maximal at two weeks postaxotomy; transport returns to normal by six weeks. In eight-week old animals two weeks postaxotomy, the leading edge of the actin and tubulin waves moved at average rates of 7.0 and 6.1 mm/d, respectively, as compared to 3.9 and 2.8 mm/d in controls. The total level of radioactivity in both of these proteins was significantly increased during regeneration (120-150% of control values). In contrast, at two weeks postaxotomy, the level of radioactivity in the neurofilament triplet was reduced to 50% of control values.

The transport velocities of actin and tubulin fell more than twofold between three weeks and one year of age. This trend was dramatically reversed during regeneration during which the velocities of these proteins exceeded those of the three-week old animals. In terms of transport velocity, this pattern resembles the profiles occurring in developing nerve cells. In addition, the level of radioactivity in the neurofilament triplet proteins was reduced by half during regeneration at the same time that the labeling of both actin and tubulin was significantly increased. Since the embryonic motor axon is characterized by an abundance of microtubules and fewer neurofilaments, this change leads to a pattern similar to that seen during development.

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242.1 LOCAL SPIKING INTERNEURONES AND THE CONTROL OF LEG MOVEMENTS IN THE LOCUST. M.Burrows and M.V.S. Siegler*. Department of Zoology, University of Cambridge, Cambridge CB2 3EJ, U.K.

The local neurones involved in the control of limb movements, and described so far in the thoracic ganglia of insects, have proved to be unusual in that they do not produce action potentials. They synapse upon the motor neurones, which they control by the graded release of transmitter. These interneurones form a population diverse in their morphology, and in their physiological effects.

We now describe a population of local interneurones in the meso- and metathoracic ganglia of the locust that normally do produce action potentials. Anatomically, they are a diverse, and probably large population, as revealed by intracellular staining. They have extensive dendrite arborizations in both the ventral neuropile, to which sensory neurones predominantly project, and in the dorsal neuropile, where motor neurones have the majority of their branches. Their cell bodies form two ventral groups; one close to the midline of the ganglion, the other lateral and close to the emergence of the first lateral nerve. No neurone that has been stained has a process that can be readily described as an axon. At a gross level, the spiking local neurones are not easily distinguishable from the non-spiking ones. Each local spiking interneurone can be activated either by specific movements of one hind leg, but no other legs, or by stimulation of specific sense organs in that leg. For example, an interneurone may respond only to extension of the femoral-tibial joint, or only to stimulation of a few hairs on the femur. By contrast, non-spiking interneurones each respond to diverse sensory inputs. Anatomical evidence suggests that there are several spiking local interneurones that respond to each of the different sensory stimuli. The interneurones spike readily when depolarized with injected current, but there is no apparent motor effect, measured either from extracellular myograms, or from simultaneous intracellular recordings in motor neurones. This is in contrast to the results of stimulating the non-spiking interneurones, where small currents produce obvious motor effects. It is possible that only the summed activity of several spiking local interneurones, which are normally activated together, is sufficient to produce a motor effect.

Local spiking interneurones represent yet another population of small neurones that must be taken into account when explaining the neural control of locomotion. Could they be responsible for the primary integration of information supplied by the massive numbers of sensory neurones?

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242.2 PROPRIOCEPTIVE DEPENDENT INHIBITORY PATHWAYS TO HINDLEG FLEXOR MOTORNEURONS IN THE LOCUST. J. D. Steeves and K. G. Pearson. Dept. of Zoology, UBC , Vancouver, B.C. , V6T 2A9 , and Dept. of Physiology, Univ. of Alberta, Edmonton, Alberta , T6G 2H7.

A distinctive behavior of many animals is a ballistic movement to avoid capture. One ballistic escape response is the jump of the locust which is produced by a stereotyped motor pattern (Heitler and Burrows, J. Exp. Biol., 66: 203-220, 1977). Initially there is a complete flexion of both hindleg tibiae, followed by a period of coactivation of flexor and extensor motorneurons (MNs). The jump arises from the sudden inhibition of the flexor tibiae MNs. This allows the isometrically contracted extensor tibiae muscles to rapidly shorten, thereby extending the tibiae, launching the animal into the air.

Recently a pair of interneurons (M-neurons) have been identified in the metathoracic ganglion (one for each hindleg) whose properties suggest they are the trigger neurons for the jump (Pearson et al., J. Neurophysiol., 43: 257-278, 1980). These M-neurons monosynaptically inhibit flexor tibiae MNs, have a high threshold for activation, and receive strong excitatory input from several sensory modalities (visual, auditory, tactile, and proprioceptive). Due to the high threshold for spike initiation in the M-neurons, the external sensory inputs alone (eg. movement within the visual field) rarely activate the M-neurons. However during the coactivation of the flexor and extensor tibiae MNs, preceeding the jump, proprioceptive feedback depolarizes the M-neurons thereby decreasing their threshold for activation. It has been suggested that only during this period could external sensory stimuli activate the M-neurons and trigger a jump (Pearson et al., 1980).

In order to test this hypothesis, intracellular recordings were obtained from metathoracic flexor MNs and the M-neurons according to previously described methods (Pearson et al., 1980). Inhibitory postsynaptic potentials (IPSPs) in flexor MNs, in response to movements within the visual field or to auditory stimuli, were only seen in the presence of proprioceptive feedback from the hindleg. Furthermore, the M-neurons were activated under the same experimental conditions. An external stimulus alone, in the absence of proprioceptive feedback, rarely produced a burst of activity in the M-neurons or IPSPs in the hindleg flexor MNs. We propose that this proprioceptive "gating" of inhibitory pathways to flexor MNs occurs at the postsynaptic membrane of the M-neuron. Proprioceptive "gating" would ensure the development of suffcient isometric tension in the extensor tibiae muscles before a successful jump is triggered in response to external stimuli. (Supported by the MRC and NSERC of Canada)

242.3 SITES OF LEECH MOTONEURON CONNECTIONS SUGGESTED BY INTRACELLULAR STAINING OF PAIRS OF NEURONS USING HORSERADISH PEROXIDASE AND LUCIFER YELLOW SIMULTANEOUSLY. B. Granzow and W. B. Kristan, Jr., Dept. of Biology, UCSD, La Jolla, CA. 92093.

We are currently employing a double intracellular staining technique in the leech Hirudo medicinalis to investigate the spatial relationships between pairs of identified swim motoneurons. One cell in a ganglion is filled with the enzyme horseradish peroxidase and another in the same gangion is filled with Lucifer Yellow. The ganglion is processed according to the method of Macagno et al., (Brain Res., in press, 1981). Using a combination of uv epi-illumination and transmitted green light, the two cells can be observed simultaneously in whole mounts, and the processes of the individual cells can be distinguished from one another even in areas where they may overlap. In this way, potential sites of synaptic contact are revealed.

We have found, for example, that one motoneuron, cell 1, which inhibits the excitor motoneuron, cell 3, appears to make direct contact with cell 3. Likewise, cell 2, also an inhibitor motoneuron, appears to make direct contact with cell 4, another excitor motoneuron. The sites of electrotonic coupling between bilateral homologues are also suggested using this technique. That these are areas of synaptic contact will require confirmation by electron microscopy.

Our anatomical results support the hypothesis that the inhibitor and excitor motoneurons are monosynaptically connected. The usual physiological tests for monosynaptic connections have been inconclusive in these cases, since the connetions are mediated by transmitter release which is influenced by D.C. changes in pre-synaptic membrane potential without requiring action potentials. In addition, the spike initiation zones of motoneurons are distant from the cell bodies so that current injected into the pre-synaptic cell body in order to drive an action potential, itself causes a post-synaptic response which masks the effect of the action potential. This precludes tests based on a one-to-one relationship between driven pre-synaptic action potentials and IPSPs.

There are two morphologically distinct areas of contact between cell 1 and cell 3. We are extending these experiments to determine whether one or both areas might be involved in synaptic transmission between the two cells: by making selective kills of each of the areas using the cell-kill method of Miller and Selverston (Science 206:702, 1979) and by investigating the ultrastructure of these areas using electron microscopy.

This research was supported by PHS Grant # NS14410.

242.4 LOCAL CIRCUIT INTERNEURONS AND MOTOR NEURONS IN THE RESPIRATORY SYSTEM OF THE CRAB. R. A. DiCaprio and C. R. Fourtner. Dept. Biological Sciences, SUNY/Buffalo, Buffalo, NY 14260.

The respiratory system in the crab, Carcinus meanas, has been described by Young (1975; J. comp. Physiol. 101: 1-37). The motor pattern consists of reciprocal activity in at least five levator and five depressor motor neurons. Simmers and Bush (1980; Brain Res. 197: 247-252) have recently described physiologically three interneurons which are either active during the respiratory rhythm or can control the frequency of the rhythm. We have extended their studies using Lucifer-yellow (Stewart, 1978; Cell 14: 741-759) filled electrodes to record from and identify motor neurons and interneurons. Of particular interest in our studies thus far are two local circuit interneurons. The first interneuron produces non-spiking oscillations, of approximately 8 mv, which are in phase with the respiratory rhythm; the depolarizing phase occurring during the levator burst and the hyperpolarizing during the depressor burst. Application of steady hyperpolarizing current slowed the rhythm by increasing the duration of both the levator and the depressor bursts. Depolarizing pulses applied at any time during the cycle could reset the rhythm. Cleared whole mounts following Lucifer-yellow injection revealed a local circuit neuron with its soma on the lateral edge of the ganglion and anterior to a highly branching dendritic field, located in an extremely lateral position in the neuropile. The second interneuron produced low amplitude, approximately 1.0 mv, hyperpolarizations which were coincident with depressor activity. During periods of no respiratory rhythm, depolarization of this interneuron started the rhythm and an increase in the depolarization increased the frequency of the rhythm; subsequent hyperpolarization stopped the rhythm. This particular interneuron had two separate dendritic areas: one similar to that of the first interneuron and the second dendritic area in a medial region of the neuropile. The cell body was anterior to the dendritic fields and mid-way between the lateral edge and the mid-line of the ganglion. Our physiological and morphological studies clearly demonstrate that local circuit, non-spiking interneurons are an integral part of the central pattern generator producing the respiratory rhythm in the crab. (Supported by grants IF32NS06277 to R.A.D.; K04NS00141 and UB H079 to C.R.F.).

242.5 REPETITIVE AND SPONTANEOUS DRIVER POTENTIALS IN CRUSTACEAN CARDIAC GANGLION NEURONS. A. Berlind. Bekesy Laboratory of Neurobiology, 1993 East-West Rd., Honolulu, HI 96822 and Biology Department, Wesleyan University, Middletown, CT 06457.

In crustacean cardiac ganglion neurons a slow depolarization, the driver potential (DP), underlies each burst of spikes. DPs can be isolated by treatment with tetrodotoxin; they have been shown by Tazaki and Cooke to involve Ca^{++} influx and to be terminated by a V-dependent K^+ current and possibly a Ca^{++}-activated K^+ current. DPs were recorded from large cells (motorneurons) in ganglia of the crabs Podophthalmus vigil and Portunus sanguinolentus. In contrast to previous results in Portunus, where a brief (20-100 msec) depolarizing stimulus is required to elicit each DP, a large percentage of Podophthalmus ganglia in TTX exhibit periodic, long-lasting (1-5 min) spontaneous trains of DPs. In ganglia that do not show spontaneous DPs, long-lasting trains can usually be elicited by a strong 3-sec depolarizing current pulse, by a rapid series of brief pulses, or by treatment with dopamine. The Podophthalmus ganglia can also be driven by 3 sec depolarizing current pulses to fire repetitive DPs, the frequency of which increases with increasing current strength. This contrasts strongly with Portunus, where long depolarizing current of any suprathreshold intensity evokes only a single DP. In Portunus, neither suppression of the V-dependent K^+-current by tetraethylammonium (50 mM), nor suppression of a fast K^+-current by 4-aminopyridine (2 mM) allows repetitive DPs in response to a long current pulse; treatment with procaine (5 mM) allows repetitive DPs. Evoked DPs in Podophthalmus and Portunus also exhibit distinct differences in form and ion dependency. The Podophthalmus DP rises much more rapidly and is of shorter duration, is less effectively suppressed by the addition of Mn^{++} to the medium, and is not increased in amplitude by raising the external Ca^{++} concentration or lowering Na^+. The results 1) show that DP mechanisms may show distinct differences in detail even in closely related species; 2) suggest that endogenous properties of individual neurons might play a considerably more important role in the generation of rhythmic output by this system than has previously been assumed.

Supported in part by NIH grant NS11808 to I.M. Cooke.

242.6 INTRACELLULAR RECORDINGS FROM IDENTIFIED MOTORNEURONS IN THE NEMATODE ASCARIS. R. E. Davis* and A. O. W. Stretton. Neurosciences Program and Department of Zoology, University of Wisconsin, Madison, Wisconsin 53706.

We have been investigating the motornervous system of the large parasitic nematode, Ascaris (Stretton, A. O. W. et al., PNAS 75:3493, 1978). The dorsal and ventral nerve cords of Ascaris are connected by a repeating pattern of single identified motorneuron processes, called commissures. A mat of muscle cells overlies the commissures. After removing a portion of this muscle with fine forceps single commissures can be visualized and penetrated with a microelectrode. The large diameter of the commissural process (10-20 μ) facilitates these electrophysiological recordings.

The dye, Lucifer Yellow (Stewart, W. W., Cell, 14:741, 1978) has been injected into motorneurons through intracellular microelectrodes and the neurons identified by subsequent fluorescence microscopy. Utilizing high resistance 3M KCl-filled microelectrodes (60-100 MΩ) we have obtained intracellular recordings from representatives of each commissural motorneuron type. Resting membrane potentials vary between -25 and -40 mV depending on the motorneuron type being investigated. Excitatory motorneurons tend to have higher resting potentials than inhibitory motorneurons.

Two excitatory motorneuron types (DE1, DE3) show rhythmic spontaneous inhibitory post-synaptic potentials (IPSPs); one excitatory motorneuron type (DE2) exhibits patterned excitatory post-synaptic potentials (EPSPs), probably from two different sources. The inhibitory motorneuron types (DI, VI) display membrane potential oscillations. Current research is being directed at the following questions: (1) the membrane properties of the motorneurons, (2) the ionic bases of their signals and (3) the physiology of the synaptic interactions known anatomically to exist between motorneurons.

Supported by USPHS Neuroscience Training Grant 5T32 GM 7507 and Grant # AI 15429.

243.1 EVIDENCE FOR A PASSIVE INFLUENCE ON NEURAL CREST CELL DISTRIBUTION IN THE TRUNK OF THE AVIAN EMBRYO. M. Bronner-Fraser* (SPON: R. Eckert). Dept. of Physiol. and Biophys., Univ. of Calif., Irvine, CA 92717.

Neural crest cells migrate along two pathways in the trunk of avian embryos: a dorsolateral route just under the ectoderm; and a ventral route between the neural tube and the somite. The mechanisms causing crest migration to defined loci are not understood. The evidence presented here suggest a role for passive environmental influences, independent of cell motility.

Previous experiments have used an injection technique to introduce neural crest and other cell types to the ventral neural crest pathway (Bronner-Fraser and Cohen, Devel. Biol., 77:130, 1980). These studies demonstrated that neural crest-derived pigment cells are capable of migrating ventrally even after differentiation; however, somite cells and skin fibroblasts do not migrate along the ventral route.

In the present study, retinal pigment epithelium cells (RPE) were injected onto a neural crest pathway by the same injection method. These cells are the only pigmented cells in the body which are not of neural crest origin. Like crest-derived pigment cells, these RPE cells distribute along the ventral neural crest pathway. Unlike neural crest pigment cells which extend processes after injection and look morphologically like migratory cells, the RPE cells remain rounded and do not appear to be actively moving. This observation raises the possibility that factors other than active migration may affect localization of the injected RPE cells (and perhaps endogenous crest cells as well). To test this possibility, monodispersed latex polystyrene beads were injected onto the ventral pathway as an inert probe of the environment. These beads are of approximately the same size as neural crest cells. The latex particles distribute along the neural crest pathway with a time course similar to that of endogenous crest cells. In the majority of cases, the beads localize near the sympathetic ganglia or adjacent to the dorsal aorta, in close proximity to endogenous crest cells. It is unlikely that the latex particles are adhering to migrating neural crest cells; in tissue culture, only a small percentage (on the order of 1%) of neural crest cells nonspecifically bind latex beads. The results suggest that there exists a passive component which influences the distribution of neural crest cells as well as non-motile substances along the ventral crest pathway. The fact that other cell types (somites and fibroblasts) do not migrate along this pathway may indicate that certain cells have intrinsic or cell surface characteristics which override the passive environmental effects.

243.2 NUCLEUS LOCUS COERULEUS: A MORPHOMETRIC GOLGI STUDY IN RATS OF THREE AGE GROUPS. S. Diaz-Cintra*, L. Cintra*, T. Kemper* and P. J. Morgane. (SPON: W. Stern). Worcester Foundation for Expt. Biology, Shrewsbury, MA 01545.

Using Rapid Golgi and Nissl techniques, three major cell types were identified in the nucleus locus coeruleus of male rats at 30, 90 and 220 days of age: fusiform, multipolar and ovoid. One hundred neurons per age group, encompassing all three cell types from all parts of the nucleus, were selected for quantitative study. The following measures were made on all cells in the three age groups: major and minor axes of the cell body, number of somatic spines, number of primary and secondary dendrites, diameter of primary and secondary dendrites, linear extent of primary and secondary dendrites and the number of dendritic spines on primary and secondary dendrites (the latter measured along a 50 micron segment near the midpoint of the dendritic length). The most striking age-related changes were that all three cell types showed significant decreases in spine density on both primary and secondary dendrites between 30 and 90 days of age with the most marked decreases seen in the ovoid cells. On the other hand, between 90 and 220 days all three cell types showed significant increases in primary and secondary dendritic spine densities. The most marked increase in dendritic spine density was seen in the ovoid cells. Perisomatic spine counts were also significantly decreased in all three cell types between 30 and 90 days with the greatest decrease (52%) seen in the multipolar cells. Only the ovoid cells showed a significant increase in perisomatic spines between 90 and 220 days of age (35%). Special neuro-vascular relations of dendrites of locus coeruleus neurons to blood vessels, such as we previously observed in the nucleus raphe dorsalis, were not seen. Relative to dendritic branching, the cells of the locus coeruleus show a typical reticular type organization with prominent dendritic fields extending widely outside the "nucleus." These dendrites extend into the central gray and dorsal nucleus of Gudden as well as into the trigeminal nucleus laterally. In the dorsal nucleus of Gudden there is considerable overlap with dendrites of neurons of the nucleus raphe dorsalis. It is of interest that the dendritic spine densities of the 3 types of locus coeruleus cells show exactly opposite developmental tendencies from those we previously described in the nucleus raphe dorsalis (Brain Res. 207: 1-16, 1981). In the nucleus raphe dorsalis each cell type showed significant increases in dendritic spine density between 30 and 90 days followed by significant decreases in dendritic spine density between 90 and 220 days of age. (Supported by NSF grant 79-22507 and NICHD grant HD-06364).

243.3 THREE DIFFERENT AND POSSIBLY COORDINATED PATTERNS OF HISTOGENESIS IN THE RABBIT'S TELENCEPHALON : AUTORADIOGRAPHIC EVIDENCE. Victor Fernández* and Rodrigo O. Kuljis (SPON: A. L. de Blas). Dept. of Physiology and Biophysics, University of Chile and Dept. of Neurobiology and Behavior, S.U.N.Y. at Stony Brook, New York 11794.

Autoradiographic studies of CNS development have consistently shown that cell migration and deposit sequences (histogenic gradients) depend on the topographic location of the structures under formation, rather than on their functional role in the adult (Fernández et al., Brain Behav. Evol. 16:113-128, 1979). Here we analyze the histogenic gradients of three functionally different areas that, being telencephalic derivatives, share a close topographic relationship in embryogenesis.

Fifty-four rabbit embryos ranging from the 15th day of gestation up to the newborn stage received a single pulse of ^{3}H-thymidine, via maternal intraperitoneal injection (5mCi/Kg). After different survival periods, development of the septal, neostriatal and neocortical areas was studied autoradiographically.

The septal area develops between the 15th and at least the 25th embryonic days (nucleus accumbens continues further), following a medial to lateral gradient. Thus, cells are laid progressively closer to the neuroepithelial layer from which they originated. The neocortical area develops between the 15th and 22nd embryonic days according to a ventro-lateral to dorso-medial gradient. In this case cells are laid progressively farther from their original matrix layer. The neostriatal area develops between the 15th and 18th embryonic days along a medio-ventral to latero-dorsal gradient. In this instance cells are laid in isochronic columns roughly perpendicular to their germinal layer, such that cells originating at the same time are laid at different distances from this layer, and they are deposited in an orderly fashion according to their time of birth (Fernández et al, loc.cit.).

Although these gradients seem to be quite different when considered individually, it is possible to view them as forming part of a common topographic-but not temporal-gradient involving the whole telencephalon. A topographical sequence can be imagined, in fact, which would start at the medial portion of the septal region, involving progressively more lateral structures. After passing through the neostriatal area, this gradient would reach the basal area of the neocortical primordium, sliding along the lateral wall of the lateral ventricle, and fanning out across the entire extent of the cortical plate. We hypothesize that this situation could be due to the modification of the simple deposit sequence (such as that seen in the septal area) by factors operating as mechanical guides along the path of cell migration and deposit at the neostriatal and neocortical levels(Kuljis and Fernández, in preparation). Fogarty 1F05 TW02947 & U.Ch. B-160-805.

243.4 ADJUSTMENT OF CONNECTIVITY IN RATS DEPRIVED OF LAYERS II-IV OF CEREBRAL CORTEX BY PRENATAL DESTRUCTION OF PRECURSOR CELLS. L. Yurkewicz, E.G. Jones, K.L. Valentino, and J.W. Fleshman, Jr. Dept. of Anatomy & Neurobiology and McDonnell Center for the Study of Higher Brain Functions, Washington University, School of Medicine, St. Louis, Missouri 63110.

Injection of the cytotoxic drugs methylazoxymethanol acetate or 5-azacytidine to pregnant rats at 15-17 days gestation leads to the birth of infants that grow to maturity lacking layers II-IV of the cerebral cortex but with earlier and later developing parts of the brain intact. In the affected animals, at maturity, we studied the structure of the sensory-motor regions with Nissl and Golgi methods, their input-output connections with anterograde and retrograde tracing methods and the receptive field properties of neurons in the cortex and ventroposterior thalamic nucleus, with electrophysiological methods.

The remaining layers V and VI are structured and subdivided as in normal rats but layer V is surmounted by a molecular layer. Pyramidal and non-pyramidal neurons are visible. Pyramidal neurons are normal except in the superficial half of layer V where many have widely divaricated apical dendritic trees and a high proportion are inverted. Layer VI cells project only to the thalamus; layer V cells, including the inverted and atypical pyramids, project into the pyramidal tract. A number of islands of aberrant cells whose migration may have been arrested, occur in the external capsule. These cells though abnormally oriented, have many of the characteristics of pyramidal neurons and project axons through the corpus callosum which is much reduced in size.

Thalamic afferents terminate in a bistratified manner; (abnormally) just superficial to the somata of the deeper layer V pyramids and (normally) at the junction of layers V and VI. Mapping of unit responses in the cortex and in the structurally normal thalamus indicates that body somatotopy is preserved and that receptive field sizes are unchanged from normal.

These findings show that the destruction of target cells in the cortex prior to the arrival of thalamic afferents does not prevent the afferents from colonizing the cortex and establishing a normal somatotopically organized pattern of connections, partly in an abnormal position. This is associated with changes in the morphogenesis of pyramidal cells though these still appear to send their axons to appropriate targets.

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243.5 QUANTITATIVE ANALYSIS OF NEURONAL MORPHOMETRY: ORDER AND ANGULAR STUDIES OF HIPPOCAMPAL PYRAMIDS FROM NORMAL AND ABERRANT HUMAN BRAIN. Albert M. Paldino. Dept. of Neuroscience, Rose F. Kennedy Center for Research in Mental Retardation and Human Development, Albert Einstein Coll. of Med. of Yeshiva Univ., Bronx, New York 10461.

Morphometric analyses have been carried out on hippocampal pyramidal neurons from six human infants. Five of these infants were preterm, with gestational periods ranging from 22-to-30 weeks; all had normally developed brains as assessed by routine gross and microscopic examinations. The sixth infant was born at 36 weeks and survived for three weeks; chromosomal studies performed on this infant confirmed the clinical impression of TRISOMY 18.

Small blocks of tissue obtained at autopsy were fixed with formalin and subjected to the Rapid Golgi process. Three dimensional coordinate data representing individual neuronal structures were obtained with a computer microscope and mathematical analyses were performed on these data by computer programs written in FORTRAN.

Preliminary branch ordering studies have been performed on these neurons by the centrifugal (somatofugal) method. Initial segments projecting from the soma were assigned order 1; mother segments of order N gave rise to daughter segments of order N+1. These studies included the calculation of the average number of segments/order/cell, total length/order/cell, and average segment length/order/cell for both apical and basilar dendritic systems. The results from normally developed cortex suggest the following: The average dendritic segment length/order/cell is small during the early stages of gestation (18-to-26 weeks) and subsequently increases during the latter stages of gestational development (26-to-30 weeks); the average number of basilar segments/order/cell systematically increases throughout the gestational period. For the infant of 36 weeks gestational age (g.a.) with TRISOMY 18, the average dendritic segment length/order/cell for both apical and basilar dendritic systems suggests a staggered level of development.

Our previous studies in normally developed human cortex demonstrated that dendritic branch and fission angles could be reliable indices of maturation. Preliminary investigations of these angular parameters in the 36 weeks g.a. infant with TRISOMY 18 and three weeks of extrauterine survival suggest that this infant attained a level of cortical development comparable with that of a normally developed infant of 26-to-30 weeks g.a. These quantitative assessments of a developmental lag in cortical neurons of an infant with confirmed TRISOMY 18 may facilitate an understanding of the neuronal basis of mental retardation.

243.6 DEVELOPMENTAL CHANGES OF THE SUBPLATE LAYER IN THE FRONTAL CORTEX OF HUMAN FETUS. I. Kostović, Dept. of Anatomy, Med. Faculty, 41001 Zagreb, Yugoslavia.

In a previous study /Kostović and Molliver,Anat.Rec.,178: 395,1974/ we have demonstrated the presence of prominent synaptic lamina /subplate layer, s.l./ below the cortical plate in the neocortex of human fetuses. Experimental studies in primates suggested that s.l. may serve as a"waiting" compartment for incoming cortico-cortical fibres during critical period of fissural development and that subplate neurons participate in the morphological rearrangements after prenatal cortical injury /Goldman-Rakic,Pregr. Brain Res., 53:3,1980/. To examine developmental changes of the subplate layer and their relation to fibre growth and gyral development, orbito-lateral frontal cortex of human fetuses ranging between 15 and 38 weeks of gestation /w.g./ was analyzed by means of Nissl, Gless silver, Golgi and acetylcholinesterase / AChE/ methods.

Several features characterize neuronal organization of the subplate layer /situated below the cortical plate and above the fetal "white" matter/: a/ presence of loose axonal network with numerous growth cones,b/ the multiplicity of neuronal types with random distribution, c/ permanent shifts in AChE reactivity. The following developmental changes were observed: a/ Gradual increase in the thickness between 15-32 w.g. /up to 10 mm/, thereafter,during rapid development of secondary gyri, there is a gradual decrease of s.l.This change starts in the orbital-inferior frontal gyrus while in the middle-superior frontal gyrus prominent s.l. was found through 31-36 w.g.,b/ AChE reactivity appears at 16 w, transient strong staining was observed at 22 e.g., later there is gradual decrease in AChE reactiviy, c/after 32 w.g. reduction of axonal network was found and many subplate neurons become incorporated into approaching fascicles of gyral "white" matter. This change begins in the orbital cortex and proceeds towards lateral frontal gyri.

In conclusion, subplate layer reaches its greatest prominence during a period /22-34 w.g./ of intensive growth of cortical fibres and formation of primary gyri in the human frontal lobe. Several weeks before birth, concomitantly with the reduction of axonal network below cortical plate and formation of secondary gyri, s.l. become less conspicuous. We suggest that s.l. is reduced by growth of its axons into cortical plate and by incorporation of subplate neurons in the gyral white matter./Support: SIZ V and RSIZ Spec.obraz./

244.1 TRIMETHYLTIN INDUCED HYPERACTIVITY: TIME COURSE AND PATTERN. P. H. Ruppert, T. J. Walsh, L. W. Reiter and R. S. Dyer. Neurotoxicology Division, Health Effects Research Laboratory, US Environmental Protection Agency, Research Triangle Park, NC 27711.

Trimethyltin (TMT), a neurotoxic organotin compound, produces structural damage primarily in the hippocampus, but also in the pyriform cortex, amygdaloid nucleus and neocortex. Limited neuropathological changes are evident as early as 2 days following a single dose of TMT. Maximal damage is seen 21 days following dosing and persists for at least 70 days post-treatment (Brown et al., Am. J. Path. 97:57-82, 1979). Swartzwelder et al. (Neurotoxicol., submitted) have reported that TMT rats were hyperactive in a 2 min open field test 40 days after dosing. The purpose of the present experiment was to determine the time course, dose dependence and circadian pattern of hyperactivity produced by acute exposure to TMT.

Adult male Long-Evans hooded rats (N=10/group) were intubated with either 0, 5, 6, or 7 mg/kg trimethyltin chloride as the base given in a volume of 1.0 ml/kg. Motor activity was automatically measured in a figure-eight maze consisting of 10x10 cm alleys that converge on a central arena (Reiter et al., Environ. Health Perspect. 12:119-123, 1975). Animals were tested for 1 hr starting 2 hr after dosing on Day 0, and again on Days 4, 8, 16 and 32. On Days 49-51, activity was measured over a 23 hr period. There were no differences in body weight between groups at any time following dosing. Animals treated with 7 mg/kg TMT exhibited hyperreactivity and spontaneous seizures which were most evident 5-10 days after dosing and disappeared by Day 16 following treatment. There were no differences in activity on the day of dosing, but on all subsequent days the 7 mg/kg TMT animals were hyperactive in comparison to all other groups. The time course for development of hyperactivity which was apparent by Day 4 and asymptoted on Day 16, parallels the time course of neuropathological changes, which first appear 2 days after dosing and are maximal within 3 wks (Brown et al., 1979). In addition to increasing the total amount of activity, TMT also changed the spatial pattern of activity; activity was increased in the "figure-eight" portion of the maze but not in the blind alleys. Activity of the 7 mg/kg TMT animals was also increased during all periods in the 23 hr test. These results demonstrate that structural damage produced by acute exposure to TMT is associated with long lasting hyperactivity.

244.2 TRIMETHYLTIN TOXICITY IN GRASSHOPPER MICE (ONYCHOMYS TORRIDUS). K.L. Hulebak*, Z. Annau. Dept. Environ. Hlth. Sci., Div. Toxicology, The Johns Hopkins Univ., Baltimore, MD 21205

Trimethyltin (TMT) is an organometallic neurotoxin which produces specific neuronal lesions in discrete brain regions. Damaged areas include the hippocampus, pyriform cortex, septal nucleus and amygdala. These lesions have been shown in the rat to be accompanied by hyperirritability and possibly increased agonistic behaviors.

The present study determined the acute toxicity (LD-50) of trimethyltin chloride in two species of mice, Mus musculus (CFW) and Onychomys torridus, and assessed the effects of chronic TMT exposure on the social behavior of Onychomys. These carnivorous rodents not only display a diverse repertoire of social behaviors, but also show predatory behavior towards prey such as crickets.

The LD-50 was determined by intubating groups of 8 mice (both CFW and Onychomys) with 0, 1.0, 2.0, 2.25, 2.5, 2.75, and 3.0 mg/kg TMT in saline. Animals were observed for a period of 14 days post-intubation. At 2.75 mg/kg, all animals died 7 days post-intubation. At 2.25 mg/kg, half the animals died three days post-intubation. Survivors showed whole-body tremors with severity dependent on dose. The tremors disappeared in the survivors by five days post-intubation.

Same-sex pairs of male and female grasshopper mice were exposed (p.o.) to 0, 0.5, and 1.0 mg/kg TMT weekly, for 3 weeks. Their performance on a variety of behavioral tasks and situations was monitored on a weekly basis during exposure, and at one month post-exposure, and compared with their pre-exposure performance. Behavioral tasks included auditory startle reflex, a test of predatory aggression (cricket-killing), and undisturbed social behavior of each pair. The latter category consisted of general maintenance behavior, agonistic behavior, and mutual social behaviors. Results indicate sex-, dose-, and time-dependent depression of the auditory startle reflex, and concomitant disruptions of social, agonistic and general activity behaviors of the mice.

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244.3 NEURONAL RESPONSES TO AN ENVIRONMENTAL POLLUTANT IN AN INTACT MARINE ANIMAL. J. E. KANZ, M. D. DUVALL. Marine Biology Dept. Texas A&M University at Galveston, Galveston Tx. 77553

Drilling fluids are heavy mixtures which include barite ($BaSO_4$), lignosulfonates, fungicides, bacteriocides and even pesticides and are used to facilitate several off-shore oil platform drilling related functions, e.g., removal of drill cutting and lubrication of drilling bits. After use, drilling fluids are usually discharged into the environment around the platform where they have been shown to adversely effect a wide range of marine fauna.

A recently developed in vivo extracellular recording technique coupled with new computer procedures for analysis of the extracellular neuronal data have been applied to determine the effects of drilling fluid on the nervous system of the marine gastropod Aplysia. Therefore, utilizing a freely behaving animal it is possible to monitor subtle changes in neuronal activity which over short-term (1-4 days) have no detectable behavioral correlates. Two types of Aplysia siphon nerve activity were studied and compared before and after cannulation of drilling fluid into the body cavity: 1) spontaneous background activity and 2) the frequency and pattern of activity making up a spontaneous burst of activity from a network of cells in the abdominal ganglion (Interneuron II). Supported by EPA grant R807137-01-0 to J.K.

244.4 AN EXAMINATION OF THE PROGRESSION OF BEHAVIORAL AND PATHOLOGICAL EFFECTS IN MICE EXPOSED TO THE PARASITE TOXOCARA CANIS. Z.S. Dolinsky, R.G. Burright‡ and P.J. Donovick, Dept. of Psychology and Center for Neurobehavioral Sciences, SUNY Binghamton, Binghamton, N.Y. 13901, and B. Summers* and R.H. Cypess‡ Dept. of Preventive Medicine, Cornell Univ., Ithaca, N.Y. 14850.

Toxocara Canis a parasitic roundworm commonly infects dogs. The eggs of the parasite are excreted in the animal's feces and may be ingested by children. The larval stage of this parasite is known to migrate through various organs in the human including liver, lung, muscle, and CNS. The long term viability of the eggs in the environment, and the difficulty in diagnosis and treatment of this parasitic infection make it a potential health problem.

We examined the time course of behavioral and pathological changes, over a period of 86 days, in mice intubated with 1000 embryonated decorticated eggs of Toxocara Canis. A battery of behavioral tests (Home cage observation, Open field, Swimming, Shock sensitivity, Balance) was administered at each of 3 post intubation periods (Period 1 -- 8-10 days post intubation, Period 2 -- 49-51 days, Period 3 -- 84-86 days). All animals were intubated on the same day, however each post intubation period was comprised of an independent group of animals. At each post intubation period animals were sacrificed at the end of behavioral testing and liver, lung, muscle, and brain were examined for histopathology.

Toxocara treated animals showed depressed levels of activity relative to controls on a select number of measures during the first test period. However, the scope and severity of behavioral changes became more severe on the subsequent two periods.

Peripheral organs showed marked pathology during the first post intubation period, followed by a decrease in severity on subsequent test periods. While larvae were present in the brain, only mild inflammatory changes were observed during the first period. However, a progressive Wallerian Type degeneration of major fiber pathways was observed on subsequent post intubation periods.

These results suggest that pathological changes in both peripheral organs and brain which are associated with Toxocara Canis infection may have behavioral consequences in mice. However, the time course of the behavioral changes, with respect to relative CNS and peripheral tissue pathology, suggest the critical involvement of the CNS in the behavioral effects associated with Toxocara Canis infection.

These results may have important implications in the etiology of behavioral disorders for children who have a known history of pica for dirt. (Supported by NSF (DAR7911233) grant to PJD & RGB)

244.5 TOXOCARA CANIS AND LEAD ALTER SENSORY REACTIVITY OF MICE. P. J. Donovick, Z. S. Dolinsky, V. P. Perdue, and R. G. Burright*, Dept. of Psychology and Center for Neurobehavioral Sciences, SUNY Binghamton, Binghamton, N.Y. 13901, and R. H. Cypess*, Dept. of Preventive Medicine, Cornell Univ., Ithaca, N.Y. 14850.

The common parasite roundworm of dogs, Toxocara canis, presents a health problem to humans. The eggs of the parasite are excreted in the dog's feces. The long-term viability of these eggs makes them a health hazard, particularly to children who may play in areas frequented by dogs. Thus, in urban settings individuals may be exposed both to parasites and other toxic elements, such as lead, as well. Previously, we found that several measures of motoric behavior were dramatically altered in mice infected with Toxocara canis. In that report we noted that combined ingestion of parasite plus lead often resulted in a less severe syndrome than when the parasite was administered alone.

In the present research we examined the effects of Toxocara canis and lead toxicity in mice on behaviors less dependent on fine motor coordination than those previously employed. Sensory reactivity as reflected in ingestion of saccharin and quinine solutions was measured in adult mice which had been administered Toxocara canis alone or in combination with lead. In addition, seizure susceptibility to ECS was assessed. Measures of taste reactivity indicated that in mice, lead or Toxocara may alter the mode of interaction with the environment. Thus lead increased sensitivity to alterations in palatability of fluid while administration of the parasite alone diminished such reactivity. The combined lead and Toxocara group fell between these extremes. While the two agents did not have an additive effect in the case of taste reactivity, the combined group exhibited the least severe electroconvulsive shock seizures. It is, however, interesting to note that the combined group did not exhibit more neuropathology than the parasite alone group.

These results suggest that Toxocara canis alone or in combination with lead can alter behaviors which have strong sensory components. They may have important health implications for urban children with known history of pica for dirt.

This research was supported in part by a grant to P. J. Donovick and R. G. Burright from NSF (DAR7911233).

244.6 SCRAPIE VIRUS AND ALTERED PUP GROWTH: EVIDENCE FOR ALTERED MATERNAL BEHAVIOR? R.F. Seegal and J.E. Hotchin. Div. Labs. & Research, New York State Dept. Health, Albany, New York 12201.

Scrapie is a fatal degenerative neural disease of sheep thought to be caused by a viroid-like agent capable of relication although it induces no antibody response in the host. It has been transferred from sheep to the mouse, hamster and primate. This agent, a member of the spongioform encephalopathies, gains in experimental and clinical importance because of its close relationship to the human disorders Kuru and Creutzfeldt-Jakob disease.

The earliest signs of infection are often behavioral and include changes in locomotor and open-field activity, sucrose-water consumption and Y-maze performance (Heitzman and Corps, 1968; Outram, 1972; McFarland and Hotchin, 1980). However, little effort has been devoted to a study of reproductive behaviors in spite of the fact that Parry and Livett (1976, 1977) noted changes in neurophysin levels in hypothalamic and infundibular regions of scrapie infected sheep brain-areas intimately involved in neuroendocrine control.

Nulliparous Nylar:nya mice were injected intra-cerebrally (IC) with either a 10^{-2} dilution of infected mouse brain containing approximately 10^{6} mouse LD_{50s} of the Chandler strain of scrapie /gram of brain tissue or a 10^{-2} dilution of normal mouse brain (NMB). These animals were mated so parturition would occur on about Day 50 post-infection. When pregnant the animals were individually housed with wood shavings provided for nest construction. Dams were inspected daily for births. All litters were culled to eight pups with cross-fostering within treatment employed to equalize litter size. No attempt was made at matching the sexual composition of the litter and only litters with 7,8 or 9 pups were included in the analysis.

No differences in sex ratio, initial number of pups born or mean weight of the pups at birth were noted. However, by two weeks post-parturition there were significant differences in the mean weight of the pups (offspring of scrapie mothers weighed significantly less than offspring of NMB inoculated mothers). These effects became more pronounced in the final week before weaning. No difference in mortality or quality of nest construction were noted between the experimental and control animals.

These results are among the earliest recorded following scrapie infection and suggest that the differences in pup body weight are a function of post-parturitional changes in behavior and/or physiology of the mother rather than in-utero changes in development of the offspring induced by the virus infection.

245.1 CONDITIONED RESPIRATORY BEHAVIOR IN RABBITS CORRELATES WITH CHANGES IN SPATIO-TEMPORAL ACTIVITY IN THE OLFACTORY BULB. G.W. Davis, W.J. Freeman, T. Whitney.* Dept. Phys.-Anat., Univ. of Calif., Berkeley, CA 94720.

The techniques of classical conditioning and multi-channel recording of neural activity have been applied to assess changes in sensory processing during conditioning of respiratory respones.

Rabbits were trained through classical aversive conditioning to give a respiratory response (sniffing) when a conditioning stimulus (CS) (amyl acetate 1/2000, or butyric acid 1/2500; dillution olfactometer) was presented followed after 2.5 sec. with shock. The instantaneous rate of respiration was monitored by a circumabdomenal pneumograph, and conditioned responses(CRs) were detected through statistical criteria executed by an Interdata 7/16 minicomputer. The neural activity of the olfactory bulb(OB) was recorded through an array of 64 electrodes implanted over the dural surface of the left OB. Six seconds of data from the array was recorded (2.5 ms digitization interval/channel) for each trial presentation of the CS or blank trials with no CS. Individual damped sinusoidal bursts of bulbar activity (35-80 Hz, 50-100 ms in duration) were selected before and after presentation of the CS. Twenty to thirty trials per session were accumulated, and as many as 30 sessions made up the total data base. Burst amplitudes (64 channels, 75 ms time series/channel) were averaged across trials according to temporal relationship to the CS: pre-CS, and post-CS bursts.

The results show that 1) the automated detection of statistical flucuations in respiration rate is an effective method for monitoring olfactory conditioning 2) approximately 300 ms after the arrival of CS, a marked decrease in burst amplitude occurs, and 3) statistically significant differences in spatial structure are detected.

The results raise several questions regarding the processing of olfactory information in the presence of behaviorally relevant stimuli. For example, does the arrival of behaviorally important odors cause the OB to alter its activity, or does the 300 ms delay between arrival of the CS and the initiation of changes in the bursts indicate that centrifugal impulses to the OB are suppressing the processing of further, possibly distracting, information.

245.2 VISUAL EVOKED POTENTIALS DURING CLASSICAL EYEBLINK CONDITIONING. M. Serdaru*, J. Rohrbaugh, K. Syndulko and D.B. Lindsley. Dept. Psychology, Univ. Calif., Los Angeles, CA 90024.

Visual evoked potentials (VEPs) to a conditioned stimulus (CS) were evaluated during preconditioning, conditioning, and extinction phases of a classical eyeblink conditioning procedure. VEPs were recorded from scalp sites overlying central (Cz) and occipital (O1) areas. Two experimental groups, with 7 subjects each, participated in 2 sessions - the first a preconditioning session, and the second session comprising conditioning and extinction trials. For one group of subjects, the CS was presented foveally, whereas for the second group the CS was presented 20° in the temporal field. For both groups, green light-emitting diodes served as the light sources, and viewing was with the right eye. The unconditioned stimulus (UCS) was an airpuff delivered to the cornea 850 msec after light onset. The reinforcement ratio during conditioning was 100%.

The VEPs to peripheral stimuli were substantially smaller than to foveal stimuli, in accord with previous findings. Both foveal and peripheral VEPs, however, showed changes during the 30 conditioning trials. These changes were centered about a negative component at about 200 msec (N200) and, to a lesser extent, a late positive component at 300 msec or later (P300), both of which were larger during the conditioning trials. Relative to preconditioning values, the VEP enhancement persisted also through the 25 extinction trials. These changes were observed both at Cz and O1, and were more pronounced on trials eliciting a conditioned blink than on trials for which no conditioned blink was obtained. A comparison of the average VEP obtained during the first 15 conditioning trials with the VEP from the last 15 trials yielded no major differences in N200, suggesting that the acquisition of the VEP changes was fairly rapid, and that they were resistant to habituation. The VEPs for voluntary responders (i.e., those subjects with short latency responses) did not differ appreciably from the VEPs of conditioned responders. No effects, either in the frequency or latency of the conditioned blink, or in the responsiveness of the VEP to the conditioning procedures, were associated with the variable of foveal versus peripheral stimulation.

245.3 RAPID CONDITIONING OF EYEBLINK REFLEX: RESPONSE TOPOGRAPHY. N.E. Berthier,* B. Betts* and C.D. Woody (Spon: B.E. Swartz) Depts. of Anatomy and Psychiatry, UCLA Med. Center, Los Angeles, CA 90024

Classical conditioning was studied in a mammalian preparation in which a discriminative response with multiple latency components could be rapidly acquired. During training, the CS (a 75 db click) was followed 340 ms later by a mechanical tap of the glabella (US) which elicited a bilateral eye blink (UR). Electrical stimulation of the lateral hypothalamus (HS) was delivered unilaterally 580 ms after the CS. A discriminative stimulus (DS; 75 db hiss) was delivered 4.4 s after the CS. Seven cats were run through series of conditioning and extinction sessions (US and HS omitted) with 10 s intertrial intervals. The orbicularis oculi EMG was rectified, filtered (Tc=5ms), and digitized with a PDP 11/44 computer to produce post-stimulus histograms with 2 ms bins. EMG deviations larger than 3 sd above the mean of the spontaneous activity in the 400 ms prior to CS delivery were defined as responses. During training, the CR increased with trials reaching asymptote (74% CRs) within 9 trials. Several response peaks could be distinguished. The most prominent of these (i.e. 5 sd above mean spontaneous activity) were classified into four windows (0-80 ms, 120-200 ms, 220-260 ms, 280-300 ms) based on their latency following CS or DS delivery. Analysis of variance indicated that cats made a comparable number of responses to the CS and DS in the shortest window (0-80 ms), but more responses to the CS than DS in the last three windows ($p \leq .006$). Responses to the CS during the last three windows increased with training. During extinction the cats initially made more responses to the CS than to the DS ($p \leq .001$), but by the 9th trial of extinction there was little responding to either the CS or DS.

This model of conditioning lends itself to electrophysiological investigation due to the speed of acquisition and the ability to characterize the rapid acquisition objectively by computer analysis of the orbicularis oculi EMG. The conditioned response was acquired rapidly within 9 trials, was discriminative for the CS, and extinguished within 9 trials when the CS was presented without US or HS. The response did not develop when the order of CS-US-HS presentations was reversed (backward conditioning), nor did a response develop within 100 pairings of CS-US or CS-HS alone. The latency of the conditioned response to the CS decreased during training, but this did not seem to represent simply a shortening of response latency. Instead, additional responses of a shorter latency were acquired while longer latency responses were maintained. Responses within the first 80 ms after CS or DS presentation seemed primarily to reflect sensitized responding to the stimulus, responses of $\geq$120 ms latency representing the discriminatively performed CRs. (Sup.by BNS78-24146&AFOSR81-0179)

245.4 NEURONAL ACTIVITY RECORDED FROM THE AMYGDALA CENTRAL NUCLEUS DURING AVERSIVE PAVLOVIAN HEART RATE CONDITIONING IN THE RABBIT. Craig D. Applegate*, Robert C. Frysinger*, Bruce S. Kapp & Michela Gallagher. Dept. of Psychology, University of Vermont, Burlington, Vermont, 05405.

Evidence in the rabbit demonstrating that (1) lesions or pharmacological manipulations of the amygdala central nucleus (ACE) attenuate vagally-mediated, aversively conditioned bradycardia (Kapp et al, Physiol. Behav., 1979; Gallagher et al., Pharmac. Biochem. Behav., 1980), (2) the ACE projects monosynaptically to dorsal medullary cardioregulatory nuclei (Schwaber et al., Neuro. Lett., 1980), and (3) electrical stimulation of the ACE produces bradycardia and depressor responses (Kapp et al., submitted) suggests that the ACE may contribute to the expression of conditioned bradycardia in this species. Based on these data, it was hypothesized that changes in ACE neuronal activity should develop to a conditioned stimulus (CS) and correlate with the bradycardia elicited to that CS during aversive Pavlovian conditioning.

To test this hypothesis multiple unit activity was recorded from 34 ACE sites in 26 New Zealand rabbits during the acquisition of a conditioned bradycardia response using an aversive Pavlovian conditioning procedure. The procedure consisted of an orienting phase in which a 1 Khz, 5.0 sec., 85 db tone (CS) was presented alone, a conditioning phase in which CS offset was coincident with a 500 msec, 2.0 mA eyelid shock (UCS), and an extinction phase in which the CS was again presented alone.

In support of the hypothesis, the neuronal activity at twelve ACE sites showed increases to the CS which emerged during the conditioning phase of the procedure, and the increased activity from two of these sites was significantly correlated with the bradycardia response. The activity from these latter placements was characterized by (1) low levels of spontaneous, pre-CS activity, (2) short latency (< 50 msec) increases in activity to the CS which were sustained for the duration of the CS and (3) a pattern of development to the CS over trials of the conditioning phase parallel to the pattern of development of the conditioned bradycardia response to the CS. Activity recorded from the remaining sites showed either no obvious changes to the CS or complex changes to the CS over the course of the procedure

The results are consistent with the hypothesis that the ACE contributes to the expression of conditioned bradycardia in the rabbit, but also suggest that neural elements of the ACE do not respond in a unitary fashion under the conditions of the Pavlovian procedure employed. (Supported by USPHS Grants RO1 MH31811 and KO2 MH00118 to BSK).

245.5 ANTERIOR LIMBIC UNIT ACTIVITY DURING DELAYED RESPONSE IN CATS. S.A. Stwertka, T.I. Lidsky, J.S. Stamm, F.C.T. Chang and S.C. Linares* SUNY at Stony Brook, NY

The anterior limbic cortex (ALC) has been reported to be involved in sequential behaviors, response suppression and attention. We investigated single unit activity of this area during the cat's performance of a task requiring some of these behaviors. Animals prepared for chronic recording performed visually cued delayed response (DR) with head immobolized, sitting in a restraint box, facing a response panel consisting of two levers each with a small light centered above them. A trial began when flickering illumination of one of the lights for 1 sec. indicated the side to be responded to after a delay of 3-5 secs. Illumination of both lights after the delay signaled that a lever press was to be made to the cued side. Correct responding delivered water to the liquid deprived cats.

Of 340 units sampled, 42% were related to aspects of DR performance. In percentages of task related cells, 56% responded with the same sign of activity (increase or decrease in rate) both to the cue and choice lights, 19% had a response to the cue which persisted throughout the delay until time of lever pressing, 8% responded during the delay only and 16% had a response to the cue which was followed by activity opposite in sign during the delay, then returning back to cue characteristic activity again to the choice lights. For all unit types both increases or decreases in activity were seen, although suppression of activity was more frequent. Differential effects for left and right trials were seen in a proportion of all unit types during their responsive phase. Latencies to on responses in cue and choice light related cells varied between 50 and 75 msec. In control tests, visual stimulation delivered with the panel lights and analysis of eye movements recorded during some sessions revealed contributions to DR task relatedness both from sensory, visually driven cells and units associated with eye movements, with activity both preceding and occurring during the movement.

These preliminary results indicate relations between the activity of ALC neurons and components of DR performance.

(Supported in part by NIMH grant MH31027)

245.6 EFFECTS OF ANTERIOR THALAMIC LESIONS ON DISCRIMINATIVE AVOIDANCE BEHAVIOR AND NEURONAL ACTIVITY IN THE CINGULATE CORTEX. M. Gabriel, Edward Orona, Kent Foster* and Richard W. Lambert*. Dept. Psych., Univ. Texas, Austin, TX 78712.

Past studies have demonstrated the development of discriminative multiple-unit activity in the cingulate cortex and in the anteroventral (AV) nucleus of the thalamus, in response to the positive and negative conditional stimuli (CS+ and CS-) during differential conditioning of locomotory avoidance behavior in rabbits (Gabriel, M., Foster, K., Orona, E., Science, 208:1050, 1980). The discriminative neuronal responsiveness developed in the deep laminae (V-VI) of cingulate cortex in an early stage of behavioral acquisition, but discriminative responsiveness in the superficial laminae (I-IV) and in the AV nucleus did not develop until a late stage of acquisition, after the development of significant discriminative behavior. These results suggested that the deep laminae subserve neural encoding relevant to original acquisition but that the late-formed code produced by the AV nucleus may contribute to behavioral retention. The late-formed code in the superficial laminae may reflect feedback from the AV nucleus informing cortex that the code is being produced in thalamus.

To test these ideas male albino rabbits (N=8) with chronically indwelling unit recording electrodes in the superficial laminae, and with lesioning electrodes in the AV nucleus, received training to a criterion on the discriminative avoidance task, followed by bilateral electrolytic lesions (1.5 ma. for 45 sec) of the AV nucleus. Controls (N=7) had lesioning electrodes implanted but they did not receive lesions. Eight days after training each rabbit was tested for retention using an extinction procedure, followed by reacquisition and reversal training. The behavioral expression of the original habit was significantly attentuated in the lesioned subjects, relative to controls in all of the test phases. Excitatory, discriminative neuronal activity occurred in the superficial laminae of the control subjects during reacquisition, and reversal of the discriminative response occurred during reversal training. However, the neuronal activity of the lesioned subjects manifested only inhibitory ("off") responses to the CSs, and no significant discriminative effects during the test phases. These results are compatible with the original hypotheses. In addition, they suggest that neurons of the AV nucleus normally produce excitatory effects in the cingulate cortex. Removal of the excitatory influence results in a predominance of inhibitory drive upon the cingulate neurons.

245.7

WITHDRAWN

245.8 MODULATION OF LONG-TERM POTENTIATION (LTP) BY AMPHETAMINE. R. L. Delanoy, D. L. Tucci* and P. E. Gold. Dept. of Psychol., Univ. of Virginia, Charlottesville, VA 22901.

The effects of amphetamine (AMPH) on the response of dentate granule cells to low frequency angular bundle stimulation before and after LTP were examined in pentobarbital anesthetized rats. Recording and stimulating electrodes were implanted in the dentate granule cell layer and the angular bundle, respectively. Test stimuli (40 V, 100 μsec) were delivered at a rate of 0.5 Hz. The baseline latencies and amplitudes of the three extremes of the waveform (1st EPSP, population spike, 2nd EPSP) were averaged from 20 consecutive traces. Saline or AMPH (0.3 or 1.0 mg/kg, IP) was then injected. The waveform was again charted 12 min later and compared with the preinjection waveform. At 15 min after injection, rats received a stimulation train (20 V, 65 μsec, 100 Hz, 1 sec) which resulted in half-maximal LTP. At 23 min after injection, rats received a second high frequency train (40 V) which produced maximal potentiation. The potentiated waveforms were charted 3 min after each high frequency train and were compared to the post-injection waveform.

In general, AMPH reduced the latencies of the unpotentiated evoked response peaks with the higher dose being more effective. AMPH did not alter the amplitude of the evoked response. LTP was characterized by decreased latencies and increased amplitudes of the evoked response peaks. Those animals which had received the lower dose, but not the higher dose, of AMPH showed enhanced potentiation on several measures. As compared to the evoked responses seen in saline-injected rats following LTP, the low-dose AMPH animals showed significantly larger decreases in the latencies of the 1st EPSP and population spikes and a larger increase in the amplitude of the 2nd EPSP. These latter AMPH effects appear to be the result of a drug effect on LTP itself because: any effects of AMPH on the evoked response prior to potentiation were subtracted prior to analysis of the waveform; the dose-related effects of AMPH on changes in latency differed in potentiated and unpotentiated evoked responses; AMPH resulted in an increase in the 2nd EPSP amplitude of potentiated but not unpotentiated responses. These results suggest that the mechanisms underlying potentiation of a monosynaptic pathway may be modulated by the concurrent activity of other neuronal systems, possibly including biogenic amines. In this regard, it may be interesting to note that the inverted-U dose-response relationship of AMPH to LTP is analogous to reported effects of pre- and post-training AMPH injections on behaviorally-assessed memory.

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245.9 LONG-TERM POTENTIATION FACILITATES THE ACQUISITION OF PERFORANT PATH STIMULATION AS A DISCRIMINATIVE STIMULUS. R.W. Skelton, A.G. Phillips, and J.J. Miller, Depts. of Psych. and Physiol., Univ. of Brit. Columbia, Vancouver, B.C., Canada V6T 1W5.

Electrophysiological investigations of long-term potentiation (LTP) have shown that brief activation of hippocampal afferent pathways can result in an enduring increase in synaptic efficacy. (Bliss, 1979). Although LTP satisfies many of the theoretical requirements of a neural mechanism for learning, direct evidence for this relationship is minimal, due to the paucity of information about the functions of hippocampal neuronal systems.

In the present series of experiments, direct electrical stimulation of the perforant path (PP) served as a discriminative stimulus (DS) signalling the availability of food pellets, contingent upon an operant response to the food cup. Population spikes, recorded from electrodes positioned in the dentate gyrus (DG) ensured that the DS produced activation of PP-DG synapses. The PP-DG system was chosen because it provides a nearly ideal preparation in which to observe or produce LTP.

In the first experiment, the current intensity of single (100 μsec) pulses to the PP of chronically implanted rats was adjusted to evoke asymptotic population spikes in the DG. At the end of training for 10 days (40 trials/day), all animals responded immediately after, and not before the brain stimulation. This distribution of responses indicated that the animals could indeed use the PP stimulation as a DS.

The second experiment was designed to determine whether LTP, produced by tetanic stimulation, would facilitate the acquisition of the discrimination. Current intensities in this study were adjusted to evoke a sub-maximal population spike. The tetanic stimulation used to potentiate these responses was delivered as 10 trains of 10 pulses (200 Hz). These trains served as the DS's of the first 10 trials for the experimental group. The control animals received the usual single pulse stimulation on these trials. All subsequent DS's for all animals were single pulses. After 14 days of testing, four of the five tetanized animals were discriminating the PP stimulation at criterion levels while only one of the control animals showed similar performance.

These data indicate that LTP can facilitate the behavioral response to information mediated by PP synapses. It is suggested that LTP may provide a neural mechanism for behavioral changes associated with learning.

Bliss, T.V.P., Trends in Neuroscience, 1979, 2, 2, 42-54.

245.10 LONG LASTING FACILITATION OF A MONOSYNAPTIC RESPONSE IN THE MAGNOCELLULAR MEDIAL GENICULATE NUCLEUS OF THE ANESTHETIZED CAT. R. A. Gerren* and N. M. Weinberger. Dept. Psychobiology, University of California, Irvine, CA 92717.

The effects of high frequency electrical stimulation on a short latency response, recorded in the medial geniculate magnocellularis (MGm) to stimulation of the brachium of the inferior colliculus (BIC), were investigated in the anesthetized (40 mg/kg sodium pentobarbital, I.P.) and paralyzed (12 mg/kg gallamine triethiodide, I.V.) cat. Test stimuli (0.2 Hz, 0.1 ms) delivered to the BIC for approximately 20 min. prior to the presentation of a high frequency train (300 Hz, 285 ms), evoked responses in MGm which were stable in amplitude (50-200 μV) and latency (0.94 ± 0.08 msec) during this baseline period. Several changes in the BIC evoked MGm response were observed following the high frequency train. Initially, either a short lasting (15-30 sec) facilitation or depression of the MGm response amplitude was observed. In either instance, the short lasting amplitude change was replaced by a long lasting facilitation of the MGm response amplitude, which reached an average maximum facilitation of 141.8 ± 10.7% of the baseline values at an average of 21.5 ± 5.2 min. following the train. The duration of this long lasting facilitation was as short as 23 min. and as long as 4.5 hr. In addition to the observed amplitude facilitation following the train, the latency of the MGm response decreased by an average of 0.036 ± 0.007 ms, 40 min. after the train.

An additional investigation indicates that single units in the MGm can be driven with short latencies (1.2-3.0 msec) by BIC stimulation. Following a high frequency train, decreases in latency and variance of latency were observed. Additionally, single units which fired only once to BIC stimulation before the train were observed to fire in short bursts (2 or 3 spikes) following the train. These effects have been recorded for more than 1 hr. These findings suggest that the amplitude facilitation and decrease in latency of the monosynaptic response following a high frequency train are directly related to changes occurring in the same population of MGm neurons, as opposed to the recruitment of another population of MGm neurons.

The implications of a long lasting facilitation of the BIC-elicited MGm response are as yet unknown. It may be that this facilitation is related to the specific physiological plasticity of the MGm during behavioral learning (Ryugo & Weinberger, Behav. Biol. 22:275, 1978). Also, this phenomenon may be related to the long-term potentiation previously observed in the hippocampus (Bliss & Lømo, J. Physiol. 232:331, 1973). We thank Bill Hopkins for his assistance.

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245.11 SINGLE-UNIT ACTIVITY RELATED TO SHORT-TERM MEMORY OF TONES (STMT), IN THE AUDITORY CORTEX OF THE MONKEY. Y. Gottlieb*, E. Vaadia* and M. Abeles* (SPONS; S. Schuetze). Hebrew Univ. Hadassah Med. Sch., Jerusalem, Israel.

Several studies of short-term memory (STM) suggest that (a) STM depends on the attention level of the subject and (b) the auditory cortex is required for STMT.

We trained a Baboon monkey to perform a task which required him to remember the frequency of a tone for 1 sec. As the monkey learnt the task, it was prepared (under anesthesia) for single-unit recordings. Beginning 1 wk later, 127 units in the auditory cortex were studied while the animal performed the STMT task. Each unit was also studied during a control session. Peri-stimulus time (PST) histograms and auto-renewal density functions were computed for the two behavioural states.

PST histograms of 50% of the cells were dependent on tone frequency for 1 sec after its presentation in both STMT and control trials.

PST histograms of 36% of the cells differed from background for 1 sec after stimulus presentation during STMT trials only.

In response to paired stimuli (one second apart), 23% of the cells could distinguish pairs with identical tones from pairs of different tones, but only during STMT trials. Clear reverberatory activity could not be demonstrated in the activity of any cell.

On the basis of the results we suggested two models for the mechanism of STMT: (a) Non-attention dependent STMT. This mechanism can store information by a firing rate code. (b) Conditioned STMT. This mechanism is conditional on the animal's attention. It explains how neural activity which reflects attention processes can facilitate STMT.

245.12 CONDITIONED MODIFICATION OF AVIAN DORSAL GENICULATE NEURONS IS A FUNCTION OF THEIR RESPONSE TO THE UNCONDITIONED STIMULUS. C.M. Gibbs*, D.H. Cohen, J. Broyles* and A. Solina*. Dept. of Neurobiology and Behavior, SUNY, Stony Brook, Stony Brook, N.Y. 11794.

Visually conditioned heart rate change in the pigeon has been developed as a vertebrate model for cellular studies of learning. In this system, the ascending visual pathways transmit the conditioned stimulus (CS) information, and the thalamic relays of at least two such pathways show training-induced modification. In contrast, the maintained and evoked discharge of retinal ganglion cells are invariant over training. Reported here are further studies of the principal optic n.(OPT), the avian dorsal geniculate homologue and thalamic relay of the thalamofugal system.

Upon isolating the activity of a single cell in a behaviorally naive animal, ten 6-sec pulses of whole-field illumination were given. Presented next were 40 such lights followed by 0.5-sec foot-shocks (conditioning) or 40 unpaired lights and shocks (sensitization). This was followed by five 50-msec foot-shocks.

All OPT neurons responded to light onset at latencies of 24-70 msec ($\bar{x}$ = 38 msec), 38 with increased (Type I) and 16 with decreased (Type II) discharge. 94% of the cells also responded to foot-shock, the unconditioned stimulus (US), at latencies of 60-300 msec ($\bar{x}$ = 109 msec), 30 with increased and 21 with decreased discharge.

We reported previously that the light-evoked response of most OPT neurons is enhanced by conditioning and attenuated by non-associative training. However, new data suggest this modifiability is influenced by the response to the US. For example, 89% of Type I units responding to the US with decreased discharge showed rapid augmentation (3-6 CS-US pairings) of their light-evoked response during conditioning, while Type I units with increased discharge to the US showed either response attenuation or only a gradual enhancement later in training. Moreover, two such cells which were unresponsive to the US showed no change. Such interactive effects were not confounded by differences in initial CS-evoked responses or by differential changes in maintained activity. Although the sample size for Type II cells precluded statistical analysis of interactive effects, similar trends were observed.

This study confirms our previous report of: (a) training-induced modification of OPT neurons, most of which are likely to be retinorecipient, and (b) the US responsiveness of most OPT cells. It further suggests that: (a) convergence of CS and US inputs is necessary for training-induced modification; (b) there are at least two sources of US input to OPT; and (c) these inputs are differentially effective for training-induced change. We are identifying the cells of origin of the US inputs to allow study of the effects of their selective interruption upon conditioned changes of OPT cells. (Supported by NSF grant BNS8016396.)

246.1 NEURAL SUBSTRATES OF THE CLASSICALLY CONDITIONED RABBIT NICTITATING MEMBRANE PREPARATION: TRIGEMINAL SYSTEM AFFERENTS.
Y. Torigoe*, W. Wenokor*, and C. F. Cegavske. Department of Psychology, SUNY-Binghamton, Binghamton, NY 13901.

The two studies reported here are part of a series of experiments designed to characterize the unconditioned response (UCR) pathway in this preparation. The studies identify the sensory projections into the brainstem from the cornea, and from the area of the face innervated by the supraorbital nerve (SON) (a subdivision of the ophthalmic part of the trigeminal system).

These projections were demonstrated with horseradish peroxidase (HRP) mixed with dimethylsulfoxide (DMSO) in normal saline solution. The HRP was injected into all parts of the cornea with a 30-gauge needle and applied to the SON by encapsulating the cut end of the nerve together with the tracer. After corneal injections the animals were sedated with Acepromazine until sacrificed. In both studies, electrical stimulation (of cornea or nerve) was used to enhance transport, the survival period was two days, and the brain tissue was processed in tetramethylbenzidine.

Excellent labelling was observed in the trigeminal ganglion and in the brainstem. In the ophthalmic part of the ganglion the cells from the cornea study (about 20% of the number seen after SON transport) occupied a sub-area of the SON-cell distribution.

In the brainstem the projections from both studies were similar in the following ways. Transport was seen in the ventral part of both the principal and spinal nuclei, and extended into the dorsal part of the spinal nucleus caudal to the obex. It was also seen in an area lateral to the motor trigeminal nucleus, adjacent to the dorsal part of the facial nerve, between the trigeminal and facial nuclei, in the reticular formation close to the spinal nucleus at all levels, in lamina I (marginal layer) at about the level of the obex, and in the lateral part of the nucleus of the solitary tract. At more posterior levels the projections became less dense and were distributed both dorsally and ventrally.

The brainstem projections were different in the two studies as follows. Relative to the cornea study the SON study had larger (usually overlapping) projection fields, had a projection area (from facial nerve to obex) that extended dorsomedially along both sides of the spinal nucleus' ventral boundary with the reticular formation, had projections in lamina IV that were ventral to those from the cornea, and had projections that extended about 19 mm caudal to the obex instead of 14 mm.

Two main conclusions of these studies are: the data are not consistent with the view that the trigeminal system has a precise somatotopic organization; and, the internuncials mediating the UCR should be found within the projection areas identified here.

246.2 FORWARD LOCOMOTION IN THE DIENCEPHALON AND MESENCEPHALON MAPPED BY ELECTRICAL STIMULATION. S. M. Parker,* R. C. Russo,* J. W. Mink and H. M. Sinnamon. Lab. of Neuropsychology, Wesleyan University, Middletown, CT 06457.

Locomotion has been reported to be elicited by stimulation in several ventral diencephalic sites including the medial and lateral hypothalamus and the subthalamic region in the area of the zona incerta nucleus. The purpose of this study was to survey these regions caudal to the anterior hypothalamus in order to determine if anatomically distinct locomotor systems existed and the course of their trajectory to the midbrain.

Long-Evans rats (N=26) were anesthetized with Chloropent (3-4 ml/kg) and subjected to stereotaxic surgery in which 3 or 6 stainless steel (125 μm) monopolar electrodes were implanted. Following at least 5 days recovery the sites were tested for locomotion elicited by constant current cathodal stimulation at 50, 100, 200 and 300 μA and 50, 100 and 154 Hz pulse frequency. The pulse duration was 0.5 msec. The test apparatus was a 27 X 9 X 24 cm chamber containing a 7 cm wide treadmill belt. Each site was tested under two separate stimulation conditions with each combination of current and frequency tested once. In the first condition, a 5 sec. train of stimulation was delivered no more often than every 20 sec. In the second condition, 20 trains of 1-sec. duration were delivered every 4 sec. In this way locomotion elicited both directly and as an aftereffect (post) could be examined. Both direct and post locomotion were scored as either absent or present at each stimulation condition.

Direct locomotion was elicited at 34 of 93 sites. Post-stimulation locomotion was elicited at 58 sites, 31 of which also showed direct locomotion. The majority of these most effective sites appeared to be concentrated among ventromedial regions of the diencephalon and mesencephalon, including the interpeduncular nucleus, the ventral tegmental area, the mammillary bodies, the pre-mammillary nucleus and the posterior, dorsomedial and ventromedial nuclei of the hypothalamus. The zona incerta nucleus and the immediately surrounding areas were also highly effective. The ventromedial thalamus and the medial forebrain bundle were effective in producing only post-stimulation locomotion. Stimulation of the fields of Forel and the area posterior to the zona incerta was ineffective, and thus the course of the presumed output to the midbrain is yet to be determined. Very few sites in either the substantia nigra or cerebral peduncle were effective. This pattern indicates that locomotion may be elicited from widespread areas in the ventral midbrain and diencephalon. Despite the large number of sites tested, simple anatomical distinctions between these effective sites are not yet apparent.

246.3 TOPOGRAPHICAL ORGANIZATION OF THE FIBERS WITHIN THE STRIA TERMINALIS IN THE CAT BRAIN. S. Beaulieu, P. Langelier*, A. Parent, L.J. Poirier and R. Boucher. Lab. de neurobiologie, Hôpital de l'Enfant-Jésus, Québec, Qué., Canada, G1J 1Z4.

Taking advantage of the property of lesioned fibers to transport horseradish peroxidase (HRP) both retrogradely and anterogradely, HRP (Sigma type VI, 50% solution) was injected (0.02 ul) directly in the stria terminalis (S.T.) at the level of the body of the caudate nucleus. The survival time was 24 hrs in all experiments. The benzidine dihydrochloride or tetramethylbenzidine was used as the chromogen. Wheat germ agglutinin-horseradish peroxidase (WGA-HRP) was used in a few animals. Tritiated leucine (20-50uCi in 0.06-0.5 ul) was injected in the bed nucleus of the stria terminalis (BNST), the nucleus of the lateral olfactory tract (NLOT) and in various areas of the amygdala in order to trace the efferent projections from these structures. Standard autoradiographic procedures were used. In other experiments lesions of the S.T. were made and, 3 days later, the acetylcholinesterase (AChE) activity within these fibers was revealed histochemically in diisopropyl fluorophosphate (DFP) pretreated (1.6 mg DFP/kg, 6-9 hrs before sacrifice) cats. Injections of HRP interrupting most fibers of the S.T. are associated with very intense labeling of the neurons of the NLOT. The medial part (magnocellular division) of the central nucleus contains a large number of labeled neurons. The lateral part of the central nucleus, the medial and cortical nuclei and part of the basal nucleus of the amygdala as well as the amygdala-hippocampal area and the BNST contain a moderate to low number of labeled cells. On the basis of injections of HRP involving different parts of the S.T. we may infer that the dorsomedial part of the S.T. contains fibers from the basal nucleus and the amygdalo-hippocampal area. A majority of the efferent fibers of the amygdala coursing in the lateral part of the S.T. originate in the central nucleus. Autoradiographic experiments complement these data. Among other things the BNST is one of the main sources of the afferent fibers to the amygdala. They course in the lateral part of the S.T. Histochemical findings demonstrate the presence of AChE-rich fibers which course predominantly in the lateral part of the S.T. and end in the amygdala. An attempt is being made to localize the exact origin of these fibers by using the Mesulam method ('76) which allows to visualize both AChE and HRP. The findings of the present study suggest that the fibers coursing within the S.T. are organized according to a complex pattern. The lateral part of the S.T. contains amygdala afferents from the BNST whereas the dorsomedial part of the S.T. contains efferents arising principally from the basal nucleus and amygdalo-hippocampal area. Also, the NLOT appears to contribute heavily to this major pathway of the limbic system.
(Supported by the MRC).

246.4 LABELING KNIFE CUTS USED TO TRACE A PATHWAY NECESSARY FOR LORDOSIS IN THE HAMSTER. C. W. Scouten and C. W. Malsbury. Dept. of Psych., Memorial University of Newfoundland, St. Johns, NF, CANADA A1B 3X9.

There has been no practical method of scanning an entire brain for the locations of cell bodies whose axons have been cut or injured. Partly because of this technical limitation, it has been difficult to assign the cause of functional deficits following a lesion to damage to specific cell groups or pathways. The cells in the zone of necrosis surrounding the lesion represent only a subset of all cells directly injured. Cells in distant locations whose axons merely pass through or near the lesion may also be directly damaged, and thus may be the cells whose damage caused the functional deficit. However, the locations of these cells are not disclosed by the standard histological analysis.

We now report a labeling knife cut technique which allows us to observe the behavioral effects of a stereotaxically-placed knife cut, and then scan the brain for all directly affected cells. An axonal transport tracer (1 ul of 2% HRP in water) is allowed to dry on the blade of an extended wire knife (DKI) prior to making a cut. Behavior is tested after 48 hours, and the animal is then sacrificed and the brain perfused and reacted in the standard manner (Mesulam, M.M., J. Histo. Cytochem. 26:1281, 1978)

We are now using this technique in the analysis of a hypothalamic pathway essential for lordosis in the female hamster. Unilateral sagittal plane knife cuts medial or lateral to the medial forebrain bundle at the level of the supraoptic commissures impair lordosis in response to stimulation of the contralateral, but not the ipsilateral, flank (Ostrowski, Scouten and Malsbury, Physio. Behav., in press, 1981). Unilateral labeling cuts were made through each of the two lateral positions in individual females. Some of the areas containing labeled cells are common to both types of cuts in females showing behavioral deficits. These include the peripeduncular nucleus, the dorsal raphe, the ventral tegmental area, the ventral premammillary nucleus, the lateral ventromedial hypothalamus, and parts of the amygdala. The areas labeled by both cuts contain populations of cells that project into or out of the hypothalamus at the level of the SOC. The cells whose axons form the pathway(s) necessary for lordosis must therefore comprise a subset of this population.

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246.5 SEPTAL LESIONS MODIFY C.N.S. CHOLINERGIC-DOPAMINERGIC PHARMACOLOGICAL RESPONSES. R. J. Carey. Veterans Administration Medical Center, Syracuse, New York 13210.

Administration of 0.5 mg/kg d-Amphetamine and 0.25 mg/kg Scopolamine produced equivalent degrees of hyperactivity in intact rats (N=8). The hyperactivity effects of these two drugs, however, proved to be strongly modified by septal lesions. Rats with septal lesions (N=8) became much more active than controls after 0.5 mg/kg Amphetamine, but did not exhibit any hyperactivity after the 0.25 mg/kg injections of Scopolamine. This differential effect of septal lesions on Amphetamine and Scopolamine was also observed across 3 dose levels of each drug (0.25, 0.5 and 1.0 mg/kg d-Amphetamine and 0.125, 0.25 and 0.5 mg/kg Scopolamine.) Thus, septal lesions appear to significantly alter cholinergic-dopaminergic balance in the brain. These observations suggested that septal lesions might modify the effects of cholinergic-dopaminergic imbalances produced by 6-hydroxydopamine (6-OHDA) lesions. Bilateral 6-OHDA injections into the nucleus accumbens (N=8) attenuated both Apomorphine (.125, .25 and .5 mg/kg) induced stereotyped behavior and Haloperidol (2.5 mg/kg) induced catalepsy. If the 6-OHDA lesions of the nucleus accumbens were made in combination with septal lesions, however, the effects of the apomorphine and haloperidol were restored to levels of control rats. Thus, a septal lesion reversed the manifestations of a dopamine deficiency, thereby providing additional evidence that this limbic system structure is important in cholinergic-dopaminergic balance.

246.6 MESOLIMBIC MECHANISMS OF BEHAVIORAL SUPERSTITION. J. A. Devenport* and L. D. Devenport. (SPON: J. A. Andrezik). Dept. Psychiat. & Behav. Sci., Univ. Okla. Health Sciences Ctr., Oklahoma City, OK 73190.

The hippocampus helps to protect against erroneous response learning and it seems to do this by promoting behavioral variability (Devenport & Holloway, J. Comp. Physiol. Psychol. 94:691, 1980) such that alternative rates, places, topographies, etc., are sampled and compared. Variability permits the detection of redundancies, superstitions, and more favorable strategies. We have adduced evidence to the effect that the hippocampus modulates behavioral variability by opposing reward-induced stereotypy (Devenport et al., Science, in press). Functional and anatomical considerations suggest that the hippocampus and stereotypy mechanism interact within the mesolimbic forebrain. If this is the case, then lesions here should mimic hippocampal lesions. Electrolytic lesions were made in various mesolimbic forebrain sites as well as in the superior colliculus and hippocampus. Rats with neocortical ablation were also prepared.

Susceptibility to superstition was assessed by daily 30 min exposures to a fixed-time 100 sec schedule of noncontingent pellet delivery. Testing occurred in operant chambers where gratuitous responses could be sampled and automatically recorded. Rats with extensive hippocampal damage and those with lesions of the nucleus accumbens (NA) and nucleus interstitialis striae terminalis (NST) developed superstitious bar-pressing and other responses. Their superstition was in marked contrast with groups bearing damage in the neocortex, olfactory tubercle, and superior colliculus. We suggest that the NA and NST are sites where the hippocampus and reward systems converge in functional opposition.

246.7 AN AUTORADIOGRAPHIC STUDY OF THE EFFERENT CONNECTIONS OF THE CENTRAL NUCLEUS OF THE MONKEY AMYGDALA. D.G. Amaral[1] and J.L. Price. Dept. of Anat. & Neurobiol., Washington Univ. Sch. of Med., St. Louis, MO 63110, [1]The Salk Institute, San Diego, CA 92138.

As part of an ongoing study of the efferent connections of the amygdaloid complex in the primate, we have injected small amounts (100 nl) of ^{3}H-amino acids into the central nucleus of the cynomolgus monkey (Macaca fascicularis). After a two-week survival period the animals were sacrificed and the brains processed for the autoradiographic demonstration of axonal projections. A small ^{3}H-amino acid injection, which is largely confined to the central nucleus, leads to the labeling of a number of brainstem structures with fibers extending caudally to the spino-medullary junction. Specifically, in the forebrain, the central nucleus projects heavily to the nucleus of the stria terminalis, the basal nucleus of Meynert, the horizontal limb of the nucleus of the diagonal band and more lightly to the substantia innominata and the preoptic area. In the hypothalamus, label is found over the dorsomedial nucleus, the perifornical region, the lateral hypothalamic area, the supramammillary region and most heavily in the paramammillary nucleus. The nucleus centralis medialis of the thalamus receives a projection as does the ventrolateral quadrant of the nucleus reuniens. In the mesencephalon, there is fairly heavy labeling within and dorsal to the substantia nigra pars compacta and to the peripeduncular nucleus; there is lighter label among the cells of the ventral tegmental area and the midbrain central gray substance. More caudally, fibers from the central nucleus travel in the lateral tegmental reticular fields and contribute collaterals to the raphe nuclei, the central gray substance and the medial pulvinar. One of the heaviest terminal zones is the parabrachial region of the pons; both the lateral and medial nuclei receive a prominent input. Only the ventral aspect of the nucleus locus coeruleus receives a substantial projection but there is also labeling of the subcoerulear region. Finally, there is very heavy labeling of the nucleus of the solitary tract and of the immediate surround of the dorsal motor nucleus of the vagus nerve. We have also determined that the lateral, basolateral and basal accessory amygdaloid nuclei and the periamygdaloid area all send intra-amygdaloid projections to the central nucleus. Thus, the present findings, taken together with recent reports of widespread projections from the temporal cortex to the amygdala, demonstrate a potentially trisynaptic route between temporal association neocortex and a variety of brainstem nuclei, many of which are related to autonomic functions.

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246.8 EFFECTS OF ELECTRICAL STIMULATION OF THE HIPPOCAMPAL FORMATION UPON THE EXTENT OF THE TRIGEMINAL SENSORY FIELD PRESENT DURING ATTACK BEHAVIOR IN THE CAT. R.E. Watson, A. Siegel, H. Edinger, C.H. Block and S.L. Stoddard-Apter. Departments of Physiology and Neuroscience, N.J. Medical School, Newark, N.J. 07103.

That the dorsal and ventral hippocampus are capable of differentially modulating hypothalamically-elicited quiet biting attack (QBA) has previously been demonstrated (Siegel and Flynn, 1968). Additionally, it has been shown that stimulation of QBA sites in the hypothalamus can modify the trigeminal somatosensory fields around the region of the lipline that are normally activated during the course of QBA, contributing to the successful completion of the attack behavior (MacDonnell and Flynn, 1966). In a recent analysis, Block, et al. (1980) demonstrated that sensory fields of the lipline supplied by the trigeminal nerve were expanded by the concurrent stimulation of amygdaloid sites facilitatory to QBA and constricted following stimulation of inhibitory amygdaloid sites. The present study was conducted to ascertain whether similar mechanisms are exerted upon the effective sensory fields following stimulation of hippocampal sites which modulate hypothalamically-elicited QBA.

Electrodes for stimulation and recording were implanted bilaterally in the dorsal and ventral hippocampal formation and hypothalamus under aseptic conditions in cats. Postoperatively, stage I of the experimental protocol consisted of identifying sites in the hippocampus which significantly modulated ($p < 0.05$) hypothalamically-elicited QBA. In stage II, the lateral extent of the lipline that, when probed, could elicit the jaw-opening component of QBA, was determined with hypothalamic stimulation alone or with the concurrent stimulation of hypothalamus and hippocampus. Preliminary results indicate that stimulation of ventral hippocampal sites which facilitate QBA significantly expanded the effective trigeminal sensory fields while stimulation of dorsal hippocampal sites which inhibited QBA had little or no effect upon the size of the trigeminal sensory fields. Latencies for the jaw-opening response were not affected by stimulation of any region of hippocampus. It thus appears that only the ventral hippocampus contributes to the modulation of hypothalamically-elicited QBA by acting upon sensory components of the response mechanism.

The neural systems metabolically activated following stimulation of hippocampal sites which effectively modulate QBA are currently undergoing examination with the use of ^{14}C-2-deoxyglucose autoradiography.

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246.9 THE NEURAL BASIS OF ESCAPE BEHAVIOR ELICITED FROM THE FELINE HYPOTHALAMUS. S. Fuchs, A. Siegel, and H. Edinger. Depts. of Physiology and Neurosciences, College of Medicine and Dentistry of N.J., Newark, New Jersey, 07103.

The feline escape response is characterized by pupillary dilatation followed by a vigorous attempt by the cat to jump out of the observation chamber. An approach involving the methods of electrical brain stimulation, ^{3}H-leucine radioautography, and ^{14}C-2-deoxyglucose radioautography was utilized to describe the neural pathways mediating flight behavior. The principal paradigm involved the initial identification of an escape response by hypothalamic stimulation with a moveable electrode. Following identification of this behavior, an injection of ^{3}H-leucine was placed through a Hamilton syringe at the position occupied previously by the electrode tip and the efferent projections from hypothalamic cell bodies were traced. In order to further describe the nature of the functional projections, ^{14}C-2-DG was injected systemically in separate animals from which escape behavior was elicited and the metabolically-activated pathways were identified.

The sites from which flight behavior was elicited were located throughout the entire rostro-caudal and medio-lateral extent of the dorsal hypothalamus. The pathways associated with escape behavior from the dorsal hypothalamus appeared to supply rostral forebrain sites which included the septum, diagonal band, bed n. of the stria terminalis, preoptic region, substantia innominata, and medial amygdala. The first-order efferent fibers supplying the amygdala appeared to reach their target by two routes-- the stria terminalis and through axons passing in a reverse direction in the ventroamygdalofugal pathway. Fibers were distributed also to the anterior, perifornical, ventromedial, and posterior hypothalamic regions. It should be noted that labeled fibers were distributed caudally to the centrum-medianum--parafascicular (cm-pf) region of thalamus and were also traced downstream to the midbrain central gray and central and ventral tegmental fields. The results point to the likelihood that structures identified previously with the control of flight behavior such as cm-pf, midbrain central gray, and specific nuclei of the basal forebrain receive first-order projections from dorsal hypothalamic sites which, upon stimulation, can elicit an escape response.

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246.10 FUNCTIONAL ACTIVATION OF THE MEDIODORSAL THALAMIC PROJECTION SYSTEM IN THE RAT: A ^{14}C-2-DEOXYGLUCOSE ANALYSIS. M. Brutus, R. Watson, S. Weiner* and A. Siegel. Dept. of Neurosciences, N.J. Medical School, Newark, N.J. 07103.

Previous studies have indicated that stimulation of the mediodorsal thalamic nucleus (MD) powerfully modulates hypothalamically-elicited aggressive behavior. In order to provide greater insight into the possible mechanisms underlying MD control of hypothalamic processes, the present study utilized the ^{14}C-2-deoxyglucose (2-DG) radioautographic tracing method to functionally characterize pathways and target structures activated following electrical stimulation of various sites within the MD.

Nembutal anesthetized (45 mg/kg) rats were injected with 2-DG prior to administration of an experimental paradigm which consisted of 45 minutes of electrical brain stimulation (200uA, 62.5 Hz, 1 msec biphasic pulses) delivered for alternating 30 sec. on and off periods. Standard procedures were utilized for the removal and processing of brain tissue for X-ray autoradiography.

Stimulation sites within MD were grouped as follows: rostral MD, lateral and medial aspects of central levels of MD, and posterior MD. Sites within control animals included the n. reuniens, reticular, ventromedial and anteromedial thalamic nuclei. In order to determine the degree to which changes reflected antidromic activation of cortical efferents, stimulating electrodes were also placed in the anterior cingulate, sulcal and medial prefrontal cortices.

Stimulation of any region within MD resulted in the activation of fiber bundles supplying the ventral aspect of the reticular nucleus, ventromedial thalamic nucleus, medial aspects of the nucleus accumbens and medial and sulcal prefrontal cortices. The most intense labeling of the sulcal cortex was associated with stimulation of central levels of MD. Stimulation of wide regions within MD also appeared to result in activation of the midline thalamic nuclei and of fibers passing ventrolaterally in the inferior thalamic penduncle to the vicinity of the far lateral preoptic region, substantia innominata and horizontal limb of the diagonal band. The data further suggests the possibility that medial MD stimulation may also activate descending fibers within periaqueductal central gray and neighboring regions of the raphe system.

The data suggests the likelihood that MD inputs into the perifornical lateral hypothalamus in the rat are indirect ones and probably involve such interneurons as the midline thalamic nuclei and such basal forebrain regions as the diagonal band nuclei, n. accumbens and substantia innominata. (Supported by N.I.H. Grant NS07941-12).

246.11 THE MEDIAL FOREBRAIN BUNDLE OF THE RAT. R. Nieuwenhuys[X], J.G. Veening[X] and L.M.G. Geeraedts[X](SPON: P.G.M. Luiten).
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The medial forebrain bundle (MFB) may be considered as the central longitudinal pathway of the limbic forebrain-midbrain continuum. Extending from the tuberculum olfactorium to the ventromedial part of the tegmentum mesencephali this bundle traverses the lateral hypothalamic area. Although at present a wealth of information is available on the manifold origins and sites of termination of the axons constituting the MFB, relatively little attention has been given to the bundle itself, i.e. to its boundaries, its fiber composition and to the spatial arrangement of its constituent components. In order to facilitate the study of these aspects the main trajectory of the MFB was analysed in normal Klüver-Barrera and Bodian stained material. A detailed atlas of the bundle and its surroundings, comprising sections at ten equidistant levels, was prepared. This atlas has provided the basis for an analysis of the extensive collection of experimental material present in the Dept. of Anatomy and Neurobiology, Washington University, St. Louis, consisting of serially sectioned brains of rats which had received a single injection of tritiated amino acids in areas known to contribute fibers to the MFB. This analysis, which has been carried out by one of us (J.G.V.), in cooperation with L. Swanson and M.W. Cowan, has revealed that most of the 20 components investigated occupy a specific and rather stable position in an overlapping pattern. Ascending components showed a clear preference for the dorsal half of the bundle. Some components (origin e.g. the medial preoptic area and the various hypothalamic nuclei) distribute their fibers diffusely over a large area, whereas others (origin e.g. olfactory tubercle, magnocellular preoptic nucleus) were found to occupy only a restricted part of the MFB. The results obtained so far suggest that the various neuron groups in the lateral hypothalamic area receive a specific input, which is directly related to their position within the MFB.

246.12 EVOLUTION OF THE HUMAN ANTERIOR THALAMIC COMPLEX: RESULTS OF MORPHOMETRIC AND ALLOMETRIC ANALYSES. E. Armstrong* and M. St. Onge*, Dept. of Anatomy, Louisiana State University Medical Center, New Orleans, LA 70117 (SPON: B. V. Updyke).

Initial quantitative analyses of Nissl stained serial sections of the dorsal thalamus among humans and apes showed that the human thalamic limbic nuclei (anterior principal (Ap) and lateral dorsal) contain more neurons than could be accounted for on the basis of brain size. The finding was interpreted as showing that during hominid evolution Ap neurons were preferentially increased (Amer. J. Phys. Anthro., 52:43, 1980). Data from an expanded series of hominoids continue to show that taxonomic grouping predicts the number of neurons in the human Ap better than does brain weight and an enlargement of an ape brain to human dimensions predicts fewer neurons than observed. Prosimian, New World and Old World monkeys were also studied. In this report the human and ape Ap is considered to be homologous, as recipient of mamillary body and subicular inputs, to the non-hominoid anterior ventral (AV), anterior medial (AM), and anterior intermedial (IAM) nuclei. Analyses based on AV data alone show the human Ap to be even more derived (less predicted by the overall size of the brain) than when the combined AV and AM data are used. Only Tarsius was observed to lack the typical non-hominoid AV and AM divisions. Comparing Ap volume to body size among primates produces a strong correlation with body weight, but with humans having a larger Ap volume than expected for a primate of our body size. In contrast, other human limbic structures such as the septum (Andy and Stephan, J. Comp. Neurol. 126:157, 1966) or the amygdala (Stephan and Andy, Acta Anat., 98:130, 1977) have a volume that is expected for a primate of our body size. Comparing Ap volume to brain weight, however, shows the human Ap to be as large as one would predict for a primate brain of human size. A given volume of nervous tissue is composed of many features and one which can be measured with relative ease is the number of neurons. The human Ap has many more neurons than the correlation of neuronal numbers to brain weight among primates predicts. The correlations between numbers of neurons and brain weight in New World monkeys came closest to predicting human values. Given the larger proportion of Am to Ap among New World monkeys than in humans and the lack of any fossil record indicating a close ancestral relationship between New World monkeys and hominids, the similar relationship between number of Ap neurons and brain weight in these two taxonomic groups is best interpreted as homoplasy. During human evolution Ap increased its numbers of neurons more than the brain expanded. Supported by the Southern Regional Education Board and NIH BRSG 449-89-5148.

246.13 EFFECT OF KINDLING AND POSTTRIAL HIPPOCAMPAL STIMULATION ON DISCRIMINATION LEARNING. Mauro Caudarella & J. B. Bryer*. Bucknell U., Lewisburg, PA. 17837

Repeated daily brain stimulation ("kindling") leads to permanent potentiation of synaptic transmission, especially in limbic structures. Behavioral effects have received less attention although Campbell & Milgram (Physiol. Behav. 1980, 24, 1115) have shown that potentiating hippocampal (HPC) stimulation facilitates acquisition of HPC self-stimulation. Caudarella, Campbell & Milgram showed that posttrial HPC stimulation facilitates passive-avoidance learning. Since this latter effect was obtained in animals that had had prior HPC self-stimulation experience, the present study tested the hypothesis that prior HPC kindling would enhance the facilitation of learning produced by posttrial HPC stimulation. Thirty-nine rats were implanted with a bipolar electrode in dorsolateral HPC (CA3). Twenty received 1 sec of 30μA sine-wave stimulation twice a day, 4-6 hr apart, for 14 days. The other 19 were handled identically but not stimulated. Then, all rats were trained in a standard Skinner-box lever-pressing task for 20 min/day with continuous reinforcement until they had performed 100 responses. The next day each rat was subjected to a discrimination training session: reward was given during only 1 of 2 alternating 30-sec light-on and light-off periods. Immediately after the 15-min session half the rats in each pretreated group (kindled and not kindled) received 80 sec of 5μA HPC stimulation in a different box; the others were handled identically but not stimulated. All rats were tested in a further session 7 days later. Discrimination ratios (no. of correct lever presses/total no. of responses) indicated a significant facilitatory effect of prior stimulation experience; furthermore, the combination of pretrial and posttrial stimulation was significantly better than either posttrial stimulation alone or no stimulation at all. Thus learning was facilitated by both types of stimulation, although pretrial stimulation (kindling) had by far the greater effect.

246.14 HUMAN AUDITORY BRAINSTEM RESPONSES DURING AUDITORY LATERALIZATION AND SELECTIVE ATTENTION TASKS. G.C. Galbraith and D.G. Kim*. MRRC UCLA Research Group, Pomona, CA. 91769

Lukas (Psychophysiol., 1980, 17, 444-452) reported an effect of selective attention on the auditory brainstem response (ABR). During visual attention Wave V latency was increased and amplitude decreased to a 2000 Hz sine stimulus, but not to an 8000 Hz stimulus. Lukas notes "...that within the inferior colliculus, neurons with best frequencies below 3000 Hz are critically sensitive to shifts in interaural phase differences and therefore may be involved with sound localization. It appears that these neurons which are involved in sound localization may be inhibited during concentrated visual attention" (p. 449).

In our laboratory we have been studying ABR correlates of auditory lateralization (stimuli presented through headphones). In previous pilot studies we found evidence for differential ABR patterns in subjects with high and low discriminability for auditory lateralization. Discriminability was quantified by means of the d' statistic derived from signal detection theory. Stimuli in the psychophysical task were brief (0.1 ms) clicks; 65 dB to the left ear, and either 65 or 70 dB to the right ear. Nineteen subjects judged whether the stimulus was lateralized to the center of the head (65-65 dB) or to the right ear (65-70 dB). During the ABR recording the left ear was stimulated at 65 dB, but the right ear received 65, 75, or 85 dB. Results showed that ABR amplitudes for high d' subjects (greater discriminability) showed a reducing pattern (decreased amplitudes) for the 65-85 dB condition.

In the above study it was appropriate to use relative intensity differences between the two ears since this is an essential cue in the localization of high frequency stimuli such as brief clicks. For lower frequency stimuli, however, such as a 2000 Hz sinusoid, phase angle differences become important. In the study to be reported 20 subjects will be required to lateralize 2000 Hz sine stimuli (2-cycle tone bursts) which differ in phase angle. As before, ABR patterns will be compared with auditory lateralization discriminability. In addition, these same subjects will perform in a selective attention task to assess possible changes in Wave V latency and amplitude. Since data will be available on the same subjects for both auditory lateralization and the effects of selective attention, it will be possible to directly test the hypothesized covariance of these behaviors.

247.1 THALAMIC PROJECTIONS TO THE SOMATOSENSORY CORTEX OF THE ECHIDNA, TACHYGLOSSUS ACULEATUS. P. S. Ulinski. Dept. Anatomy and Committee on Neurobiology, Univ. Chicago, Chicago, IL. 60637.

Evoked potential studies (Lende, 1964, J. Neurophysiol., 27: 37; Allison and Goff, 1972, Arch. ital. Biol., 110: 195) have shown that echidnas have a single, topographically organized somatosensory area (SMI) that spans a large, mediolaterally oriented sulcus called sulcus alpha. The representation of the trunk and proximal extremities lies on the gyrus caudal to sulcus alpha, but the representations of the distal limbs and beak point rostrally and extend onto the rostral bank of the sulcus. This study examines the cytoarchitecture and thalamic afferents of SMI in Tachyglossus.

The physiologically defined SMI contains two cytoarchitectonic fields. The caudal field extends across the gyrus posterior to sulcus alpha and onto the floor of the sulcus. It has a well developed layer 4 and a relatively small number of medium sized pyramidal cells in layer 5. The rostral field extends from the floor of sulcus alpha onto its rostral bank. It also has a well developed layer 4, but has a larger number of large pyramidal cells in layer 5. Layer 4 thins as it is followed onto the crown of the gyrus rostral to sulcus alpha. The remainder of this gyrus contains a single cytoarchitectonic field with a thin layer 4 and a layer 5 that is heavily populated with large pyramidal cells. This field corresponds to the physiologically defined motor cortex.

Thalamic afferents to SMI were examined by placing pressure injections of horseradish peroxidase (HRP) into the two cytoarchitectonic fields. An injection that involved both fields retrogradely labelled neurons throughout the ventrobasal nucleus of Welker and Lende (1980, in Comparative Neurology of the Telencephalon). Neurons of all sizes were labelled and their axons could be traced to the injection site in a well ordered pattern. An injection restricted to the caudal field labelled a band of neurons that extended rostrocaudally throughout the ventral part of the ventrobasal nucleus. An injection restricted to the rostral field labelled a band of neurons situated dorsally in the ventrobasal nucleus. No other thalamic groups contained labelled neurons comparable to the labelling seen in the intralaminar or posterior nuclei following an HRP injection into SmI of some marsupial or placental mammals.

These results suggest that the ventrobasal nucleus in Tachyglossus may contain two zones, each of which projects to a distinct cytoarchitectonic field in SMI. (Supported by NSF Grant INT 81-02645 and funds from the School of Anatomy of the University of New South Wales)

247.2 CORTICAL AND THALAMIC CONNECTIONS OF RAT BARRELFIELD CORTEX. J.L. Uhr*, J.K. Chapin, C.-S. Lin and D.J. Woodward. Dept. of Cell Biology, The Univ. Tx. Hlth. Sci. Ctr., Dallas, Tx. 75235.

Neurophysiological studies in this laboratory have shown that sizes of receptive fields (RF's) in the rat primary somatosensory (SI) cortex are much larger in layers V and VI than in layer IV. The aim of this study was to establish an anatomical basis for this phenomenon.

Cortico-cortical and thalamocortical projections were determined by iontophoretic HRP injections (2.5 μamp for 30 min-1 hr) into electrophysiologically identified sites in the whisker region of either SI barrelfield cortex or VB thalamus. To increase HRP uptake and transport, 1-3% lysophosphatidyl choline was added to a 20-30% solution of HRP before injection. Brain sections were pretreated with 0.5% $CoCl_2$ before reacting with DAB to enhance visualization of both dendritic and axonal branching patterns of labeled cells.

Injections into the thalamus resulted in large numbers of orthogradely labeled axons in layer IV and lower layer III, in a pattern corresponding to the aggregates of granule cells defining the centers of barrels. Observations of individual axons, easily seen in less densely labeled areas, showed that a few axons terminated in layer I. Some axons projected to more than one barrel by branching in the subcortical white matter.

Injections which included layers V and VI of the barrelfield region (electrophysiologically identified according to the whisker(s) represented), produced retrogradely labeled pyramidal cells in layers III, IV, and Vb of the columns adjacent to the injection sites. The layer III cells were located at the periphery of the adjacent columns, whereas the layer IV cells were clustered at the centers of the columns. Layer Vb cells were also aggregated in the centers of the columns. Axons of layer III cells could be followed down to layer V of the same column, where they collateralized and gave off terminals before extending to the injected region. Axons of layer V cells extended into the white matter, with collaterals projecting through layers V and VI to the injection site. HRP filled axons were also observed which extended longer distances (up to 3 mm). Axons connecting adjacent columns or more distant regions generally traveled in layer V or VI. Apical and basal dendrites of labeled cells spread up to 100 μm, but never extended as far as centers of adjacent barrels.

In conclusion, this data suggests that the larger size of RF's in layer V could be best explained by intracortical projections from cells in layers III, IV and V to layer V cells in adjacent columns. Supported by NIAAA AA-0390 and the Biological Humanics Foundation.

247.3 THALAMIC PROJECTIONS OF THE POSTERIOR PARIETAL AREA IN THE DOG: AFFERENT ORGANIZATION AND ACETYLTHIOCHOLINESTERASE HISTOCHEMISTRY. R.S. Lewis, S.T. Sakai, and D. Tanaka Jr., Departments of Anatomy, Psychology and Neuroscience Program. Michigan State University, East Lansing, Michigan 48824

The posterior parietal association area (PPA) has been shown to maintain connections with a large number of divergent thalamic nuclei and multiple cortical areas, suggesting that it may be involved in the integration of complex sensorimotor information. The present study in the dog forms part of a continuing investigation into this cortical association area in different carnivore species. Thalamic connections were mapped using the horseradish peroxidase (HRP) tracing technique. Thalamic nuclei were defined by both Nissl stains and acetylthiocholinesterase (AChE) histochemistry. In some cases, an additional series of sections was histochemically processed for both HRP and AChE.

Following HRP injections of the PPA, labelled neurons were distributed in a patch-like configuration throughout much of the dorsolateral extent of the ventral anterior (VA), ventral lateral (VL), lateral posterior (LP), and Pulvinar (Pul) nuclei. The thalamic projections to PPA were topographically organized such that injections into rostral PPA labelled a greater number of cells in VA-VL while caudal PPA injections labelled cells primarily located in LP and Pul. In addition, injections of the lateral gyrus resulted in patches of labelled cells located slightly more dorsolateral to those observed following injections located in the middle suprasylvian gyrus. HRP labelled neurons were also observed in the central lateral (CL), paracentral (Pc), lateral dorsal (LD) and lateral part of the mediodorsal (MD) nuclei.

Moderate AChE staining was observed in VA-VL, LP, and Pul while Pc, CL, and part of MD displayed a denser AChE stain. HRP positive neurons were found principally in thalamic areas displaying a positive AChE reaction.

The convergence of projections from multiple thalamic nuclei upon the PPA in the dog is by and large consistent with data reported in the cat and raccoon and supports the hypothesis that the PPA is involved in sensorimotor integration.

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247.4 CROSS-MODALITY CONVERGENCE AMONG MONOSYNAPTIC THALAMOCORTICAL INPUTS TO AREA 3a. P. Zarzecki, Dept. of Physiology, Queen's University, Kingston, Ontario, Canada K7L 3N6.

A convergence of inputs from deep and cutaneous afferents upon individual neurons of area 3a was concluded from a previous study using extracellular recordings (Neurosci. Abstr. 1979). In that study subthreshold inputs were detected only indirectly by the examination of changes in neuronal excitability and it was not possible to determine the synaptic coupling from the thalamus. We have now examined, by intracellular recording in Nembutal anesthetized cats, the responses of neurons of area 3a to stimulation of several forelimb muscle and cutaneous nerves. Intracellular recordings were made within the zone of cortex where the surface potential evoked by the contralateral deep radial nerve had its peak amplitude. All recorded neurons were histologically confirmed to be within cytoarchitectonic area 3a. Latencies of PSPs were measured from the incoming thalamocortical volley recorded from the cortical surface and evoked by each nerve. Monosynaptic thalamocortical connections were presumed for PSPs with intracortical delays of 1.0 ms or less.

Nineteen neurons of layer III were tested for synaptic inputs from each of the different stimulated nerves. EPSPs with intracortical delays of 1.0 ms or less were recorded from 15 neurons. Thirteen of these neurons had presumed monosynaptic thalamocortical EPSPs evoked from group I muscle afferents and also from low threshold (2.0 x T) cutaneous afferents. Thus, monosynaptic thalamocortical inputs to layer III neurons were common, but modality specificity was rare (2 of 15 neurons). Among 11 neurons of layer V only 4 had EPSPs with intracortical delays of 1.0 ms or less and only 2 had monosynaptic EPSPs evoked by both low threshold muscle and cutaneous afferents. On the other hand, convergent inputs to all 11 layer V neurons were demonstrated with stronger stimulation of peripheral nerves or in longer latency EPSPs.

In conclusion, layer III neurons of area 3a commonly receive cross-modality convergence by a monosynaptic path from the thalamus. Layer V neurons may receive their longer latency convergent inputs via cortical interneurons or a more slowly conducting direct thalamocortical path.

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247.5 DEVELOPMENT OF NEOCORTICAL CIRCUITRY: GABA-TRANSAMINASE REPLACES AChE IN THE BARREL CENTERS. D.A. Kristt and J.V. Waldman*. Dept. of Pathology, The Johns Hopkins Univ. Sch. of Med., Baltimore, MD 21205.

This study was undertaken to examine the developmental time course for GABA-t staining in somatosensory cortex of the rat. Rats, ranging in age from 6 dpn to adulthood, were examined in this study. This method, described by Van Gelder [1965], was used to demonstrate GABA-t in 10 μm frozen sections. Coronal and tangential sections through primary somatosensory cortex (SmI) were examined. No "staining", i.e., reaction product, was apparent when the incubation medium lacked γ-aminobutyric acid (GABA). Also, specific GABA-t inhibitor ethanolamine-o-sulfate similarly prevented the reaction from taking place. In some animals, alternate sections were stained with cresyl violet to aid in evaluating laminar staining patterns in relation to cytoarchitectonics. Before 22 days of age, cortex has a uniform light blue color which represents the reaction product. At this time, the neostriatum is quite darkly stained. The barrels are not identifiable in GABA-t stained material. In contrast, they can be seen in preparations stained for Nissl [Rice and Van der Loos, 1977, mouse], AChE [Kristt, 1979], or succinic dehydrogenase [Killackey and Belford, 1979], beginning at 3 dpn. By 22 days of age, in coronal sections stained for GABA-t, one can clearly see darker foci approximately 200 μm in width separated by a clear space of approximately 50 μm. These foci extend for approximately 300 μm in the depth of layer IV. They can be followed in serial coronal sections. In addition, they appear to correspond to roughly circular, similarly stained foci noted in tangential sections through the SmI barrel field. In short, the dimensions, localization among the cell layers, and distribution in the barrel fields would suggest that these GABA-t positive foci correspond to the neuropil in the barrel centers. Unfortunately, there was too little information available to be able to conclude that these fibers are GABAergic, and GABA-t is not exclusively localized to GABAergic synapses. However, what is particularly interesting is the observation that the early-appearing AChE positivity of fibers within the barrel centers disappears in the same period that GABA-t staining of fibers first appears in the same foci. Future work is needed to determine whether this represents a cholinergic-GABAergic developmental interaction.

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247.6 EVIDENCE FOR INCOMPLETE REORGANIZATION OF THE S-I FOOT REPRESENTATION FOLLOWING SCIATIC NERVE SECTION IN THE ADULT RAT. J. T. Wall, C. G. Cusick*, and J. H. Kaas. Depts. of Psychology and Anatomy, Vanderbilt University, Nashville, TN 37240.

Studies of chronic peripheral deafferentation in adult mammals demonstrate that somatosensory neurons normally representing the denervated skin become activated from neighboring skin areas having normal innervation. Studies from adult primates indicate that cortical representations of denervated skin areas become entirely reactivated by cutaneous inputs within several weeks, but it remains unclear whether such complete reorganization is a general feature of post-injury adjustment. The present study investigates the cortical organization of adult rats which had chronically undergone partial denervation of the hindfoot to determine if the representation of the denervated skin becomes completely reactivated by cutaneous inputs within the time period suggested by the primate results.

As established by peripheral nerve recordings from normal rats, most of the hindfoot innervation is supplied by the sciatic nerve with a relatively smaller contribution from the saphenous nerve. Partial denervation of one hindfoot was produced in adults (> 3 months old) by cutting and ligating the sciatic nerve following which the organization of the hindfoot representation was determined. Rats were anesthetized with ketamine hydrochloride and receptive fields were defined with light tactile stimuli for clusters of neurons located in the middle cortical layers at approximately 80 vertical penetration sites spaced 150-300 μ apart.

Compared to 5 normal adults, results from 5 rats deafferented for 20-157 days indicate the following. (1) The area of cortex representing the skin of the saphenous nerve (range = .32-.68 mm^2) is larger than the comparable area in normal rats (range = .08-.20 mm^2). (2) The area of cortex representing the skin of the saphenous nerve is smaller than the combined areas of representation for the saphenous and sciatic nerves in normal rats (range = .86-1.19 mm^2). (3) The receptive fields are located almost exclusively over skin regions coinciding with the normal saphenous distribution.

These observations suggest that deafferentation in adult rats results in an incomplete cutaneous reactivation of the cortical representation for the denervated skin during the first few months after injury. From receptive fields defined with light tactile stimuli it does not appear that the sciatic skin became reinnervated to any major extent. If results from rats and primates can be compared, these studies indicate that variables in addition to the time following denervation contribute to the degree of central reorganization following peripheral injury.

247.7 SELECTIVE EFFECTS OF ALCOHOL ON RESPONSES OF SINGLE UNITS IN SOMATOSENSORY CORTEX. John K. Chapin and Donald J. Woodward. The Univ. Tx. Hlth. Sci. Ctr., Dallas, Tx. 75235.

This study was conducted to determine the effect of alcohol intoxication on sensory responses of single units in the somatosensory (SI) cortex of awake, freely moving rats.

One aim was to compare the effects of alcohol with those of other anesthetics. Post-stimulus time histograms of units recorded in the SI forepaw area were generated either by natural touch of the forepaw with a hand held probe or by stimulating through bipolar electrodes surgically implanted under the skin of the paw. Histograms derived from both these methods were quite similar. These generally featured a short latency excitatory response which was divisible into an initial peak (Ela; from 7-15 msec) and a second peak (Elb; 17-45 msec). Experiments involving lesions of the cuneate nucleus indicated that the Ela peak is derived from sensory information reaching the cortex via dorsal column- medial lemniscal pathways, whereas the Elb peak arrives via extralemniscal pathways. Under halothane or nembutal anesthesia the Elb peak was selectively abolished. Administration of alcohol (1.5 gm/kg body weight by I.P. injection of 10% saline solution) produced active staggering behaviors in rats. This dose produced a slight diminution in the amplitude of the Ela peaks of the histograms produced by paw stimulation, but completely abolished the Elb peak. Thus, although the animals were still awake, alcohol produced an effect in the SI cortex similar to doses of anesthetic agents which caused unconsciousness.

A second aim was to observe the effects of alcohol intoxication on the selective inhibition of somatic sensory input to the cortex. In previous studies we have shown that transmission of cutaneous sensory information from the paw to the cortex during movement is subject to a time varying pattern of sensory suppression which selectively "gates in" only certain sensations. As a consequence of this phenomenon, histograms generated by random stimulation of the paw during running show a marked decrease in the overall sensory responsiveness of the cortical neurons compared with histograms obtained from similar stimulation during resting behavior. After alcohol administration, however, histograms of the sensory responses of single cortical units to paw stimulation, even during intense motor activity, were nearly the same as during rest.

Our hypothesis is that alcohol may compromise function in a specific system which, when it is functionally suppressed, leads to failures in transmission of long latency sensory responses in the cortex and also of selective cortical sensory gating mechanisms. Supported by NIAAA grant AA-0390 and an award from the Biological Humanics Foundation.

247.8 REGIONS OF THE SOMATIC CORTEX THAT AFFECT SUPERIOR COLLICULUS CELLS IN THE CAT. H.R. Clemo and B.E. Stein, Dept. of Physiology, Medical College of Virginia, Richmond, Va 23298.

Visual corticotectal fibers are known to account for many of the specialized features in the superior colliculus. It has been assumed that the somatic corticotectal system would function in a similar fashion. Despite this expectation a previous study demonstrated that while cooling primary visual cortex had a profound effect on the activity of visual cells in the superior colliculus, cooling primary somatic cortical areas (SI-SIII) did not change the activity of somatic cells here (Stein, 1978). It now seems possible that the descending somatic input to the superior colliculus originates predominantly from areas outside the 'primary' somatic cortices. In the present study an attempt was made to compare the effects of stimulation of different somatic cortical regions upon cells in the superior colliculus. Particular attention was paid to the anterior ectosylvian sulcus which may be a significant source of descending somatic input to the superior colliculus.

The response of somatic cells in the superior colliculus to single electrical pulses (0.2 msec, 10-900 μA) delivered to different somatic cortical regions were recorded with glass-coated tungsten microelectrodes. Superior colliculus neurons were excited by stimulation at 341 cortical sites with an average latency of 3.01 msec ± 1.21. They were activated by stimulation of primary somatic areas SI and SII with average latencies of 5.0 and 2.8 msec respectively, at stimulus thresholds of 200-900 uA. However, the shortest latencies ($\bar{x}$=2.5 msec), lowest stimulus thresholds (less than 100 uA) and greatest number of effective stimulation sites (242/341) were found in the anterior ectosylvian sulcus. These were located primarily in a 2 mm^2 area deep in the superior wall and fundus of the anterior ectosylvian sulcus. A somatotopic map (SIV) has been located in the anterior ectosylvian sulcus which consists of relatively small, discrete, receptive fields on the contralateral body (see Clemo & Stein, 1980). In the fringe areas of this region somatic neurons have large receptive fields which are often bilateral. It was in this fringe area that sites with the lowest stimulus thresholds were located. Preliminary observations indicate that the receptive fields of these corticotectal neurons are larger than, but encompass, the receptive fields of the collicular neurons they activate.

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248.1 CHANGES IN RESPONSES OF LATERAL GENICULATE CELLS FOLLOWING THE ABOLITION OF THE VISUAL CORTEX IN RATS. S. Molotchnikoff, F. Tremblay*and F. Leporé. Département de Sciences biologiques, Université de Montréal. Montréal, Québec, Canada.

Numerous histological investigations carried out on most mammals have demonstrated that the Lateral Geniculate Nucleus (LGN) and Visual Cortex (VC) have reciprocal relationships. The role of the corticogeniculate fibers is far from being defined. The aim of the present study was to analyze the contribution of VC on responses of LGN cell's in anesthetized and paralyzed hooded-rats. Unitary activity was recorded with glass micropipettes filled with NaCl (2 M). Receptive fields were mapped on a X-Y display screen positioned 30 cm from the eye. Cortical functions were abolished by a topical application onto its surface of KCl (3 M). The elimination of EEG activity and visual evoked potentials confirmed that the VC was unresponsive to light flashes. Receptive fields were mapped prior to and following cortical depression.

An analysis of the results indicated that the on response of the surround of most (6/2) OFF-center cells diminished or was completely abolished. In contrast the off-response remained unmodified or even slightly enhanced. The reverse was observed in half of the cells with ON-center receptive fields. In the remaining on-center cells the center response also declined. Thus, the differential effect of cortical depression is to modify the concentric organization of receptive fields. This differential reduction of on or off discharges was often accompanied by a broadening of the area from which the unaltered response was elicited. In geniculate cells that exhibited non-concentric organization (N = 14) their responses were virtually unaffected by cortical depression.

These results are very similar to those obtained on rabbits (Brain Res. 193: 383-399, 1980; J. Neurosci. Res. 5: 419-429, 1980) and cats (Exp. Brain. Res. 32: 345-364, 1978). Consequently, it appears that in all three species corticofugal influences affect center-surround relationships.

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248.2 EFFECTS OF CORTICOGENICULATE FEEDBACK ON CENTER-SURROUND BALANCE AND SPATIAL TUNING OF MONKEY LGN CELLS. J. W. McClurkin*, R. T. Marrocco, and R. A. Young*. Dept. of Psychology, Univ. of Oregon, Eugene, OR 97403.

We have recently shown (Marrocco et al., 1979, Soc. Neurosci. Abstr; Marrocco et al., in press) that the responsivity of monkey lateral geniculate cells (LGN) can be altered by visual activation of the corticogeniculate pathway. A radial grating not directly exciting the LGN cell produced either an inhibition or excitation (or no change) in the response to a small, central flashing spot. The grating effect was abolished by cortical cooling. The present report examines whether the grating effect has differential effects on center and surround and if the changes in balance are reflected in the spatial selectivity of the LGN cells.

Using standard electrophysiological recording techniques, LGN cell responses were measured with the radial grating either stationary or moving radially. The grating was absent from a central aperture whose diameter was chosen to be slightly larger than the receptive field surround. The LGN cell receptive field was directly stimulated with light or dark spots or annuli, or with drifting sine wave gratings presented in the radial grating window. In each case we compared cell responses in the presence of the moving grating to those obtained with the stationary radial grating.

Using spots and annuli inside the moving radial grating, three types of effects were found: I) decreased central response, no effect on surround; II) decreased central response, increased surround response; III) increased central response, decreased surround response. These changes in center/surround balance were paralleled by changes in spatial selectivity. Cells showing Type I effects had uniformly depressed contrast sensitivity functions; Type II cells showed selective inhibition to low spatial frequencies; Type III cells showed uniformly increased (higher sensitivity) spatial selectivity. If it is assumed that the dimensions and relative strengths of center and surround are prime determinants of spatial selectivity, then the changes found with spots and annuli accurately predict the alterations in the contrast sensitivity functions. Our results thus suggest that LGN cell spatial selectivity may be determined by cortical input as well as retinal influences. Supported by NIH grants EY 01286-06 and 5T32 GM07257.

248.3 EXCITATORY AND INHIBITORY EFFECTS OF LUMINANCE SHIFTS ON LGN NEURON RESPONSES UNDER SIMULATED EYE MOVEMENT CONDITIONS. D. Impelman*and B.A. Brooks. Dept. Phys., UTHSC, San Antonio, Texas.

Both local and global masking effects reduce visual sensitivity during eye movements; foveal sensitivity is reduced by luminance changes localized to the central retina as well as by changes in luminance limited to the peripheral retina, ten to twenty degrees eccentric to it(B.A. Brooks et al, ARVO Abstracts). Peripheral luminance shifts evoke transient excitatory responses in retinogeniculeate neurons. Center responses to test flash stimuli in the fixated eye are reduced by temporal interactions with the periphery response(Exp. Brain Res, 31:235-248). The excitatory periphery effect is characteristic of Y neurons and appears to be related to the shift mechanism of Y subunits that responds to changes in luminance rather than luminance levels both within and beyond their receptive field boundaries(J.P. 289:299-310). During saccades across full field vertical gratings Y neurons respond with a burst discharge similar to the shift response(J.P. 250:579-95). The response of complex cells during saccades over patterned visual fields is also a brisk excitation while simple cells respond with an inhibition which persista throughout the duration of the eye movement(Vis. Res. 20:553-56). These findings suggest that full field shift effects reduce visual responses during saccades either by temporal interactions of excitatory responses and/or by inhibition produced by activation of Y neurons which then inhibit X cell responses. In the present study we have looked at excitatory and inhibitory shift effects in cat LGN neurons under simulated eye movement conditions to find out to what extent excitatory and/or inhibitory interactions in X andY neurons contribute to masking effects during saccades under full field conditions. Responses to displacements of peripheral(.4c/d) and full field(.4-2c/d) gratings at saccadic velocities were obtained. (The space average luminance of the gratings was .1logfL and background luminance was -1 logfL.) Test flash stimuli were chosen to demonstrate either excitatory or inhibitory effects. 91% of the X cell responses to saccadic displacements of full field gratings were center responses to local luminance levels during and after the movement. Both excitatory and inhibitory response attenuated excitatory test flash responses. Weak inhibitory shift effects observed in 9% of the X cells also reduced test flash responses. Although excitatory shift responses were recorded from Y neurons a secondary burst discharge during saccades was not. The neural mechanism for the shift effect and the secondary burst discharge may not be the same. It appears that in most LGN X cells under full field conditions, center responses to local luminance levels may mediate their sensitivity changes during saccades.

248.4 VISUAL RESPONSES OF SINGLE UNITS IN THE LATERAL PULVINAR OF MACAQUE MONKEYS. G. Felsten, D. Burman*, K. Yoshida and L. A. Benevento. Department of Anatomy, University of Illinois, College of Medicine, Chicago, Illinois 60680.

Single unit recordings were made throughout the lateral pulvinar (PL) of paralyzed macaques maintained on 70% N_2O/30% O_2. This report does not include results from the retinotopically organized subdivision of the lateral pulvinar (PLα), which is adjacent to the inferior pulvinar, but includes those from the portion of PL which forms the lateral aspect of the caudal thalamus, including subdivision PLγ (Neurosci. Abst. 3 (1977) 573). Approximately 50% of the units responded to moving or stationary visual stimuli. Most had uniform receptive fields, 30% of which were smaller than 15°, while 50% were larger than 35°. Some were as large as 150° (long axis), and a few units could be influenced by a visual stimulus placed almost anywhere in the visual field. Over half the receptive fields were bilateral, the rest ipsi- or contralateral. There was no apparent retinotopic organization; many receptive fields overlapped and most were clustered about the area of central vision. Although most units were binocular, the contralateral response was dominant in 41% of the units; the ipsilateral response was dominant in 31%. In some penetrations, nearly all of the units were predominantly or exclusively driven by one eye alone. Binocular interactions were varied and complex, and often unpredictable from the monocular responses. For example, a unit could be inhibited by one eye, unaffected by the other and excited by binocular stimulation. A minority of units were orientation selective. On the other hand, several classes of cells with unusual or distinctive properties were found. Of the units sensitive to tangentially moving stimuli, one subgroup gave sustained excitation or inhibition to static levels of luminance or darkness, and another subgroup was sensitive to stimuli which moved in depth, i.e., toward or away from the eyes. For many units, the response to one class of stimuli was seemingly unrelated to the response to another class. It may be that different wiring diagrams describe these different afferent inputs. Color sensitive neurons exhibited opponent-process properties. One group was excited by some monochromatic lights, but inhibited by complementary wavelengths. Another group was excited by white light, but inhibited by monochromatic lights. Several latency classes were recognized which correlate with the response properties mentioned. For example, color units fell into the short (45-65 msec) and middle (85-120 msec) latency range, while units with unusual movement properties or binocular interactions had the longest latencies (155-235 msec). These differences are taken to reflect midbrain versus cortical inputs. (Supported by grant EY 2940 and Fellowship PHS EY 5504.)

248.5 EFFECTS OF SUPERIOR COLLICULUS AND STRIATE CORTEX LESIONS ON THE VISUAL RESPONSE PROPERTIES OF INFERIOR PULVINAR NEURONS IN THE MONKEY. D. B. Bender* (SPON: C. J. Smith). Division of Neurobiology, Physiology Dept., SUNY/AB Med. Sch., Buffalo New York 14226.

In the macaque, we have previously shown that the inferior pulvinar contains a complete representation of the contralateral hemifield. Both striate cortex and the superior colliculus project topographically onto this nucleus in a manner consistent with its retinotopic organization. We have also shown that about 2/3 of inferior pulvinar neurons are sensitive to stimulus orientation or direction of movement, while about 1/3 lack such sensitivity, responding equally well to all orientations and directions of movement.

In the present study, we wished to determine whether the input from cortex or colliculus is essential for the visual properties of pulvinar neurons. Accordingly, inferior pulvinar cells were studied in the anesthetized, immobilized macaque following lesions of either striate cortex or the superior colliculus.

Following unilateral striate cortex lesions, cells in the ipsilateral inferior pulvinar were no longer visually responsive. The range of spontaneous activity, however, appeared normal. There was no recovery of visual responsiveness up to four months following the lesion.

By contrast, large bilateral superior colliculus lesions did not eliminate the visual responses of pulvinar cells. Following colliculus lesions, the retinotopic organization in the inferior pulvinar was unaffected. Furthermore, directional, oriented, and non-oriented cells were all present.

These results suggest that striate cortex, but not the colliculus, is essential for the visual responsiveness of inferior pulvinar cells in the immobilized monkey.

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248.6 EFFECTS OF SUPERIOR COLLICULUS (SC) AND PULVINAR (P) LESIONS ON VISUAL SEARCH IN CYNOMOLGUS MONKEYS. M. Azzato*, C. Leiby*, C. Butter, D. Bender*, and D. Shirley*. Neuroscience Laboratory, University of Michigan, Ann Arbor, MI, 48109, Dept. of Physiology SUNY Medical School, Buffalo, NY, 14226.

To determine whether the SC and P participate in integrative functions underlying visual search, four monkeys were tested in a visual search task before and after receiving electrolytic SC, sham SC, or P lesions. The task was performed as follows. After a small, dim light spot appeared on the center panel, the monkey pressed it to initiate each trial. The target pattern (a circle) was then projected at varying eccentricities (5-55° from the center panel) on one of two screens located on either side of the center panel. Varying numbers of irrelevant patterns (0, 1, 3, 5, 7, 9 or 39) were also presented on the screens. All the patterns were either high contrast (16.1 cd/m^2) or low contrast (0.4 cd/m^2) relative to the ambient illumination on the screens (0.3 cd/m^2). Water reward was given only for pressing the screen on which the circle appeared. After the monkeys learned to perform the task well, their reaction times (the time from pattern presentation to the choice response) increased with a) number of irrelevant patterns and b) target eccentricity on one or both screens, suggesting that the monkeys systematically searched for the target. The slopes of these RT functions were somewhat steeper when low contrast, as opposed to high contrast patterns were presented. One monkey received a sham lesion, after which it showed no performance changes. This monkey and a second one then received SC lesions. Postoperatively, as before surgery, their RTs increased both with target eccentricity, suggesting that their search patterns were unaltered, and with number of irrelevant patterns. However, the slopes of these functions were steeper than they were preoperatively, whether the patterns were high or low contrast. Both monkeys with SC lesions also showed a striking increase in errors (pressing the screen on which the target did not appear), but only when eccentric targets and many irrelevant patterns were presented at low contrast. Errors did not increase when no irrelevant patterns were presented, so that the task was simply light detection, or when the target was presented on the part of the screens near the center panel. The RT changes could be due to deficits in saccadic movements. However, the SC-lesioned monkeys' selective increase in errors to eccentric, low contrast targets surrounded by many irrelevant patterns suggests an impairment in identifying the target or selecting it under these conditions as a stimulus for a saccade. No reliable performance changes were found in two monkeys after P lesions intended to include the tectorecipient zone.

248.7 THE ORIGIN OF THE CROSSED CORTICO-COLLICULAR PROJECTION IN THE CAT. B.R. Payne and N. Berman. Departments of Physiology/Biochemistry and Anatomy. Medical College of Pennsylvania. PA.

The superficial layers of the superior colliculus of the cat receive a direct visual input from the two retinae and indirect visual input via the two visual cortices. These projections have been shown to maintain correct retinotopic fidelity with each other (Payne & Berman, Anat. Rec. 199). We were interested in determining the exact location of the cells which form the crossed cortico-collicular pathway from the visual cortex. In addition, since the superior colliculus has been shown to contain cells which respond to other sensory modalities besides vision in the deeper layers, we were interested in determining which cortical regions send a projection to the contralateral superior colliculus. The retrograde transport of horseradish peroxidase (HRP) was used to achieve this goal. Since the needle passed through the cortex on its way to the superior colliculus it is possible that leakage of HRP into the cortex could result in labelled cells in the contralateral cortex. To overcome this possibility the corpus callosum was cut. Electrophysiological recording techniques were used to map receptive fields and to guide the placement of the injection needle into the rostal portion of the superior colliculus. Between half and one microliter of a 50% solution of HRP was injected at 4 sites. The tissue was reacted with tetramethyl-benzidine and hydrogen peroxide. In the visual cortex the largest numbers of labelled cells were observed in the lateral suprasylvian visual areas, although labelled cells were also seen in areas 17,18 and 19. Large numbers of labelled cells were also observed at several other contralateral cortical locations including the dorsal part of the posterior ectosylvian gyrus, the dorsal and anterior ectosylvian gyrus, the anterior sylvian gyrus and the lateral bank of the sylvian and rhinal sulci. In addition, large numbers of labelled cells were observed in the medial bank of the presylvian sulcus, ventral bank of the cruciate sulcus and on the medial surface of prefrontal cortex. Electrophysiological recordings in many of these regions have shown that the cells respond to more than one stimulus modality and have bilateral receptive fields. Electrical stimulation of these areas also induces eye or head movements. These results show that the superior colliculus receives inputs from a large number of regions of the contralateral cerebral cortex and the regions with the heaviest projections are those involved in orienting movements.

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248.8 CORTICAL AND INTERTECTAL INFLUENCES ON VISUALLY-GUIDED BEHAVIOR. B.E. Stein, S. Hardy* and S.B. Edwards. Department of Physiology, Medical College of Virginia, Richmond, VA 23298 and Department of Anatomy, University of Virginia School of Medicine, Charlottesville, VA 22908.

Extensive lesions of the occipitotemporal cortex produce neglect of stimuli in the contralateral visual field. This orientation deficit is ameliorated by destruction of the opposite superior colliculus or by splitting the intertectal commissure (Sprague 1966, Sherman 1974). In the present investigation we sought to determine the specific areas of cortex and colliculus responsible for this phenomenon. The visually-guided behavior of normal and lesioned cats was assessed using a perimetry device (Sprague & Meikle, '65). Ablations were made in various areas of the right cortex. When an orientation deficit in the contralateral (left) visual field was observed a lesion was placed in the superior colliculus contralateral to the cortical ablation. Visually-guided behavior was evaluated once again. We found that primary visual cortex need not be disrupted to produce a complete contralateral visual neglect. Only a band of cortex along the middle suprasylvian gyrus and sulcus had to be removed to produce this behavioral dysfunction. Furthermore, only partial lesions of the colliculus were necessary to restore visually guided orientation. Surprisingly, the reinstatement of visually-guided orientation was not predictable by referring to the region of the visual field represented in the lesioned portion of the colliculus. Thus, removal of the posterior ¼ of the left superior colliculus reinstated visually-guided behavior in all portions of the left visual field. Electrophysiological recordings confirmed that the remaining portion of the lesioned colliculus was functional.

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248.9 SOMATOSTATIN-LIKE IMMUNOREACTIVITY IN THE RODENT VISUAL SYSTEM. L. K. Laemle, S. C. Feldman, and E. Lichtenstein*. Dept. of Anatomy, CMDNJ-New Jersey Medical School, Newark, N.J. 07193 and the Hospital for Joint Diseases, New York, N.Y. 10035

The presence of somatostatin-like (SRIF) immunoreactivity was investigated in the central visual pathways of three species of rodents: mouse, guinea pig, and rat.

Brains of adult animals were fixed in Bouin's solution, embedded in paraffin, and sectioned in either the coronal or saggital plane. SRIF was localized using a highly specific antiserum to the peptide and the unlabeled antibody enzyme technique of Sternberger. Staining was judged specific by the absence of DAB reaction product in neuronal elements following adsorption of the antiserum with synthetic SRIF. Data was evaluated in terms of SRIF-synthesizing neurons as judged by the presence of DAB reaction product specific for the peptide, in the perikaryon and in neuronal processes, SRIF-containing fibers, and neurons which appeared to be innervated by SRIF-containing fibers.

Immunoreactivity was observed in the lateral geniculate nucleus, pretectal area, superior colliculus, and visual cortical areas. In the superior colliculus, a dense plexus of SRIF-containing fibers and innervated neurons was present throughout the superficial layers. In the intermediate and deep layers, SRIF fibers and innervated neurons were moderately dense adjacent to the midline, and sparsely distributed otherwise. SRIF-synthesizing cells were observed in all collicular layers. In the visual cortical areas, SRIF-containing fibers and innervated neurons were most abundant in the superficial layers. SRIF-synthesizing neurons were present in all cell layers and in a variety of cell types including pyramidal cells in layer V of the striate cortex. In the lateral geniculate nucleus we were unable to locate either SRIF-synthetic or SRIF-innervated cells, and only sparsely distributed SRIF-containing fibers. We decided, therefore, to examine similar preparations of prenatal rat brain. In the prenatal brain, just before birth, SRIF-synthetic neurons were observed in the lateral geniculate nucleus in contrast to the adult. In addition, SRIF-containing fibers and SRIF neurons were more prominent in the superior colliculus.

This is the first demonstration of somatostatin in the central visual pathway. (Supported by NIH grant 5R01 EY01174 to Dr. Laemle, and a grant to Dr. Feldman from the Foundation of the College of Medicine and Dentistry of New Jersey).

248.10 ANATOMICAL MAPPING OF REPRESENTATIONS OF THE VISUAL FIELD IN AFFERENT PROJECTIONS TO THE VISUAL CORTEX OF THE CAT. K.Albus and G. Meyer*. Dept.Neurobiol., MPI Biophys.Chem., 34Göttingen, FRG.

Small amounts of horseradish peroxidase(0.1-0.4µL) were injected into various parts of the representations of the visual field (VF) in areas 17,18 and 19. VF maps were then prepared in subcortical and cortical areas on the basis of the distribution of HRP labelled cells.

The N. lateralis post. thalami (LP) contains two complete VF representations. One occupies the middle third of the nucleus, the other the lateral third and adjacent parts of the Pulvinar(PUL). In both representations the upper hemifield(UF) is located dorsomedially, and the lower hemifield(LF) ventrolaterally from the area centralis(AC) and the horizontal meridian(HM). The vertical meridian(VM) forms the border between both representations.

The PUL contains one complete representation of the VF, which occupies only the lateral half of the nucleus. The projection columns in PUL for AC, LF and HM run from caudoventral to rostrodorsal; therefore, the vertical dimension of the VF is represented in a caudodorsal to rostroventral direction. The VF periphery extends medially from the VM, which is represented at the very lateral border of PUL.

The caudal third of the Claustrum (CL) contains a complete representation of the VF. The upper VM occupies a centre position in the caudal third of the visual CL. The AC is represented in the dorsomedial corner at the transition between caudal and middle third, and the lower VM extends through the rostral third of the visual CL, being located 0.5-1.0mm below the dorsal margin of the nucleus.

The medial part of the lateral suprasylvian area (LSAmed)contains a complete representation of the VF, and this extends over the medial bank of the middle suprasylvian sulcus(SSSmed) and the lateral third of the adjacent gyrus. The AC is represented in the caudal end of the SSSmed and continues rostrally into a large representation of the HM. Still further rostrally and proceeding from lateral to medial, are found the lower VM, the LF periphery, the UF periphery and the upper VM.

The lateral part of LSA(LSAlat), and the areas 21 and 20 contain only partial representations of the VF. In LSAlat the UF is not represented, whereas in areas 21 and 20 larger parts of the LF are lacking. It has been found, that area 20 sends projections only to area 19, whereas LSAlat, LSAmed and area21 send projections to both areas 18 and 19.

Some differences were noted between the anatomical maps and maps derived from physiological recordings. These might reflect differences in sensitivity between the methods used and/or differences in the interpretation of data.

248.11 THALAMIC PROJECTIONS TO VISUAL AREAS I AND II IN THE RABBIT. Theodore G. Weyand and Harvey A. Swadlow. Dept. Psychol.(U-20), Univ. Conn., Storrs, Conn. 06268

Thalamo-cortical projections to visual areas I and II (V-I, V-II) were studied using horseradish peroxidase (HRP) as a retrograde label. Tetramethyl benzidene was used as the chromagen. Multiple-unit mapping procedures were utilized to guide the injection of small quantities (0.04-0.15 µl) of HRP into specified regions of V-I or V-II of Dutch rabbits. As found by Karamanlidis and Giolli (Exp. Brain Res., 29: 191-199, 1977), injections restricted to V-I revealed a prominent topographical projection from the dorsal lateral geniculate nucleus (LGNd)*. Consistent projections were also found from the ventral lateral nucleus (VL), posterior thalamic nucleus (Po) and pulvinar (Pul). Injections restricted to V-II revealed consistent projections from VL, Po and Pul. In addition, following injections into V-II, a narrow zone of fibers interfacing the LGNd with the Pul contain significant numbers of labeled cells.

Our results therefore indicate that VL, Po and Pul each project to both V-I and V-II. Our data also indicate that the projection from VL to V-II is more extensive than that to V-I. Studies using double retrograde labels would prove useful in determining whether individual neurons within these nuclei project to both V-I and V-II.

* We have adopted the nomenclature of Giolli et al. (J. Comp. Neurol., 180: 743-752, 1978)

248.12 ASSESSMENT OF VISUAL PATHWAY MISROUTING IN HUMAN ALBINISM. P. A. Apkarian, D. Reits*, H. Spekreijse* and D. van Dorp*. The Netherlands Ophthalmic Research Institute, 1005 EK Amsterdam, The Netherlands.

Abnormal decussation of temporal retinogeniculostriate projections associated with albinism can be detected in the potential distribution of the visual evoked potential (VEP). Following monocular stimulation misrouted optic pathway projections produce VEP asymmetry across the occipital left and right hemispheres.

Under optimized stimulus and recording conditions a quantitative range of VEP asymmetry values was obtained in albino observers (N>50) and in normally pigmented controls. Pattern appearance/disappearance and pattern reversal VEPs were recorded from five electrodes positioned at equal intervals of 3 cm in a horizontal row across the scalp. The resultant potential distributions of the significant parts of the response were fitted with a polynomial. The difference in the location of the extreme of the polynome upon left and right eye stimulation was used as an objective indicant of asymmetry. In the controls this difference proved to be less than 1 cm; in albinos differences greater than 6 cm were typically found. As concomittant oculomotor disorders are characteristic in albinism, eye movements also were recorded and the cross spectra between left and right eye movements were obtained.

Asymmetry was found to be specific to particular VEP components within a given response profile. Under pattern appearance/disappearance conditions, e.g. the asymmetry is primarily evident in the first positive component; later components typically yield symmetric responses and/or reverse asymmetry. The degree of asymmetry was found to vary as a function of several stimulus determinants including retinal eccentricity, pattern size and orientation. Asymmetry was also examined in relation to age, acuity and albino phenotype. Left and right half field stimulation can mimic in normal observers the asymmetries found with full field stimulation in albinos.

248.13 EXTRAGENICULATE VISUAL PATHWAYS TO RETROSPLENIAL CORTEX IN THE RAT. Scott M. Thompson,* Steven C. Baker,* and Richard T. Robertson. Department of Anatomy, College of Medicine, University of California, Irvine, CA 92717. (Sponsored by Earle A. Davis, Jr.)

The extrageniculate visual projections from the pretectal complex to retrosplenial cortex via the lateral dorsal nucleus (LD) of the thalamus were examined using retrograde axonal transport of horseradish peroxidase (HRP). Small iontophoretic injections of HRP were placed at various locations within the dorsal or ventral subdivisions of retrosplenial cortex or within LD. Following 2 day survival times, frozen sections were processed for HRP histochemistry using benzidine dihydrochloride as the chromagen.

HRP injections in retrosplenial cortex produced retrogradely labelled cells within the anterior, lateral dorsal, midline, and intralaminar thalamic nuclei. Projections to retrosplenial cortex originate from LD in a partially overlapping, topographical arrangement. The ventral subdivision of retrosplenial cortex (vRSP, area 29c) receives a projection primarily from the medial half of the anterior 2/3's of LD. The dorsal subdivision of retrosplenial cortex (dRSP, area 29d) receives projections primarily from the posterior 2/3's of LD. Further, rostral injections of dRSP result in labelled cells within the rostral and medial portion of this posterior zone of LD, while caudal injections of dRSP result in labelled cells within the caudal and lateral portion of this posterior zone.

Injections of HRP within LD result in labelled cells within the pretectal complex. The number and distribution of labelled cells vary with the location of the injection. HRP injections in the caudal and lateral parts of LD (i.e., the portion of LD that projects to the caudal parts of dRSP cortex) produce the largest number of labelled cells within the pretectum. These labelled cells tend to be concentrated in the retino-recipient regions of the pretectum. HRP injections in the rostral and medial portions of LD (i.e., the portion of LD that projects to the vRSP and rostral dRSP cortices) produce relatively few labelled pretectal cells, which are located primarily in the non-retino-recipient regions of the pretectum.

These data suggest that visual information from the pretectal complex is relayed primarily via the caudal and lateral regions of the lateral dorsal nucleus to the posterior parts of dorsal retrosplenial cortex.

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248.14 CONNECTIONS OF THE PRIMARY VISUAL CORTEX WITH THE DORSAL PARASUBICULUM IN THE RABBIT. H. Holländer. Dept. Neuromorphology, Max-Planck-Institute of Psychiatry, 8000 Munich 40, F.R.G.

The rabbit parasubiculum (Brodmann's area 49) is a narrow strip of cortex intercalated between the presubiculum and the entorhinal cortex. The region was studied cyto- and myeloarchitectonically in transverse, parasagittal and horizontal series of normal material. By means of anterograde transport of horseradish peroxidase and tritiated leucine it was found that there is a projection from the primary visual cortex to the dorsal part of the parasubiculum, mainly to its first layer. Medium sized nerve cells which were retrogradely labelled with HRP were found in the same region in the lower external principal layer and the upper lamina dissecans. This finding indicated that there is also a projection back from the dorsal parasubiculum to the primary visual cortex.

248.15 DIFFERENTIAL PROJECTIONS FROM CORTICAL VISUAL AREAS TO THE NUCLEI OF THE ACCESSORY OPTIC SYSTEM IN THE CAT. R. R. Marcotte and B. V. Updyke. Dept. of Anatomy, Louisiana State University Medical Center, New Orleans, LA 70112.

In the course of studying cortico-thalamic projections of the lateral suprasylvian visual areas in the cat, we noted differential projections from these areas to the nuclei of the accessory optic system. Areas AMLS, ALLS, PMLS, PLLS, DLS, 20a, 20b, 21a and 21b, as identified by Palmer et al. (J. Comp. Neurol. 177:237, 1978) and Tusa and Palmer, (J. Comp. Neurol. 193:147, 1980) were identified by electrophysiological mapping prior to injecting tritiated proline. Additional autoradiographic material from cases involving areas 17, 18, 19, the crown of the middle suprasylvian gyrus and the splenial sulcus were also examined for evidence of projections to the accessory optic system.

The presence of terminal labelling within the accessory optic nuclei was detected in 16 of 53 injection cases. Terminal labelling was most often associated with large injections (> 30 µCi) of high specific activity precursor (> 100 Ci/mmol) and after survival of 40-48 hours.

Injections of areas AMLS, PMLS, 21a and 21b resulted in terminal labelling within the medial, lateral and dorsal terminal nuclei. Injections of area PLLS resulted in labelling only within the lateral terminal nucleus. Large injections of the 17/18 border resulted in terminal labelling only within the medial terminal nucleus. No terminal labelling was detected following smaller injections restricted to area 17, 18 or 19. Large injections of high specific activity label in areas ALLS, DLS, 20a, 20b, the crown of the middle suprasylvian gyrus, and splenial sulcus also failed to yield terminal labelling within the accessory optic nuclei.

These results suggest that only selected cortical visual areas influence activity of the accessory optic nuclei via descending projections. The accessory optic system has been implicated in the control of visual reflexes. Thus direction-specific deficits in horizontal optokinetic nystagmus following bilateral aspiration of visual cortex (Wood, Spear and Braun, Brain Res. 60:231, 1973) may be related, in part, to disruption of the corticofugal projections described above.

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248.16 THE PROJECTION FROM STRIATE AND PRESTRIATE VISUAL CORTEX ONTO THE PONTINE NUCLEI IN THE MACAQUE MONKEY. W. Fries+ (SPON: E. Pöppel) Dept. of Anatomy, University College London, and Institute for Medical Psychology, University of Munich (FRG).

The neocortex of mammals is linked with the cerebellum via the pontine nuclei (PN). Early studies on the visual cortical projections to the PN demonstrated only a minor, if any, contribution of the striate cortex. The main source of the visual cortical input to the PN appeared to be the prestriate cortex. Recently, the prestriate cortex of monkey has been described to consist of several distinct visual areas which are regarded as functionally specialized (Zeki, Nature 274,1978). We studied how these areas contribute to the pontine projection by injecting H^3-proline or HRP into the prestriate cortex for anterograde tracing.

Terminal labelling was located in the dorso-lateral part of the rostral two thirds of the pons. The density and the rostro-caudal extent varied depending on the cortical area injected. Confirming earlier reports, no projection was found from the central 10^{o} of the striate cortex (V1), but a small projection appeared after more peripheral injections. There was again no projection from central V2, nor from the V2/V3 border representing about 30^{o} eccentricity. A fairly strong projection was found to originate from V3A in the anterior bank of the lunate sulcus. The V4-complex had only a minimal projection from its rostral border region. However, the anteriorly adjoining area V5 had a powerful projection to the PN. These results suggest that all visual areas, with the possible exception of V2, take part in the pontine projection, but there are areal differences. It appears that the cortical representations of central parts of the retina are spared from this projection. However, HRP injections into V5 (the motion area) which resulted in strong terminal labelling in the pons, showed that the injected area received input from foveal striate cortex. HRP injections into V4 (the colour coding area) which similarly received input from foveal striate cortex, produced virtually no labelling in the pons. It might therefore be that topographical factors are less relevant than functional properties in determining the strength of the visual cortico-pontine projection.

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248.17 THE VISUAL CORTICAL PROJECTION TO THE STRIATUM IN THE GOLDEN HAMSTER. D. C. Kuo, J. D. Polcer*, R. W. Rhoades, S. E. Fish, T. J. Voneida and D. K. Goodman*. Dept. of Anatomy, CMDNJ-New Jersey School of Osteopathic Medicine and Rutgers Medical School, Piscataway, NJ 08854; Neurobiology Prog., Northeastern Ohio Universities College of Medicine, Rootstown, OH 44272.

Large injections of radioactive amino acids into the posterior neocortex revealed that in hamster, as in rat (Webster, J. Anat., 165, 1961), this region projected to the caudal striatum. Smaller, electrophoretic deposits of [^{3}H]-leucine into areas 17, 18a and 18b demonstrated further that all of these cortical areas sent axons to the posterior striatum. These small deposits also showed that the visual corticostriatal projection was topographically organized, at least along the superior-inferior axis of the cortical visual field representation. Caudal visual cortical deposits yielded labelling in the most posterior part of the striatum while anterior deposits resulted in more rostral striatal labelling. The autoradiographic experiments, revealed, however, that the visual corticostriatal projection was not strictly point-to-point. Even the smallest [^{3}H]-leucine deposits resulted in several discrete patches of striatal labelling.

In a complementary series of experiments, small deposits of horseradish peroxidase (HRP) were made into the caudal striatum. These injections produced HRP labelled neurons in areas 17, 18a and 18b. The labelled cells were, in most cases, pyramidal neurons located in the upper portion of lamina V. The HRP data revealed a topographic organization for the corticostriatal projection similar to that observed in the autoradiographic material.

In cat (Jayaraman, Brain Res., 195, 1980) it has been shown that neurons in the caudate, putamen and globus pallidus send axons to the auditory cortex. To determine whether or not the hamster's visual cortex was innervated by the striatum we made injections of HRP into the visual cortex, and [^{3}H]-leucine deposits into the striatum. Neither series of experiments provided any evidence for a striato-visual cortical projection in the hamster.

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248.18 COMPARTMENTALIZATION OF CORTICAL INPUTS TO THE THALAMIC LATERAL GROUP OF THE HAMSTER. Roberto Lent* (SPON: Walter Rosenblith). Department of Psychology, M.I.T., Cambridge, MA 02139.

Hodological and histochemical work in cats and monkeys has led to the notion that the thalamic lateral group is a complex structural entity whose inputs are compartmentalized in distinct regions. I was interested in testing the generality of this idea, beginning with an investigation of the pattern of projections from posterior cortical regions to the lateral group in hamsters.

I have made focal cortical lesions in the left hemisphere and restricted injections of tritiated proline in the right hemisphere of 10 adult hamsters. After adequate survival times, the animals were sacrificed and perfused with fixative. Four series of adjacent sections were collected after the brain was frozen and cut coronally, to be processed respectively by the autoradiographic technique, the Fink-Heimer procedure I, the Gallyas myelin stain and the Nissl (cresyl-violet) technique.

Results have revealed that both the nucleus lateralis anterior (LA) - often referred to as lateralis dorsalis or simply lateralis - and nucleus lateralis posterior (LP) receive afferents from ipsilateral posterior cortical regions. In both nuclei, inputs from the striate cortex (V_1), area 18b (V_M) and the parietal cortex (areas 7 and 2) are partially segregated in different subregions. Thus, V_1 projects to a laterodorsal part of LA, while V_M projects to a small medial band and areas 7 and 2 to a ventromedial region continuous with dorsal aspects of the somatosensory thalamus. In LP, V_1 projects topographically to the rostral half of the nucleus occupying its whole depth, while V_M projects to a restricted lateral band situated within the caudal tecto-recipient zone of LP. The parietal cortex input occupies only the ventral half of rostral LP, largely overlapping the V_1 terminal fields.

These findings suggest that compartmentalization of inputs in the thalamic lateral group may be a general mammalian feature. They also point to the possibility of using this feature to establish homologies of extrageniculate pathways in mammals. It is known that LP is part of an ascending tectothalamic pathway that reaches the visual cortex, while LA participates in a pretectothalamocortical pathway that instead reaches the limbic cortex. The existence of compartmentalized cortical feedback to these thalamic nuclei may mean that these ascending systems convey specialized, multi-channeled sensory information to their cortical target areas.

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248.19 CORTICAL CONNECTIONS FROM THE STRIATE CORTEX IN THE GRAY SQUIRREL: DEFINITION OF EXTRASTRIATE CORTICAL VISUAL AREAS. V.M. Montero and K.D. Cliffer. Dept. Neurophysiology, Univ. Wisconsin, Madison, WI 53706, and J.F. Bell Museum Nat. History, Univ. Minnesota, Minneapolis, MN 55455.

The distribution of cortical connections from area 17 to the ipsilateral cortex in the gray squirrel was studied to define the location of extrastriate cortical visual areas. From single injection sites of 3H-proline in area 17, autoradiograms show labeled projections in several extrastriate cortical areas. These areas occupy the following positions with respect to the striate cortex, and are named accordingly: posterior (P), posterolateral (PL), mediolateral (ML), laterolateral (LL), anterolateral (AL) and anteromedial (AM). In addition, there are projections to retrosplenial (RS), dorsal pararhinal (PR) and anterotemporal (AT) cortices. Areas P, PL, ML and AL are located from caudal to rostral in architectonic area 18, area LL is in architectonic area 19, and area AM is in anteromedial peristriate cortex. Area AT is in a region rostral to 19 and dorsal to architectonic area Ta. Projections from different retinotopic regions of area 17 are established into each of the extrastriate areas with varying degrees of convergence, and projections from a single striate cortex site diverge into multiple patches within most extrastriate areas.

The similarities in the distribution of striate cortical projections in the gray squirrel (family Sciuridae) with those found in rodents of families Muridae and Octodontidae (rat, Montero et al. Brain Res. 53:202,'73; Octodon degus, Kuljis et al. Neurocirugia, in press), and in the rabbit (Montero and Murphy, Anat. Rec. 183:483,'76), suggest a general pattern of visual cortex organization in Rodentia and Lagomorpha. We call this a "rodent type" of visual cortex organization. This pattern is different in several respects from the distribution of striate cortical connections seen in the cat (Montero, Brain Behav. Evol. 18,'81) and in rhesus and owl monkeys (V.M.M. unpublished). For example, besides other differences, in the cat and monkey there are no direct striate cortical projections to the retrosplenial cortex. In the gray squirrel, however, there is a higher degree of divergence in these connections than in the rat and rabbit. This property is similar to the divergence found in striate-extrastriate connections in the cat and monkey (Montero, J. Comp. Neurol. 189:45,'80).

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249.1 PROPERTIES OF JUNCTIONAL AChR THAT NEWLY APPEAR AFTER DENERVATION IN VITRO. A. Olek and N. Robbins. Anat. Dept.,Case Western Reserve Med. Sch., Cleveland, O. 44106

In rat diaphragm muscles the number of junctional acetylcholine receptors (AChR), as assayed by ^{125}I-alphabungarotoxin (*BTX) binding, increases by as much as 80% in diaphragm strips after 42 hrs. in organ culture. The presence of a 2 cm. long nerve stump prevents this increase in AChR (Olek and Robbins, 1981, Neuroscience, In press).

By exposing existing surface AChR to unlabelled alphabungarotoxin (BTX) prior to the expected increase in junctional AChR, we were able to characterize certain properties of the population of AChR that appeared after denervation: 1) The new population of junctional AChR was functional. Bath application of acetylcholine (.5mg./ml) produced a membrane depolarization at the endplate in diaphragm strips without a nerve stump (-N), while no acetylcholine response could be elicited in strips with a 2 cm. nerve stump (+N), even at 2-5 times the dosage. The acetylcholine response in -N strips was maximal at the endplate and declined to 0 at distances greater than 75 micra. The maximal endplate response in -N strips to a given dose of acetylcholine was approximately 50% of that produced in a freshly dissected diaphragm. In -N strips demonstrating a junctional acetylcholine response, the binding of *BTX was found to be 40-80% that of innervated muscle. Both the response to acetylcholine and *BTX binding in the new population of junctional AChR could be blocked by unlabelled BTX. 2) The appearance of new junctional AChR was rapid. After cold block of pre-existing AChR, a detectable junctional response to acetylcholine developed within a 2 hour time period, as did the appearance of junctional *BTX binding sites. 3) The turnover of the new population of junctional AChR was slow relative to that of extrajunctional AChR. The new population of AChR was labelled with *BTX and the number of junctional AChR was then monitored at 0, 8, and 15.5 hours in organ culture. Little *BTX binding (< 15%) was lost from the endplate between 0 and 15.5 hours, indicating that the new junctional AChR turned over more slowly than typical extrajunctional AChR.

Since there is close correspondence between the appearance of new *BTX binding sites and physiological sensitivity, it is likely that a mechanism exists for rapid insertion of functional AChR at the motor endplate. Since this insertion is nerve stump dependent, a novel mechanism for the rapid neural regulation of junctional AChR may be indicated.

This work supported by a grant from the Muscular Dystrophy Assoc. and the Nat. Inst. of Aging AG00795.

249.2 ALTERATIONS IN ENZYMATIC ACTIVITIES AS A RESULT OF CROSSING OF ELECTRICALLY SILENT NERVES TO FAST AND SLOW MUSCLES. L. Eldridge*, K. Baldwin*, M. Liebhold* and W. Mommaerts (SPON: E. Decima). Depts. Physiol., Univ. of California, Los Angeles, California 90024 and Univ. of California, Irvine 92717.

To assess the ability of nerves to specify fast and slow properties of skeletal muscle by trophic signals, some biochemical and histochemical effects of nerve-crossing were examined in a cat preparation in which the electrical activity of the motoneurones had been permanently silenced by a surgical spinal cord isolation (SI). In the SI cats, all dorsal roots between two cord transections enclosing the 5th lumbar through the 3rd sacral cord segments were severed. Control cats underwent either bilateral dorsal rhizectomy including L5 through S3, cord transections at the borders of the region L5 through S3, or no spinal surgery. The nerves to fast (F) flexor hallucis longus and slow (S) soleus muscles were crossed (X) in one leg of each cat. In the contralateral leg (U), the nerves to these muscles either remained intact or were severed but reconnected in their original positions. All surgical operations on a given cat occurred within the same day. Eight months after surgery, the F and S muscles were removed for testing. They were assayed biochemically for the activities of lactate dehydrogenase, α-glycerophosphate dehydrogenase citrate synthase, and Ca^{++} activated myosin ATPase and for the susceptibility of myosin ATPase to inactivation by alkaline preincubation. The muscles were examined histochemically with regard to the proportion of fibers staining heavily for ATPase activity after alkaline preincubation. A complex pattern of results emerged from analysis of the 6 assays on 2 muscle types after 2 arrangements of nerve connections and 4 spinal treatments. Comparison of the U muscles of normal cats to those of the cats with spinal surgeries showed significant changes in enzyme activities as a result of the surgeries themselves, especially the SI. Nerve crossing was effective in all treatment groups. In 47 of the 48 comparisons between X and U means, the X mean was closer to that of normal muscles of the opposite type (F vs. S) than was the U mean. The fact that all 12 of the differences between the X and U means in the SI cats were in the same direction as the X-U differences in the normal cats indicates that the nerve can influence enzyme properties by some means other than its own electrical activity or the electrical and contractile activity it evokes in its muscle. However, that the effects of both the spinal surgery and the nerve crossing varied greatly with enzyme muscle, and spinal treatment suggests that activity is also heavily influencing enzyme activity, and that the 4 enzymes studied differ as to the types and relative amounts of control exerted by activity and trophic factors. Supp. by MDAA and NIH.

249.3

WITHDRAWN

249.4 SENESCENT NEURONS PROMOTE NON-NEURONAL PROLIFERATION IN DISSOCIATED CENTRAL NERVOUS SYSTEM CULTURES. R.J. Riopelle. Queen's University, Kingston, K7L 2V8, Canada.

One of the delayed responses of the central nervous system to a variety of insults is glial proliferation or gliosis. In certain conditions, there would appear to be a reasonable correlation between the degree of neuronal loss and the degree of gliosis. This observation might suggest that neuron death is one trigger of glial proliferation.

Mechanically dissociated eight-day embryo chick spinal cords were incubated for varying periods of time in embryo gut extract-supplemented, but otherwise chemically defined medium, on a polylysine substrate (Riopelle, R.J. and Cameron, D.A., J. Neurobiol. 12, 175-186, 1981). Polylysine inhibits attachment, and the chemically defined culture medium inhibits proliferation, of non-neuronal cells (Bottenstein, J.E. et al, Exp. Cell Res. 125, 183-190, 1980). The neurite bearing cells were allowed to incubate without changing the culture medium for periods of 12-15 days.

In all neuron cultures, some non-neuronal cells are seen, but their location on the culture dish is confined to areas beneath neuronal processes. As the cultures age, neuron morphology changes with the processes becoming smaller in diameter and beaded or varicose. As process varicosity becomes prominent, there is an increase in the number of non-neuronal cells in juxtaposition to the processes. Process beading is a prelude to the disappearance of neurons, and as the latter occurs, large numbers of non-neuronal cells appear and eventually comprise the only surviving cell types.

Non-neuronal cells cultured in the absence of neurons and in the presence of gut extract-supplemented but chemically defined medium show very low plating efficiency, and no proliferation, and have disappeared by 7-10 days.

These data suggest that non-neurons can survive in limiting conditions in the presence of intact neurons but undergo a proliferative burst as the neurons become senescent and disappear.

This work has been supported by MRC, Canada.

249.5 IN VITRO SYNTHESIS OF NERVE GROWTH FACTOR SUBUNITS BY MOUSE SARCOMA LINE S180. J. R. Perez-Polo, E. Barklis* and K.Werrbach-Perez*. Department of Human Biological Chemistry and Genetics, Marine Biomedical Institute, University of Texas Medical Branch, Galveston, Texas 77550.

The Nerve Growth Factor (NGF) is necessary for the differentiation and maintenance of vertebrate sympathetic and embryonic sensory neurons. When isolated from mouse submaxillary gland, human placenta at term or guinea pig prostrate, NGF is a subunit containing protein. In mouse submaxillary gland and human placenta at term, NGF is made up of three dissimilar subunits called α, β and γ. The β subunit expresses arginyl-esteropeptidase activity and has been suggested to process larger pro-β forms into the active β species.

In order to better define the processing properties of NGF and study regulatory features of the synthesis and processing of NGF, we studied the synthesis of NGF by the mouse S180 sarcoma line in vitro. NGF subunits synthesized de novo in the presence of ^{35}-methionine were isolated from cell pellets by monospecific antibodies bound to sepharose. We were able to show that the S180 line synthesized α, β and γ molecules indistinguishable from α, β and γ NGF isolated from mouse submaxillary gland in terms of antigenic properties, physicochemical properties as determined by dynamic isoelectric focusing, as well as biological activity. This is the first demonstration that the subunits of NGF are synthesized and secreted by a single cell as a 7S complex similar to that isolated in the mouse submaxillary gland. Taken together, these results are consistent with the hypothesis that assembly of NGF as an $\alpha_2\beta\gamma_2$ oligomer is an intracellular event and that the oligomer is secreted as an inactive storage form of NGF activity.

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249.6 BIOCHEMICAL AND MORPHOLOGICAL CHARACTERIZATION OF SYMPATHETIC NEURONS GROWN ON CHEMICALLY DEFINED MEDIUM. L. Iacovitti, T.H. Joh, M.I. Johnson, and R.P. Bunge Dept. of Anat. & Neurobiol., Washington University School of Medicine, St. Louis, Missouri 63110 Lab. Neurobiol. Cornell Univ. Med. Coll. New York, N.Y.

Individual sympathetic neurons derived from perinatal rat superior cervical ganglion (SCG) express a mixed adrenergic-cholinergic phenotype when grown under certain culture conditions (Higgins, et al. J. Neurosci. 1:126-131, 1981). Transmitter expression in these cells is critically influenced by a number of undefined components present in the culture medium. In the present study we sought to determine whether SCG neurons grown on a chemically defined serum-free medium similarly express properties of two transmitter systems.

To address this issue, principal neurons were dissociated from perinatal rat SCG and established in culture for up to 7 weeks in the absence of all other cell types. Cultures were maintained in 7% CO_2 at 35°C on chemically defined serum-free medium (Bottenstein and Sato, PNAS 76: 514-517, 1979) supplemented with 2.5S NGF.

Under these culture conditions, SCG neurons continued to express a number of characteristic adrenergic properties. Tyrosine hydroxylase (TH) activity increased linearly with time in vitro reaching levels similar to those found in serum and embryo extract-supplemented cultures. Dopamine-β-hydroxylase activity and endogenous norepinephrine levels also rose in culture but reached only 30% and 2% of those values measured in supplemented medium. Ultrastructural examination of neurons in serum-free cultures revealed a predominance of dense-cored vesicles in synaptic profiles following $KMnO_4$ fixation. In addition, immunocytochemical localization of TH with specific antibodies demonstrated that essentially all neurons contained this adrenergic enzyme for up to 7 weeks in vitro.

SCG neurons grown on a defined medium, unlike those grown on supplemented medium, did not acquire cholinergic properties in culture. These neurons did not contain detectable levels of the specific acetylcholine-synthesizing enzyme, choline acetyltransferase, and synaptic profiles contained few vesicles with a clear morphology. In addition, evidence has been presented elsewhere that these neurons do not form cholinergic synaptic interactions (although frequent electrotonic junctions are observed) (Higgins, et al. this volume).

We conclude that SCG neurons, grown under serum-free culture conditions, develop properties characteristic of adrenergic neurons and do not express a mixed adrenergic-cholinergic phenotype. (Supported by NIH Research Grant NS14416 and Training Grant NS07027).

249.7 RESPONSES OF PRIMARY RAT MUSCLE CULTURES TO SOLUBLE EXTRACTS FROM CENTRAL NERVOUS TISSUE SEEN BY LIGHT AND ELECTRON MICROSCOPE AUTORADIOGRAPHY. M. M. Salpeter, S. Spanton* and T. Podleski*. Section of Neurobiology and Behavior, Cornell Univ., Ithaca, NY 14853.

Soluble factors extracted from brain and spinal cord cause an increase in the site density and number of high density clusters of acetylcholine receptors (AChR) on both cloned L_5 muscle cells (Podleski et al., PNAS 75:2035, 1978) and cultured chick primary muscle cells (Jessel et al., PNAS 76:5397, 1979). Conditioned medium from neuroblastoma-glioma hybrids also causes an increase in the number of AChR clusters on rat primary cells but little increase in site density (Christian et al., PNAS 75: 4011, 1978). We examined the responses of primary rat cells to fetal or newborn rat central nervous tissue extract using quantitative light and electron microscope autoradiography. We found that: (1) As with neuroblastoma-glioma conditioned medium, primary rat cells respond mainly by the formation of high density clusters although a 1.5 to 2-fold increase in average site density was seen by light autoradiography which appeared to involve, at least in part, a slight decrease in receptor degradation rate. (2) Both a high (>70,000 daltons) and a low (<10,000 daltons) molecular weight fraction induced the clusters on primary cells, whereas an intermediate weight fraction did not. (3) By EM autoradiography, we determined that in controls the average AChR site density and the number of clusters were higher at the bottom of the cell, whereas in extract treated cultures, both were higher at the top of the cell. (4) Extracts caused no cellular specialization except possibly an increase in basal lamina profiles and frequent bulges (or membrane out-pocketing) in the region of the induced AChR clusters. (5) By labeling the receptors prior to the addition of extract, we could demonstrate a redistribution of preexisting receptors within 3 hrs. (6) In receptor clusters on control muscle, the highest receptor density (averaged over the unit tabulation area of $\sim 40\mu m^2$) did not exceed $\sim$5,000 sites/μm^2, whereas within the clusters induced after extract treatment, AChR site density reached values of 15,000-21,000 sites/μm^2.

Thus, in primary rat muscle cultures, the soluble nerve extract mimics innervation in its ability to cause a rapid redistribution of AChR's, reaching site densities equal to that at innervated nmj's.

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249.8 THE EFECTS OF UNILATERAL IXth NERVE DENERVATION ON THE DEVELOPMENT OF RAT CIRCUMVALLATE TASTE BUDS. M. A. Hosley and B. Oakley, Neuroscience Lab. Bldg., Div. of Biol. Sci., Univ. of Michigan, Ann Arbor, MI 48109.

The effects of early glossopharyngeal (C.N. IX) nerve denervation on the formation of taste buds was investigated by performing unilateral denervations in young rat pups (Rattus norvegicus) at age 3 d post-partum (p.p.); attempts at bilateral denervations were unsuccessful due to a 100% mortality rate. There were no discernible differences in the mortality rates of normal, sham operated or unilaterally denervated animals. Experimental animals were sacrificed at 5, 10, 15, 21, 33 and 45 days p.p. and control animals at 0, 3, 5, 10, 21 and 33 days p.p. with circumvallate (CV) taste buds counted to assay for the effects of denervation.

Unilateral denervation in adult animals results in a 10%-12% loss of taste buds (other researchers) while complete (bilateral) denervation results in a 100% loss of taste buds due to the trophic dependency of the taste buds upon an intact nerve supply. At 21 days p.p., experimental animals had a 50% deficiency in the CV taste bud population in comparison with controls ($p \leq 0.01$). This deficiency in the taste bud population indicates the developmental need for the entire nerve supply and the limited trophic capacity of an individual IXth nerve to act in the forming and maintaining CV taste buds. Since the taste buds which do form are distributed throughout the CV papilla, the deficiency does not result from an inability of the remaining IXth nerve to reach the areas of the taste bud precursors. Additional studies indicate that equivalent deficiencies in taste bud numbers can be produced by unilateral denervations performed at 1, 2, 4, 5, or 6 days p.p. The deficiency in taste buds remains through at least age 45 d p.p., indicating that the deficiency is permanent in nature and that a "critical period" for the induction of taste bud formation may exist. This period would represent a time span during which the nerve supply must interact with the taste bud precursors to establish the precursors' potential to differentiate into taste buds.

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249.9 DETERIORATION OF GUSTATORY RESPONSES AND NERVE ULTRASTRUCTURE FOLLOWING SPECIFIC ACTION OF COLCHICINE UPON GUSTATORY AXONS. H. E. Sloan, S. E. Hughes* and B. Oakley. Div. Biol. Sci., Neuroscience Lab Bldg, Univ of Mich, Ann Arbor Mi 48109

A silastic nerve cuff containing 1% colchicine was placed around the combined lingual-chorda tympani nerve of the Mongolian gerbil (Meriones unguiculatus). Three days of nerve exposure to colchicine reduced the summated gustatory responses recorded from the chorda tympani nerve in the middle ear by at least 60% and produced detectable ultrastructural changes in the lingual-chorda tympani nerve. There was a loss of microtubules, an increase in neurofilaments, and some loss of the longitudinal orientation of the neurofilaments. The axoplasm was shrunken distal parts of the colchicine treated axons; axon cross-sectional area was significantly less than in control cuffed nerves ($p<.001$). The axoplasm also showed an increase in electron density as well as the presence of floccular material. Nerve cuffs lacking colchicine had little effect on chorda tympani taste responses or nerve ultrastructure. We believe colchicine acted on the nerve trunk, and not the taste buds, to reduce taste responses because ^{3}H-colchicine levels were indistinguishable on the two sides of the tongue whereas functional and structural impairment was always restricted to the ipsilateral side. Normal taste responses were obtained 8 and 15 days after application of cuffs containing 1% lumicolchicine, an isomer of colchicine which lacks tubulin binding properties. This suggests that colchicine acted by a specfic disruptive effect upon axonal tubulin. After 8 and 15 days of colchicine treatment, ipsilateral chorda tympani taste responses and fungiform taste buds were nearly absent and substantial pathological changes were evident in nerve ultrastructure. There was additional shrinkage and densification of the axoplasm, deterioration of mitochondria, disruption of myelin, and a further increase in neurofilaments accompanied by greater disorientation. We conclude that colchicine had a specific action upon tubulin of gustatory axons which led to a loss of taste responses and taste buds. Supported in part by NIH Grant NS-07072.

249.10 ACETYLCHOLINE RECEPTOR AGGREGATION PROTEIN DEFICIENCY: A CLONALLY INHERITED DEFECT IN SYNAPSE FORMATION. N.A. Busis, M.P. Daniels, H.C. Bauer, P. Sonderegger*, A.E. Schaffner, and M. Nirenberg. Lab. of Biochem. Genetics, NHLBI; and Lab. of Devel. Neurobiol., NICHD, NIH, Bethesda, MD 20205.

Neuroblastoma x glioma NG108-15 hybrid cells form synapses with cultured rat skeletal muscle cells and release a protein into the medium that induces nicotinic acetylcholine receptor (AChR) aggregation on myotubes. Four additional synapse-forming neuroblastoma or hybrid cell lines and 12 lines that form few or no synapses with myotubes but synthesize and release acetylcholine (ACh) were cocultured with myotubes and myotube AChR aggregates were visualized with rhodamine-conjugated α-bungarotoxin. Thirteen cell lines, including all lines that form many synapses, induced AChR aggregation. N4G-Ba, N4G-Ca, and 141-3 cells, derived by fusion of N4TG-3A mouse neuroblastoma cells with C6BU-1 rat glioma cells, form few synapses and did not induce AChR aggregation, nor did the parental N4TG-3A or N4TG-1 neuroblastoma cells. Medium conditioned by N4G-Ba, N4G-Ca, 141-3, N4TG3-A, or N4TG-1 cells and homogenates of the cells also lacked AChR aggregation activity. Cells without AChR aggregation activity did not inhibit AChR aggregation induced by other cell lines. Ultrastructural studies revealed that all cell lines examined that induce AChR aggregation contain large dense core vesicles 100-200 nm in diameter and small clear vesicles 60 nm in diameter. In contrast, small clear vesicles but few or no large dense core vesicles were detected in N4G-Ba, N4G-Ca, and 141-3 cells. Large dense core vesicles were also not detected in N4TG3-A and N4TG-1 cells. N4G-Ba cells formed few synapses on myotubes with low miniature end-plate potential (mepp) frequencies as detected by intracellular recording from the myotubes. Coculture of N18TG-2 cells, which release AChR aggregation protein but have low choline acetyltransferase activity and do not form synapses on myotubes, with N4G-Ba cells and myotubes increased the average mepp frequency by 227%, the proportion of myotubes innervated by 153%, and the number of AChR aggregates per myotube by 354%. These results demonstrate cooperative interactions between cells with different kinds of defects in synapse formation and suggest that N18TG-2 cells release the AChR aggregation protein from large dense core vesicles thereby increasing myotube sensitivity to quanta of ACh secreted by N4G-Ba cells. Thus, both molecules that specify neurotransmitter receptor position and neurotransmitters may be released from one or more presynaptic cells, and may be required for the formation and function of certain classes of synapses.

249.11 FATE OF AFFERENTS TO THE DENTATE GYRUS FOLLOWING DESTRUCTION OF DENTATE GRANULE CELLS WITH COLCHICINE. Richard B. Goldschmidt * and Oswald Steward. (SPON: Lennart Heimer) Dept. of Neurosurgery and Anatomy, University of Virginia School of Medicine, Charlottesville, VA 22908.

Injections of colchicine into the dentate gyrus (DG of adult rats preferentially destroy dentate granule cells (Goldschmidt & Steward, PNAS 77: 3047, 1980). In the present study, we evaluate the fate of some of the normal afferents to the granule cells to determine whether they survive the destruction of their normal synaptic targets, and whether they form "ectopic" connections similar to those described after destruction of granule cells in developing animals (Laurberg & Hjorth-Simonsen, Nature 269:158, 1977; Gerbrandt et al. Exp. Neurol. 62:122, 1978).

Adult male rats were given two injections into the DG of one side consisting of 3ug of colchicine in 0.6ul of deionized water. Entorhinal and commissural afferents to the DG were traced by the aid of anterogradely transported HRP following injections into the ipsilateral entorhinal cortex (EC) and contralateral hippocampus 60-70 days following the colchicine injections. Animals survived two days after the HRP injection and the brains were processed according to the TMB and Hanker-Yates methods.

Despite the absence of granule cells and shrinkage of the DG molecular layer (terminal zone of EC and commissural afferents), the injections in both sites resulted in labeled terminal fields in what remained of the dentate molecular layer. The density of labeling and width of the terminal zones appeared considerably reduced, particularly in the free blade of the dentate gyrus, where the molecular layer was markedly shruken. Commissural and EC projections to the hippocampus proper, as well as fibers passing through the region of the colchicine injection, appeared intact. Ectopic terminal fields similar to those observed after granule cell destruction in developing animals were not observed, and there was no noticeable increase in the density of the normally sparse pathway from EC to the contralateral DG.

The preferential distribution of labeling in the molecular layer of the hidden blade, near where hilar interneurons are located, suggests that there may be a preferential survival of terminals which are able to maintain synaptic contact with some neuron (hilar interneuron).

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249.12 TURNOVER OF ACETYLCHOLINESTERASE IN INNERVATED AND DENERVATED RAT DIAPHRAGM S. G. Younkin, Dept. of Pharmacology, Case Western Reserve University, Cleveland, Ohio 44106

The aims of this study were 1) to compare the rate of degradation of junctional and non-junctional AChE in innervated muscle, and 2) to determine if denervation alters the degradation of junctional and/or non-junctional AChE. There is very little junctional AChE in rat diaphragm so AChE could not be pulse labelled with radiolabelled amino acids and followed in the conventional way. A label for AChE was needed which ideally would 1) be specific for AChE, 2) be easily measured, 3) form a stable complex with AChE, and 4) have minimal effect on the degradation of AChE. Echothiophate meets these criteria reasonably well, and was administered intravenously through the tail vein to label the AChE in rat diaphragm. Echothiophate diethylphosphorylates the active site of AChE thereby inactivating it. Labelled (diethyl-phosphorylated) AChE was measured at various times after labelling by removing the diaphragm, solubilizing and assaying AChE, and then determining the increase in AChE activity caused by treatment with pralidoxime, a drug that rapidly and completely reactivates diethylphosphorylated AChE. In this procedure labelled AChE is measured by determining the AChE activity revealed by pralidoxime treatment so the label is easily measured and is as specific as the AChE assay. The stability of the labelled AChE was evaluated by labelling muscle homogenate with echothiophate, dialyzing overnight to remove echothiophate, and measuring the rate of reappearance of AChE. Under these conditions label was lost spontaneously with a half-time of 27 hours. If echothiophate altered the degradation of AChE, a change in the level of total AChE would be expected. The measurement of label with this procedure requires the measurement of total AChE, and total AChE did not change. The AChE in innervated and denervated hemidiaphragms was labelled as described above and the forms of labelled junctional and non-junctional AChE were evaluated as described earlier (Takach and Younkin, abstract this session). In innervated muscle junctional AChE (both asymmetric and non-extractable) was degraded very slowly; label was lost at very nearly the rate of spontaneous dissociation measured in homogenates. . During the rapid loss of junctional AChE 1-2 days after denervation the rate of degradation of junctional AChE increased dramatically, but by 3-4 days after denervation it was again like that in innervated muscle. Non-junctional AChE was lost with a half time of about 1 day in innervated muscle, but no change was observed during the loss of non-junctional AChE 1-2 days after denervation.

249.13 INHIBITION BY CEREBRAL NEURONS OF [^{3}H]-THYMIDINE INCORPORATION INTO CHOROID PLEXUS CELLS IN VITRO. G. R. Hanson, L. M. Partlow* and P. L. Iversen. Dept. of Pharmacology, College of Medicine, University of Utah, Salt Lake City, UT 84132.

Neurons prepared from the cerebral hemispheres of 10-day chick embryos by a method similar to that of McCarthy and Partlow [Brain Research 114 (1976) 391-414] have been reported to be mitogenic for embryonic chick glial cells isolated from various parts of the nervous system. In contrast, we have found these neurons inhibit the proliferation of cells isolated from the choroid plexus of 10 day chick embryos. Thus, the presence of postmitotic cerebral neurons resulted in a decrease of up to 77% in thymidine incorporation by choroid plexus cells. This inhibitory effect was found to be directly dependent on the concentration of cerebral neurons. The lack of a similar inhibitory effect as a result of addition of similar numbers of either optic lobe and sympathetic neurons suggests that this interaction is a specific cell-cell phenomenon. The neuronal inhibition of thymidine incorporation into choroid plexus cells did not involve the release of a diffusable factor into the culture medium.

Addition of the sonicate of cerebral neurons to choroid plexus cultures resulted in an inhibition of thymidine incorporation of up to 54%. The inhibitory factor released by sonication was found to be heat stable. Similar inhibitory factors were not found in disrupted sympathetic and optic lobe neurons.

These results demonstrate that embryonic cerebral neurons can exert both stimulatory and inhibitory influences on dividing cells present in the developing cerebral hemispheres. Thus, these neurons might have an important regulatory role in mediating the development of the brain.

249.14 THE DIFFERENTIATION AND INNERVATION OF OTOCYSTS TRANSPLANTED TO OPTIC TECTA OF CHICK EMBRYOS. Mark C. Whitehead. Dept. of Anat. UConn Health Ctr., Farmington, Connecticut 06032.

Developing cochlear and vestibular ganglion cells become morphologically distinguished and form very different synaptic endings in the ear while their axons form distinctive synapses centrally on specific medullary neurons. The present study investigates the extent to which ganglion cells and their peripheral connections can develop when otocysts (embryonic inner ears) are confronted with inappropriate or absent central targets.

Otocysts with their associated ganglia and surrounding mesenchyme were removed from quail or White Leghorn chick embryos at 2½-3 days of incubation. They were transplanted either to the chorio-allantoic membrane or to the superficial or ventricular surfaces of the optic tecta of 2½-5 day host chick embryos. At the times of transplantation, vestibulocochlear ganglion cells have not connected with the brain or the undifferentiated epithelium of the ear. Neither have the superficial tectal layers received the bulk of their retinal input.

In all locations, the otocysts developed organotypically; vestibular cristae were distinguished from cochleae. These areas were histotypic as well, with thick receptor epithelia containing hair cells adjoining thinner areas of lining epithelia. Compared with normal ganglia, only a variable fraction of ganglion cells survive in the transplanted otocysts. However, these cells, in reduced silver stains, have fibers which gather as fascicles and innervate, selectively, only receptor epithelia, sometimes after wandering for long distances below the lining epithelia. Different ganglion cell types differentiate; the largest of these send "colossal" fibers into the cristae where they form calycine endings. In the Feulgen-stained quail-chick chimaeras, tectal and ear tissues did not intermix, but in both superficial and deep implants, neurons of the deep tectal layers became apposed to areas of donor epithelium. Afferent and efferent connections of the ectopic ears were seen with silver stains. Tectal axons from deep layers were similar to the fibers of ganglion cells in that they sent endings only into sensory epithelia, often after streaming over the cartilage of the otocysts and below the basal lamina of their lining epithelia. Optic axons, despite their proximity, never innervated the otocysts. Axons from the ectopic ganglion cells sometimes grew out of the otocysts and, because of their relative thickness, could be followed into deep tectal layers.

These findings demonstrate that ganglion cell types and at least some appropriate peripheral endings can differentiate in the absence of normal central targets. Nonspecific but, nevertheless, selective factors result in afferent and efferent connections of the ectopic otocysts with deep tectal laminae.
(Supported by PHS grant 5 R01 NS 14354).

249.15 FORMS OF ACHE IN RAT DIAPHRAGM AFTER DENERVATION P.L. Takach*, S.G. Younkin and T.L. Rosenberry*, Depts. of Anatomy and Pharmacology, Case Western Reserve Univ., Cleveland, O. 44106. (Sponsor: L.H. Younkin).

Innervation has a profound influence on mammalian skeletal muscle acetylcholinesterase (AChE), but it is not known if this influence is exerted at the level of synthesis, assembly, secretion, or degradation. There are 3 collagen-tailed asymmetric forms (16S, 12.5S, 8.5S) and 3 globular forms (10S, 6.5S, 4S) of AChE that can be extracted from mammalian skeletal muscle. Skeletal muscle also contains non-extractable AChE. This AChE has previously been ignored but is of interest because it has properties that suggest it is predominantly associated with the basal lamina at the NMJ. To clarify the nature of the neuronal control of AChE, we examined the forms of AChE in rat diaphragm after denervation. Diaphragms were removed, divided into endplate-containing (+EP) and endplate-free (-EP) regions, and subjected to a sequential extraction procedure. Globular forms were first extracted into buffered detergent at low ionic strength and asymmetric forms were then extracted into buffered detergent at high ionic strength leaving the non-extractable AChE behind. Individual forms were then analyzed by velocity sedimentation on 5-25% isokinetic sucrose gradients. Non-junctional AChE was measured directly in -EP muscle and junctional AChE was evaluated by subtracting non-junctional AChE from the total AChE in +EP muscle. Of the total AChE in innervated muscle 27% is junctional AChE (12% asymmetric forms, 7% globular forms, and 8% non-extractable AChE) and 73% is non-junctional AChE (12% asymmetric forms, 58% globular forms, and 3% non-extractable AChE). Junctional asymmetric, globular, and non-extractable forms each have a distinct response to denervation which differs both in time course and magnitude from that seen in non-junctional forms. Each component of non-junctional AChE decreases in the first day after denervation whereas the corresponding components of junctional AChE do not. Non-junctional asymmetric, globular and non-extractable AChE fall to 38%, 50%, and 77% of normal respectively by 3 days after denervation whereas the fall in the corresponding junctional forms is to 23%, 9%, and 42% respectively. Analysis of individual asymmetric forms in +EP muscle revealed that the 8.5S form, which is barely discernible in innervated muscle, actually rises transiently between 1 and 3 days after denervation while the 16S and 12.5S forms are decreasing dramatically. Junctional and non-junctional asymmetric and globular forms slowly return toward normal 3-14 days after denervation. These results are consistent with the hypothesis that denervation causes a transient increase in the rate of degradation of junctional AChE.

249.16 THE APPEARANCE OF MUSCLE DERIVED PROTEINS IN NERVE AND THEIR ENHANCEMENT WITH α-BTX IN VIVO. Katrina Gilmer-Waymire* & Stanley H. Appel (SPON: J.C. Waymire). Dept. of Neurology & Prog. in Neurosci., Baylor College of Medicine, Houston, TX 77030.

Skeletal muscle from young rats possess protein(s) which enhances neuron cell survival, neurite extension, and acetylcholine synthesis in cultured spinal cord. The present experiments were designed to complement these in vitro observations, namely to assess whether protein synthesized in muscle can be taken up by nerve endings and can migrate by retrograde axonal flow in vivo. The sciatic nerves of Sprague-Dawley rats were ligated and injected with 100 μCi ^{35}S-meth into the adjacent gastrocnemius. Five days later the muscles and proximal and distal sides of the ligated sciatic nerve were removed. In all experiments a 2-3 fold increase of acid precipitable radioactivity was detected in total homogenates below the ligature compared to above the ligature. Two-thirds of the radioactivity and homogenates below the ligature was in a 32,000 gxhr pellet and one-third was in the supernatant. On SDS polyacrylamide gel electrophoresis, the supernatant radioactivity below the ligature migrated as two distinct peaks of 60,000 and 90,000 daltons. To support the muscle origin of the below ligature radioactive proteins, dialyzed supernatant extracts of muscle injected with ^{35}S-meth were injected into rats whose sciatic nerves had been ligated but had not previously been injected with ^{35}S-meth. These rats injected with ^{35}S-labelled muscle proteins also exhibited an uptake of radioactivity into sciatic nerve. Animals were injected with α-BTX (2 μg) immediately prior to the injection of ^{35}S-meth. α-BTX was chosen because it reacts specifically with the muscle nicotinic AchR and has been demonstrated to induce nerve sprouting. Animals injected with α-BTX demonstrated a 60% increase in ^{35}S-meth incorporation into proteins extracted from muscle and a 2-fold increase in radioactive proteins below the ligature compared to rats not injected with α-BTX. As a confirmation that α-BTX had a functional effect on nerve metabolism, the effect of daily gastrocnemius injection of α-BTX on nerve choline acetyltransferase (CAT) activity was assessed. After 1 week rats injected with α-BTX contained 2 times the level of CAT in their sciatic nerves compared to controls. Thus, proteins derived from muscle do appear to be taken up by nerve and pass in a retrograde fashion. α-BTX enhances the incorporation of radioactivity of such proteins in muscle, increases their appearance in nerve, and enhances nerve CAT activity. These data suggest that the effects of α-BTX on sprouting noted by Holland & Brown and the enhanced CAT activity noted by us may be explained by proteins derived from muscle which are taken up by nerve and migrate by retrograde axonal flow.
(Supported by the Hartford Foundation 470-G09281).

249.17 ROLE OF PERIPHERAL TARGET IN DEVELOPMENT OF SENSORY NEURONS. March D. Ard and D. Kent Morest. Dept. of Anatomy, Univ. of Connecticut Health Center, Farmington, CT 06032.

Neuronal cell bodies have been counted in the auditory and vestibular ganglia following fixation in a formaldehyde mixture, embedding in JB-4 plastic (Polysciences), sectioning at 5μm, and staining with toluidine blue. Counts were corrected for cells split between sections. In the combined auditory and lagenar (cochlear) ganglion of 3 two-week hatchling chicks (P14) there were 9140 ± 960 neurons($\bar{X}$ ± SD), and in the vestibular ganglion, 8610 ± 108. In 3 eight-day embryos (E8) there were 11170 ± 330 cochlear neurons and 12687 ± 2111 vestibular neurons. This cell loss, 18% in the cochlear and 32% in the vestibular ganglion, was significant at $p<0.05$ (Student's t-test). Mean size of neuronal perikarya increases during this period by 89% among the cochlear and 100% among the vestibular cells. The percent loss of cochlear ganglion cells is identical to that in the cochlear nucleus (nucleus magnocellularis) of the chick between E11 and E13 (Rubel et al., JCN '76).

Stato-acoustic ganglia at E3½-4 have been explanted and cultured for two weeks either alone or in combination with the developing receptor epithelium (otocyst) or in combination with otocyst and medulla. Surrounding mesenchyme was preserved in the explants. The medium was Dulbecco's modification of Eagle's medium with approximately 40mM [K^+], 10% newborn calf serum, 10% chick embryo extract, and ferrous sulphate, ascorbic acid, cortisone, and penicillin in 5% CO_2 in air. Observations of such cultures consistently revealed histological differentiation of sensory neurons and receptor epithelium. In three explants of single ganglia only 460-550 neurons survived. In three explants, each containing an otocyst in addition to the ganglion, 1710-2020 neurons survived. Including pieces of medulla containing the cochlear and vestibular nuclei with the otocyst and ganglion did not increase the survival of ganglion cells in four cultures. Thus the preliminary results suggest that the peripheral target tissue plays a role in trophic support of the stato-acoustic ganglion cells. (Supported by USPHS grant no. 5 RO1 NS14354 and Univ. of Conn. Research Foundation.)

249.18 THE DEVELOPING HIPPOCAMPUS IN CELL CULTURE W.Seifert, B.Ranscht*, H.J.Fink*, H.W.Müller*, F.Förster

Friedrich-Miescher-Labor.,Max-Planck-Institute, Tübingen, W-Germany.

The hippocampus consists of only few major classes of neurons. At a certain developmental stage of the fetal rat brain, only pyramidal and basket neurons will survive together with glial cells and develop in dissociated cell cultures. In this system we are studying:1) morphological and biochemical development of neurons, 2) synapse formation and the neurotransmitter Gaba and Glutamate, 3) trophic interactions between glial and neuronal cells.

Identification of cell types in culture was carried out by immunofluorescence with specific antisera directed against neurons, astrocytes or oligodendrocytes. For oligod. we used a monoclonal antibody developed in our laboratory (B.Ranscht,P.Clapshaw,W.Seifert - Hoppe Seyler's Z.Phys.Chemie, 361, 1327, 1980). In addition the activity of 2'3'-CNPase was followed both in vivo and in vitro and the enzyme localized in specific cell types (H.W.Müller,P.Clapshaw,W.Seifert, J.Neurochem., June 1981).

By using serum-free, hormone supplemented media we have obtained about 90 % pure neuronal cultures. In these cultures we studied the uptake of Gaba and Glutamate by specific cells. We also followed the pattern of gangliosides during neuronal development. As shown previously in our laboratory, gangliosides may exhibit trophic effects on neuronal cells and significant changes in their pattern during development (H.J.Fink and W.Seifert, Hoppe-Seyler's Z.Phys.Chemie, 361,1280,1980, W.Seifert- "Gangliosides in Nerve Cell Cultures" in 'Gangliosides in Neurol.Function...',Raven Press,1981).

We also demonstrated a trophic interaction between glial cells and neurons in this culture system: astrocytes seem to produce a soluble neurotrcphic factor, which stimulates outgrowth of processes from these hippocampal neurons (W.Seifert, N.Buckley, B.Ranscht - Hoppe-Seyler's Z.Phys.Chemie 361, 333, 1980; manuscript in preparation).

250.1 TWITCH AND TETANIC RESPONSES OF SKELETAL MUSCLE FROM PARALYZED CHICK EMBRYOS. B.T. Stokes, P.J. Walters* and P.J. Reiser*. Dept. of Physiology, Ohio State University, Columbus, Ohio 43210.

The isometric contractile properties of 18 chick posterior latissimus dorsi (PLD) muscles have been studied at day 19 of embryonic development. These embryos were paralyzed from day 8 of incubation by injections of 3.0 mg/250 µl of d-tubocurarine (Sigma, Inc.) or 100 mg of α-bungarotoxin (Miami Serpentanium) given every two days until day 16. Activity measurements made in a humidified-temperature controlled environment at the time of injection and at day 18 confirmed the absence of embryonic motility. All muscles were studied in vitro in a modified chick ringer solution maintained at a bath temperature of 24.2±0.2°C (mean ± S.E.). The beak length and yolk absorption of all embryos confirmed they were at stages consistent with 19 days of incubation. Blotted mass of these muscles was, however, decreased by over 300%. Alterations in normalized peak tension. (P_{ot}), time to peak tension and ($t_{P_{ot}}$) and time to half relaxation ($t_{\frac{1}{2}R}$) were characterized in twitch responses; normalized peak tension (P_o) and time to half peak tension ($t_{\frac{1}{2}P_o}$) in tetani. Twitch force (P_{ot}) in muscles from curarized embryos remained the same, $t_{P_{ot}}$ increased by over 110%; $t_{\frac{1}{2}R}$ increased by greater than 700%. Tetanic force (P_o) was depressed by 50% while $t_{\frac{1}{2}P_o}$ decreased by 13%. The major effects of such manipulations therefore is to drastically increase the time to half relaxation during a twitch response. Since we have previously shown that $t_{\frac{1}{2}R}$ becomes progressively less from day 15 to 19 in embryonic development, it appears that inactivity markedly affects the development of this parameter. Muscle activity may therefore be a primary determinant of the development of calcium sequestering ability by the sarcoplasmic reticulum. The effects of inactivity on the maximum velocity of shortening in these muscles is currently under investigation.
(Supported in part by the Muscular Dystrophy Association and the National Science Foundation.)

250.2 INFLUENCE OF ELECTRICAL INACTIVITY AND DENERVATION ON THE FORMATION OF ACETYLCHOLINE RECEPTOR CLUSTERS IN EMBRYONIC RAT MUSCLES. L. Ziskind-Conhaim. Dept. of Physiology, University of California Med. Sch. San Francisco, CA 94143.

I have studied the role of electrical activity and of activity-independent neural effects in regulating the distribution of acetylcholine receptors (AChRs) on intercostal muscle fibers of rat embryos. Segments of thoracic body wall including intercostal muscles, ribs and the adjacent segments of spinal cord (S.C.) were explanted from 15-day rat embryos and maintained in organ culture for up to 6 days (Ziskind-Conhaim, L., and Dennis, M.J. Develop. Biol. 84, 1981). At embryonic day (ED) 15 the distribution of AChRs, determined by autoradiography after labeling the AChRs with ^{125}I-α-bungarotoxin, was uniform along the entire length of muscle fibers. However, after culturing ED 15 muscle-S.C. explants for one day, a single AChR cluster was found at the midregion of each fiber at the site of nerve-muscle contact. This junctional AChR cluster was maintained for at least 4 days in organ culture. Thus, AChR clusters can form at synaptic sites in vitro. The time course of their formation is similar to that seen in vivo.

I compared the formation of such AChR clusters to that which occurred: a) in the presence of tetrodotoxin (TTX) which blocked nerve action potentials and b) in muscles cultured without the S.C. After one day in the presence of TTX, 72% of the fibers had a central AChR cluster and 8% had one or more extrajunctional clusters. By the second day in culture clusters were detected on all muscle fibers, and by the fourth day 70% of the fibers had multiple AChR clusters. The pattern of AChR aggregation was different on muscles cultured without the S.C. After one day in culture 67% of the fibers did not have any pronounced clusters and 33% had one or more receptor aggregates (compared to 93% seen with S.C. and 80% with S.C. in the presence of TTX). By the second day 40% of the fibers still did not have any clusters, 15% had a single AChR cluster, and 45% had more than one site of receptor accumulation. Thus, the initial appearance of AChR clusters on aneural fibers was delayed and then when aggregates did develop, they were usually in multiples.

My results indicate that innervation has two distinct regulatory effects on formation of AChR clusters. First, a primary AChR cluster is formed beneath the motor axon terminals. The formation of this cluster requires the presence of nerve terminals, but is independent of neural electrical activity. Second, electrical activity is necessary to prevent the formation of secondary AChR clusters that develop at random sites along inactive muscle fiber surface. These appear after the time the primary cluster normally forms. Supported by NIH grant NS-16033.

250.3 EFFECT OF A DAILY REGIMEN OF TETANIC CONTRACTIONS, INDUCED VIA PERIPHERAL NERVE STIMULATION, ON PROPERTIES OF IMMOBILIZED RAT SOLEUS MUSCLES. P.F. Gardiner, M. Lapointe*, and D. Gravel*. Département d'éducation physique, Université de Montréal. Montréal, Québec, Canada H3C 3J7.

It is generally assumed that at least part of the loss in muscle mass, and alterations in muscle enzyme proteins that occur in the slow-twitch soleus muscle as a result of imposed neuromuscular disuse is attributable to the concomitant decrease in the quality and/or quantity of the mechanical events normally occurring at the muscle. In an attempt to determine the importance of the lack of tension-generating episodes in the soleus muscle changes associated with disuse, female Sprague-Dawley rats were treated to either a unilateral hindlimb immobilization condition (gp. I) or an immobilization condition, supplemented with electrical stimulation-induced tetanic contractions (gp. IS). Hindlimb immobilization was induced using plaster casts (knee=110°, ankle=90°), for 4 wks. In group IS, soleus muscles were stimulated to contract in the cast by means of fine-wire electrodes, chronically implanted around the sciatic nerve at the level of the sciatic notch. Daily stimulation, performed with the animal under Na pentobarbitol anesthesia, consisted of trains of impulses (DC square waves, .2 ms duration, 50 Hz) lasting 0.5 s, at a frequency of 0.4 Hz, for 20 minutes. The degree of soleus atrophy, compared to control (C) muscles, was not different in comparing group I (65.2±3.3%) and group IS (61.9±2.3%). Histochemical analysis of soleus muscles revealed that group I muscles contained a significantly smaller proportion (58.9±2.0%) of slow-twitch fibers, compared to C (75.4±2.1%), while the corresponding proportion from group IS (65.9±2.7%) was not different from C. Isometric contractile properties of the soleus measured in situ at 37±.5°C revealed that immobilized muscles had significantly faster time-to-peak tensions (I=30.7±1.1; IS=30.8±0.7; C=34.0± 0.2 ms), and were less fused at 15 and 25 Hz. Immobilization had no significant effects on specific tetanic tension (N/g muscle), on twitch or tetanic dP/dt estimates, half-relaxation time, or on the form of the force-frequency curve. On a 20-minute stimulation regimen similar to the daily protocol used, no differences existed among I, IS and C muscles in relative force measured at 4 and 20 minutes. The results suggest that supplementation of high-tension contractions to an immobilized soleus muscle does not significantly attenuate the progress of muscle atrophy, but may play a role in reducing the fiber-type changes that occur. This latter phenomenon may be an effect of muscle mechanical activity, or of alteration of motoneurone metabolism caused by the electrical stimulation. (Supported by Muscular Dystrophy Association of Canada, and NSERC).

250.4 EMG RESPONSE OF FAST AND SLOW EXTENSORS IMMOBILIZED AT DIFFERENT LENGTHS. M. Fournier*, R. R. Roy*, H. Perham* and V. R. Edgerton (SPON: M. A. B. Brazier). Dept. of Kinesiology, Univ. of California, Los Angeles, CA 90024.

Limb immobilization is often used as a model of disuse. However, the adaptation of skeletal muscle to immobilization is largely dependent upon the position at which the joints are fixed. Therefore, the purpose of this study was to determine the effects of hind-limb immobilization in different positions on the electrical output of slow and fast muscles.

Nine female rats had the soleus (SOL) and the medial gastrocnemius (MG) muscles chronically implanted with bipolar recording electrodes, and were randomly assigned to one experimental group of bilateral immobilization: ankle extensors in a shortened (S), lengthened (L), or neutral (N) position. An external brace was used to fix the knee and ankle joints at the desired angle. Electromyographic (EMG) activity of SOL and MG was recorded simultaneously during a 15 minute period each hour for 24 hours, before and 7, 17, and 28 days after immobilization. Raw EMG data was rectified and integrated (IEMG, time constant=200 msec.), and summated for each 24 hours. Pre-immobilization data for each group was used as a control value for comparison with post-immobilization data (Table 1: expressed as % change from control value). Group S showed the largest decrease in IEMG for both muscles and no recovery over time. Group L had the smallest decrease in IEMG and showed different recovery patterns for the SOL and MG. The MG of groups N and L showed a tendency to return to a normal level of activity after 28 days. These results suggest that the EMG response to limb fixation is dependent upon the type of muscle (fast or slow), the length at which the muscle is immobilized and the duration of immobilization. These factors may be important to consider when using immobilization as a model of disuse.

Table 1. % of IEMG Activity Change from Control Value

	DAYS				DAYS		
SOL	7	17	28	MG	7	17	28
N	-44	-34	-53	N	-35	-35	- 5
L	-27	-32	-42	L	-11	+ 6	+11
S	-66	-77	-74	S	-64	-64	-57

250.5 FIBER TYPE GROUPING IN THE SOLEUS MUSCLE OF CATS FOLLOWING SPINALIZATION OR CROSS-REINNERVATION. H. W. Goforth Jr.* and V. R. Edgerton*. (SPON: S. H. Chandler). Dept. of Kinesiology, UCLA, Los Angeles, CA 90024.

The spatial arrangement of fiber types in a mammalian skeletal muscle has been shown not to be randomly distributed. Normal mixed muscles have been shown to exhibit uniformly distributed fiber type patterns (James, J., Neurol Sci., 14:381, 1971) while pathological muscles often demonstrate varying degrees of fiber type grouping. These findings suggest the existence of regulatory mechanisms which produce a non-random (uniform) fiber pattern in normal mixed muscles. The present study was designed to determine the spatial distribution of fast twitch fibers found in the normally homogeneous slow twitch soleus muscle of cats subjected to chronic spinalization (low thoracic; 4 to 18 months, n=3) or cross-reinnervation (via flexor hallucis longus nerve; 18 months, n=4). Spinalization and cross-reinnervation resulted in the conversion of 32 ± 4 and 14 ± 9%, respectively, of the fibers to fast twitch as determined by histochemical staining for myosin ATPase (preincubation at pH 10.4 and 4.2). Measurements of distances between nearest neighbor fast twitch fibers were made from photomicrographs containing an average of 839 ± 178 fibers. The ratio of the observed distance to the expected distance, the dispersion index R, was used as an index of deviation from a random distribution where; R= 1.0 random, R<< 1 grouping and R>> 1 uniform (Clark and Evans, Ecology, 35:445, 1954).

Both spinalization and cross-reinnervation produced significant ($P < .001$) fast twitch fiber type grouping (R= 0.150 and R= 0.342, respectively). Spinalized cats demonstrated significantly greater ($P < .001$) fiber grouping compared to cross-reinnervated cats.

This study demonstrates that alterations in the spatial distribution of fiber types produced by spinalization or cross-reinnervation can be quantitatively described and statistically compared. Regulatory mechanism(s) exist in normal mixed muscles of rabbit and guinea pig which maintain a uniform fiber type distribution pattern (James, J., Neurol. Sci., 14:381,1971). The results of this study indicate that when an originally homogeneous slow twitch muscle is converted into a mixed muscle by spinalization or cross-reinnervation the mechanism(s) regulating uniform distribution do not appear to operate (even after 18 months).

250.6 MATURATIONAL CHANGES IN MOTOR NERVE ENDINGS IN NORMAL AND EXERCISED RATS. K.E. Stephens* and C.D. Tweedle. Dept. of Anatomy, Michigan State University, E. Lansing, MI. 48824.

Morphological change in neuromuscular axonal terminals was examined using a combined silver-cholinesterase stain in 100 male, Sprague-Dawley rats. Predominantly slow-twitch (adductor longus and soleus) and fast-twitch (gastrocnemius and rectus femoris) muscles were taken from sedentary animals at 6-28 weeks of age as well as from rats subjected to 4-16 weeks of intense endurance exercise. The exercise program initiated at 12 weeks of age (post-pubertal) was progressive in nature with the final group of rats running a total of 2 hours per day, 5 days per week. Motor nerve endings were categorized into defined morphological classes independently by the two investigators and the data were subsequently statistically analyzed. The number of accessory endings (an axon with one or more thin branches ending in one endplate on a single muscle fiber) increased significantly in slow-twitch muscles from 6 to 16 weeks, at which time it plateaued in the soleus and slightly decreased in the adductor longus. In fast-twitch muscles the increase ceased at 12 weeks of age, with a subsequent leveling off after that. Double endings (one axon giving rise to two endplates on one muscle fiber) also increased in the predominantly slow muscle up to between 20 and 24 weeks. Conversely, fast-twitch muscle displayed no such increase and, indeed, displayed less than 1% double endings after puberty. The number of branched endings (2 muscle fibers innervated by a single axon) appeared to decrease in the adductor longus muscle between 6 and 20 weeks of age, and thereafter increased slightly. The soleus muscle showed a similar significant increase between 16 and 20 weeks of age, as well as between 24 and 28 weeks of age. Branched endings in fast-twitch muscle decreased to below 1% of endings present between 6 and 16 weeks of age and remained at that level. Simple endings (one axon ending in a single endplate on a muscle fiber) decreased significantly between 6 and 20 weeks in slow-twitch while in fast-twitch muscles the decrease was seen between 6 and 12 weeks of age. Examination of exercise data indicated few changes could be brought about by functionally overloading the muscles. This study indicates that 1) significant differences exist in the neuromuscular axonal terminals of post-pubertal fast-twitch and slow-twitch muscle: 2) there develops with post-pubertal maturation an increase in the complexity of these terminal patterns in slow-twitch muscle not present in fast-twitch muscle: 3)exhaustive endurance exercise appears to have little effect on the modification of motor nerve endings. This indicates that normal maturational nerve ending changes are not due to alterations in neuromuscular activity. (Supported by NSF).

250.7 THE EFFECT OF UNILATERAL SCIATIC NERVE CRUSH ON ASCENDING PATHWAYS IN THE SPINAL CORD. L. J. Fisher* and M. W. Luttges. Department of Aerospace Engineering Sciences, University of Colorado, Boulder, CO 80309.

Gerren and Luttges (1979) reported that following sciatic nerve crush, time-dependent electrophysiological alterations occurred in both the spinal cord and the contralateral, undamaged sciatic nerves. Cross-spinal responses from damaged to undamaged nerves were similarly affected. The present experiments focus upon the possibility that supraspinal projections also may be altered by unilateral sciatic nerve damage.

Threshold levels of stimulation, 1-3 volts, elicited compound nerve responses with several components. The shortest latency component exhibited an estimated conduction velocity of 20 m/sec. Increasing stimulation voltage to 15 volt suprathreshold levels produced reliable decreases in the response latencies. Compared to control nerves, damaged nerves exhibited larger decreases in response latencies at 6 days postcrush. In contrast, a smaller latency shift was observed for damaged nerves at 9 days postcrush. The latency shift across voltage of both damaged and undamaged nerve responses did not differ from control when spinal influences were removed by nerve transection.

Nerve stimulation elicited thalamic responses which were recorded from indwelling electrodes bilaterally placed in the ventral posterior lateral region of the thalamus. Suprathreshold levels of stimulation as compared to threshold levels produced reliable decreases in thalamic response latencies. Thalamic responses ipsilateral to stimulation originating from either damaged or undamaged nerves showed larger latency shifts than the same pathways in control animals at 6 days postcrush. These latency decreases remained at 9 days postcrush. Similarly, the contralateral response from the undamaged nerve exhibited exaggerated response latency decreases which persisted from 6 to 9 days postcrush. The contralateral responses from the damaged nerve did not differ from control over the investigation time periods.

These results indicate that electrophysiological changes occurring at segmental levels are influencing thalamic responses following unilateral damage. This includes spinal alterations both ipsilateral and contralateral to nerve crush. All of the observed alterations, to date, are closely correlated to the time elapsing following unilateral nerve damage. Clearly, the spinal cord exhibits more comprehensive alterations following nerve damage than is generally acknowledged.

250.8 PLASTICITY OF MOTONEURON RECRUITMENT ORDER FOLLOWING SPINAL CORD TRANSECTION. R.G. Durkovic. Dept. Physiology, Upstate Medical Center, Syracuse, N.Y. 13210.

Caudal to spinal cord transection increases in recurrent inhibition (Goldfarb & Sharpless, Neuropharm. 10:413, 1971) and in monosynaptic EPSP size (Nelson & Mendell, J. Neurophys. 42:642, 1979) have been reported. These changes subsided within several weeks or months following transection. In the present study similar transient changes were observed in motoneuron recruitment order in the flexion reflex following spinal transection. The experimental preparation was the unanesthetized decerebrate cat with a T-10 spinal transection made either three months, two weeks, or immediately before decerebration.

Single electrical stimuli to cutaneous superficial peroneal or saphenous nerves were used to evoke the flexion reflex. Tibialis anterior (TA) motoneuron recruitment order was measured as a function of stimulus intensity two ways:

(i) Pairs of single motor unit EMG's were recorded by means of a fine bipolar needle electrode inserted into the belly of the TA muscle, and

(ii) Pairs of single TA nerve action potentials were recorded with bipolar hook electrodes from filaments of the cut and teased TA muscle nerve.

Over 400 determinations of recruitment order were made from pairs of motor unit EMG's and over 300 determinations from pairs of axon spikes, and the two methods gave the same results:

In acute spinal cats the smaller of the two action potentials of a pair was activated at a lower stimulus intensity in 80% of the cases.

In the two week chronics there was a reversal of recruitment order with smaller spikes having lower recruitment thresholds in only 43% of unit pairs.

In three month chronics normal recruitment order returned with smaller units activated at lower stimulus intensities in 82% of unit pairs.

Both EMG spike amplitude (Goldberg & Derfler, J. Neurophys. 40:879, 1977) and axonal action potential amplitude (Clamann & Henneman, J. Neurophysiol. 39:844, 1976) are thought to be related to motoneuron cell size. Thus, the present results suggest that a basic neurophysiological property (orderly recruitment of motoneurons from small to large) exhibits a transient alteration following spinal transection.

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250.9 Dendritic Retraction Induced by Axotomy of Frog Spinal Motoneurons: A Golgi-Computer Reconstruction Study. B. M. Rosenthal* W.L.R. Cruce, and R.C. Carlsen. Neurobiology Dept., N.E. Ohio Univ. College of Medicine, Rootstown, OH 44272 and Dept. of Human Physiology, Univ. of California, Davis, CA 95616.

In a study of the physiological changes in synaptic input which follow frog lumbar motoneuron axotomy (Carlsen, Cruce, and Mendell, 76), we found that by 28 days after axotomy there was an increase in central reflex times for both the lateral column-axosomatic pathway and the dorsal root-axodendritic pathway. We suggested that this was due to diminished synaptic efficacy. In a subsequent electron microscopic investigation (Cruce and Kinney 79) we showed that synaptic coverage of the soma is reduced after axotomy. Preliminary to investigating synaptic coverage of the dendrites we decided to see if there was any retraction or shrinkage in the dendritic tree since this had been reported to occur in rat hypoglossal nucleus (Sumner and Watson, 71). The same cords which had been used in the physiological experiments were fixed by immersion in formalin for no more than 6 months, impregnated by the Golgi-Kopsch modification of Braitenberg, embedded in epon, and cut into 80 micra thick sections. (These cords had been prepared by transecting spinal nerve 9 or 10 prior to the experiments.) Only large ventral horn cells in the lateral motor column of the segment serving the axotomized root and which showed good silver impregnation were selected for study. The dorso-lateral dendritic tree, which arborizes in the region of dorsal root termination (Bregman and Cruce, 80) was traced in the X, Y, and Z planes with the aid of a computer-assisted microscope. Where necessary, we followed branches into adjacent serial sections. The unoperated side of the spinal cord provided control neurons. After 35 days the radial distance from the center of the neuron to the distal tip of the tree was reduced by 25% (from 640 $\pm$ 66 um to 480 $\pm$ 52 um). The radial distance to the peak of dendritic branching was reduced by 29% (from 340 $\pm$ 46 um to 243 $\pm$ 28 um) (Both significant at .025 level, paired t-test) It is possible that the Golgi impregnation did not fill the distal tips of the axotomized dendritic trees and therefore what we are measuring is a staining artifact, however the maximum number of branches did not change significantly (7.0 $\pm$ 2.28 to 7.4 $\pm$ 2.15) nor did the total length of the trees (2271 $\pm$ 587 um to 2018 $\pm$ 590 um). Our interpretation is that the entire dendritic tree has coiled in closer to the cell but has not lost any significant branches or length.

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250.10 EFFECT OF SPINAL CORD ISOLATION ON α-MOTONEURON POPULATION IN QUIESCENT SEGMENTS. M. Liebhold* and L. Eldridge* (SPON: D. Junge), Dept. of Physiology, University of California, Los Angeles, California 90024.

The effect of prolonged deprivation of excitatory input on the α-motoneuron population of cat lumbosacral spinal cord was studied by using a spinal isolation (SI) procedure. In the SI, all dorsal roots between two cord transections enclosing the 5th lumbar through the 3rd sacral cord segments were severed intradurally. To maximize the health of the isolated region, the ventral artery of the cord was left intact. In order to prevent direct stimulation of the cord by pressure on the back or by movements of the cat, the width of the laminectomy was restricted to 2 mm, except in the areas of the transections, which were protected by adjacent dorsal spinous processes. This silencing procedure was effective: the muscles were in flaccid paralysis at all times, with no voluntary, reflex, or other activity detectable. Cats were sacrificed at 5 (N=2), 8 (N=3), 24 (N=1), 28 (N=1), and 36 (N=1) months after the SI surgery, and their cords compared to those from 8 normal cats. All cats were perfused intravitally with aldehydes. The entire 6th lumbar segment was sectioned at 10μ, and every tenth section Nissl-stained for study. Motoneurons of greater than 25μ mean diameter with a nucleolus visible were counted. The α-motoneurons in the left and right halves of the cords were counted separately to check for possible asymmetrical effects of the surgery, which was always performed from the left side of the cat.

There were gross pathological changes in the SI cords, including progressive atrophy and gliosis. By 24 months, the cords had lost more than 50% of their volume dorsal to the central canal; that remaining was primarily connective tissue and cysts. The ventral and lateral areas normally carrying descending tracts were occupied by non-reactive astrocytes. The α-motoneuron population, however, was almost completely normal. There was no statistically significant loss of α-motoneurons, even in the 24 to 36 month cats. The mean count per section was 12.3 (S.D. 2.61) for the SI cords and 13.0 (S.D. 1.34) for the intact. The left and right sides did not differ. The largest α-motoneurons in both SI and intact cords were 80μ. The Nissl substance in the SI cats looked normal, except in the 36 month cat, in which the grains were smaller. There was no evidence of vacuolization or pyknosis. We conclude that the α-motoneurons are very resistant to prolonged inactivity in an isolation preparation in which the cord retains the major elements of both its circulation and its bony protection.

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250.11 MOVEMENT OF OPTIC TERMINALS OUT OF A LOCAL POSTSYNAPTICALLY-BLOCKED REGION IN GOLDFISH TECTUM. J.T. Schmidt. Dept. of Biol. Sci., SUNY Albany, Albany, N.Y. 12222.

Alpha-bungarotoxin has previously been shown to block the synapses of all three classes of retinal fibers in goldfish tectum, severely decrementing the field potentials produced by either photic or electrical stimulation. Quantitative current source-density analysis demonstrated that this was due to a blocking of synaptic currents into radially oriented tectal cells. Presynaptic volleys in the field potentials remain largely unaffected. Lack of a presynaptic effect was confirmed by unit recordings before, during and after the block. Small restricted zones of synaptic blockade were created by slow, low-pressure microinjection of toxin-Ringer solutions from a micropipette. The localized nature of the block was demonstrated by showing that outside the region field potentials were undecremented. Persistence of the bound toxin was demonstrated radioautographically after injection of iodinated toxin. Six days later it was still highly localized and concentrated in discrete synaptic lamina of the tectal neuropil. The lack of substantial diffusion is in agreement with its tight binding (~ 2 day half time of dissociation) and high affinity for the receptor (most of it probably rebinds nearby instead of diffusing away). At six to eight days, few if any optic terminals can be recorded in the toxin treated zone. Extensive mapping at 200 μm intervals however could show no scotoma in the visual field. Instead, terminals with receptive fields within the corresponding area could be recorded from surrounding tectal areas, along with the usual units recorded there, producing large multi-unit receptive fields. Thus all visual areas were still represented in the map. Apparently the optic terminals within the toxin blocked areas moved outward to colonize neighboring normal areas. By two weeks however, they can once again be recorded in the injection zone and the organization of the map is normal.

An actual movement of the terminals (rather than an electrical silencing of those branches in the toxin zone) can be demonstrated anatomically via anterograde filling of the fibers with HRP. Filling the entire optic nerve 5 to 8 days afterward demonstrated that the injected area of the tectum received a very sparse innervation. Examination at high power showed that the solidly filled fibers within that zone tended to run longer distances without branching and that fewer grape-like clusters of boutons emanated from them. After several weeks, the density of innervation was once again uniform. Control injection of Ringers alone produced neither a diminution of innervation in the injected zone anatomically nor a silent zone electrophysiologically, ruling out any damage due to the injection process itself. Supported by NIH grant EY03736 to J.T.S.

250.12 LOCALIZED βBTX APPLICATION PRODUCES A DENERVATED ZONE IN THE GOLDFISH OPTIC TECTUM WHICH IS INVADED BY SURROUNDING OPTIC TERMINALS. C.A. Lemere* and J.T. Schmidt (SPON: David Carpenter) Dept. of Biol. Sci., SUNY Albany, Albany, N.Y. 12222.

Local surgical denervation produces sprouting both peripherally (neuromuscular junction and sensory innervation) and centrally in the hippocampus, tectum, etc. In this study, sprouting was produced by local injection of a presynaptically-acting toxin, β-bungarotoxin (βBTX), a 22,000 MW basic protein. To localize the injection, units were recorded and their receptive fields plotted relative to the optic disc. Two sites, 400 μm apart, were injected with 1 μl of 10^{-7} M βBTX in Ringers at a depth of 150 μm below the surface. Low pressure to a micropipette of 10-15 μm tip diameter delivered this volume over 3 to 5 minutes. As at the neuromuscular junction, the presynaptic units (optic terminals) in the injected area fired spontaneously, decreased in amplitude, and gradually disappeared within one half hour. Optic terminals could be recorded caudal to the injection sites, indicating that βBTX did not interrupt conduction in axons travelling through the toxin-treated zone. Thus, the retinotectal map recorded at this time showed both a silent zone on the tectum and a corresponding scotoma in the visual field. Between 4 and 9 days later, optic terminals could once again be recorded at all points on the tectum (200 μm grid spacings). However, a scotoma, corresponding to the injection sites, remained in the visual field map, because units recorded in the injected zone had receptive fields outside the appropriate area of the visual field. By 15 days normal maps with no scotoma in the visual field were recorded.

Anterograde HRP filling of the optic nerve was used to visualize the optic fibers and terminals. The tectal surface was reconstructed from camera lucida drawings of transverse sections. Filling on day 0 showed two distinct uninnervated regions corresponding to the βBTX injection sites. These regions contained labelled degenerating debris rather than solidly filled fibers. Filling on day 9 showed 2 regions of sparser innervation, in an otherwise solidly filled tectal projection, corresponding to the region injected with βBTX. The electrophysiological results, in conjunction with these suggest that the toxin-induced degeneration of presynaptic terminals causes them to lose their postsynaptic sites to invading healthy terminals, 4 to 9 days after βBTX injections. But by 15 days, the damaged terminals regenerate to reclaim their appropriate zone on the tectum, pushing the invading terminals back to their original sites.

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250.13 ELECTRICAL OR CHEMICAL STIMULATION OF VISUAL CORTEX PREVENTS OCULAR DOMINANCE SHIFT IN MONOCULARLY DEPRIVED KITTENS. C. Shaw and M. Cynader, Department of Psychology, Dalhousie University, Halifax, Nova Scotia B3H 4J1.

The mechanism(s) underlying the ocular dominance shift after monocular eyelid suture remain unexplained, but most models propose changes in lateral geniculate nucleus (LGN) afferents representing the sutured eye. Recently, Cynader & Mitchell (1980) suggested that post-synaptic changes in cortical cells may underlie this plasticity. We tested this possibility using electrical or chemical stimulation to alter the firing rate of neurons in visual cortex areas 17 and 18 during monocular deprivation beginning on day 49-54. Two kittens (electrical stimulation (ES)) had two pair of stimulating and ground electrodes implanted unilaterally into the visual cortex ipsilateral to the sutured eye. Two other kittens (glutamate (GL)) had a 0.5 M L-glutamate solution (pH 7.3-7.7) applied to the visual cortex ipsilateral to the sutured eye through a 30 gauge cannula attached to an osmotic minipump (0.5-1μL/hr; Alzet); the control cortex was infused with saline solution. The ES kittens were kept in the dark after surgery and allowed light exposure only during electrical stimulation (100 cps biphasic, 100-150 μA; 2ms duration), receiving 80 to 168 hours of exposure before unit recording on postnatal day 70-73. The GL kittens were kept on a normal light/dark cycle until just before the minipumps were exhausted (6 or 13 days) and then placed in the dark until unit recording on postnatal day 59-69. The results from all experiments were similar: Control cortices showed the usual shifts in ocular dominance; experimental cortices showed greater retention of binocularity (ocular dominance (OD) groups 2-6). The percentage of binocular neurons in the experimental cortices closely resembles that reported for normal kittens (Olson and Freeman, 1978), and neither orientation nor direction selectivity were altered indicating maintenance of pre-deprivation properties. The results suggest that stimulation of the visual cortex decreases the possibility for correlation between LGN input and cortical cellular response, thus largely preventing the shift in ocular dominance following eyelid suture. Further, the specificity of glutamate in activating cortical neurons, but not afferent fibers (Hess and Murata, 1974), suggests that this occurs post-synaptically.

Cortex	Kitten	No. of Neurons	% Binocular Neurons (OD Groups 2-6)
Control	ES	119	45
	GL	42	53
Experimental	ES	215	72
	GL	84	82

250.14 EFFECTS OF ENVIRONMENTAL MANIPULATIONS ON THE STELLATE CELL BRANCHING INDEX. B. Sonnier, A. Riesen, and T. Patterson. Dept. of Psychology, University of California, Riverside, CA 92521.

Percheron (1,2) proposes that stability of numerical parameters within a neuronal type indicate a central coding of neurons and that these parameters are important to specific functions and to the organization of behavior. Using Golgi and light microscope techniques, we investigated the use of the "branch index" as a tool for the evaluation of spineless stellate cell development in laminae II and IV of stumptail macaques reared in one of four environments providing differential amounts of motor and/or somatosensory stimulation. Samples were taken at 3 and 6 mons. of age from visual, S-I, S-II, M-I, M-II and premotor frontal cortex. These areas were also examined in monkeys reared for 3 mons. in the most restricted condition followed by 3 mons. in the colony control rearing condition (4), ie., reversal.

Preliminary data in Table I indicate the index is fairly stable within a narrow range (3.0-4.0) at 6 mons. in the colony control rearing condition, but it does vary with age and/or experimental condition. The index is lower at 3 mons., and it is higher in M-I and S-I in the control rearing condition. Index values for reversal animals tend either to lie between those of normal and experimental animals at 6 mons. or to be similar to those in the control condition at 3 mons. The similarity of the index values over all brain areas in the control condition is remarkable, and this is the only condition where normal behavior patterns developed. Manipulations of the environment appear to increase the variance of stellate index values between the brain areas of animals within a given experimental condition, and to increase the probability of developing abnormal behaviors.

TABLE I: LAMINAE IV STELLATE CELL BRANCH INDEX VALUES

	M-I	M-II	S-I	S-II	Visual	Frontal
Con 1-6 mons.	3.41	3.23	3.47	3.69	3.56	4.00
Con 2-6 mons.	3.60	3.38	3.67	3.78	3.82	3.97
Con 3-6 mons.	3.97	3.52	4.46	3.61	3.74	3.95
Control-6 mons.	3.97	3.76	3.85	3.60	3.76	3.82
Reversal	3.78	3.22	3.56	3.30	3.94	3.39
Con 1-3 mons.	3.35	2.79	3.42	2.76	3.70	3.06
Control-3 mons.	3.48	3.18	3.73	3.30	3.99	3.23

(1,2) Neurosci. Letters, 14: 287-293, 295-302, 1979.
(3) TIT J. Life Sci., 2: 129-140, 1972.
(4) Aggressive Behav., 5: 199-200, 1979.*
* Contains a more comprehensive description of rearing condition procedures.

250.15 EFFECTS OF ENVIRONMENTAL MANIPULATIONS ON DENDRITIC DEVELOPMENT IN SPINELESS STELLATE CELLS OF STUMPTAIL MONKEYS: A PRELIMINARY REPORT. T. Patterson, B. Sonnier, and A. Riesen. Department of Psychology, University of California, Riverside, CA 92521.

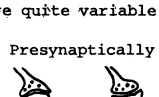
In our laboratory, we have been investigating effects of environmental manipulations on neural and behavioral development in stumptail monkeys. Rearing conditions were designed to provide graded amounts of somatosensory stimulation and/or motor activity without visual deprivation. All monkeys were reared in one of the following conditions from one week after birth to 3 or 6 mons., except for reversals, which were reared in Con 1 for 3 mons. followed by 3 mons. in Con 4. Con 1: A 12" by 12" transparent Plexiglas cube with a grille floor, an attached bottle and food hopper, all of which provided minimal opportunity for object manipulation; Con 2: A 48" by 48" transparent Plexiglas cube, equiped as in Con 1; Con 3: A cube, as in Con 2, with the addition of ladders, a trapeze and various play objects; Con 4: A large colony room immediately adjacent to the other rearing conditions, where control animals were reared in full view of other experimental animals by their mothers, who were members of a socially stabilized mixed sex group.

Typically, monkeys in Con 1, 2 and 3 exhibit primate social isolation impairments by 3 mons., and their bizzare and stereotyped posturings show some correlation to degree of deprivation. Decreases in these behaviors were observed in reversal during the last 3 mons. in Con 4. There were significant effects of rearing on ratio and number of dendritic branches in S-I, M-I and M-II at both ages, but not in S-II, visual or frontal cortex, ruling out a generalized growth harmone effect. All measures tended to be larger at 6 mons., with the exception of primary branching. This was greater at 3 mons. and in reversal, but there were no rearing effects present at either age. Some loss of primary branches with maturation is implied by the decreases observed at 6 mons., however reversal animals did not show decreases. Other data for reversals tend either to fall somewhere between that of Con 1 and 4 animals at 6 mons. or to be similar to data from Con 4 at 3 mons. Con 3 had the most extensive branching in S-I, and these animals were comparable to those in Con 4 in other areas, with the exception of M-II. Branching trends in lamina II were similar to those found in IV. Con 3 animals engaged in bizzare behavior almost as much as those in Con 1 and 2, so too much growth may be as deleterious to some behavioral functions as too little. Over all, we feel our results support the idea that functional interactions with the environment play an important role in the modulation, control and validation of growth changes, and thus influence subsequent behavioral organization.

250.16 DIFFERENTIAL POST-SYNAPTIC CURVATURE IN OCCIPITAL CORTEX FOLLOWING DIFFERENTIAL REARING IN RATS. Fen-Lei F. Chang, Janice M. Wesa*, William T. Greenough and Roger W. West, Depts. of Psychol. and Anat. Sci. and Neur. and Behav. Biol. Prog., Univ. IL, Urbana-Champaign, IL 61820 and Dept. Psychol., Memorial Univ., St. John's, NFLD, Canada.

One way in which potential correlates of synaptic plasticity have been examined has been to observe whether they are altered following differential experience. Recently, Dyson and Jones (Br. Res., 183:43, 1980), proposed that the curvature of synapses, which shifted from presynaptic convexity toward presynaptic concavity in the cerebral cortex in rats as they matured, might be an indicant of plastic capacity of synapses. To examine effects of experience, independent of age, on this measure, we examined curvature of synapses in layers 1, 3, and 4 of occipital cortex of male littermate triplet sets of rats reared in complex (EC), social (SC), or isolated (SC) environments (rearing details: Greenough, West & DeVoogd, Sci., 202:1096, 1978). Micrographs randomly positioned within the layers (avoiding somata) were printed at 41,800x and coded to prevent experimenter bias. All transversely sectioned synapses in which the postsynaptic membrane and cleft were clearly visible were measured in 8 micrographs per layer from 11 triplet sets. Curvature was assessed as the inverse radius of the circle passing through the membrane at the 2 ends of the post-synaptic thickening (PST) and through the point of maximum deviation of the membrane from a straight line through the 2 end points, using a data tablet. Synapses with subsynaptic plate perforations (SSPP's, Greenough et al.) were analyzed separately from those with continuous PST's and those with complex curvatures (e.g., biphasically curving or invaginated) were excluded from analysis (2964 individual synapses were included).

Non-SSPP synapses on average were significantly more presynaptically concave (expressed as 1/r, in cm. at 41,800x below) in EC than in IC rats, while SC values were intermediate (Table). This is the type of synapse measured by Dyson & Jones. SSPP synapses were quite variable and did not differ statistically in curvature.



	Non-SSPP	SSPP	Combined
EC	0.243	0.125	0.232
SC	0.196	0.231	0.191
IC	0.177	0.136	0.173
	P<.05		.05<P<.10

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250.17

WITHDRAWN

250.18 INHIBITORY MODULATION OF LTP IN THE HIPPOCAMPAL DENTATE AREA OF AWAKE RATS. G. V. Goddard and M. Riives. Psychology Department, Dalhousie University, Halifax, Nova Scotia, Canada, B3H 4J1

Long-term potentiation (LTP) of the population spike response among granule cells of the facia dentata to input from the perforant path following high frequency synchronous activity in the perforant path fibers was prevented or greatly diminished by preceding or coincident high frequency stimulation of the contralateral hilus in awake freely moving rats. The extent to which LTP was diminished was greater when the onset of the contralateral train preceded the onset of the perforant path train than when they were nearly coincident, and there was no consistently measurable effect when the onset of the contralateral burst was delayed by 4 msec or more after the onset of the perforant path train.

A series of 29 rats with chronically indwelling electrodes were tested at varying intervals between contralateral and perforant path train onset. The population spike height during the conditioning trains as well as the amount of LTP induced by those trains were expressed as percentages of the values observed when perforant path trains were delivered alone. When the amount of LTP was plotted against the interval between contralateral burst onset and perforant path burst onset, the function was a smooth and gradual sigmoid increase from -8 msec (contralateral burst preceding) to +4 msec (perforant path burst preceding). This function did not parallel the function obtained when the population spike evoked during these conditioning trains was plotted against the same intervals. The population spike was blocked when the contralateral train onset preceding the perforant path train onset by as little as 1 msec, and was not blocked when the contralateral train onset followed the perforant path train onset by more than 1 msec.

Since the ability of the contralateral activity to diminish LTP at the various intervals did not correlate with its ability to diminish the population spike at these intervals, LTP is seen to be independent of action potentials in the post-synaptic neurons. In this sense, the LTP is not based on Hebb-type synapses in which the post-synaptic action potential is a necessary agent of change. On the other hand, since LTP of the perforant path was dependent on interaction between activity arriving from different sources, it involves an associational mechanism, like a Hebb synapse but, dependent on a mechanism of comparison that does not entail action potentials in the post-synaptic neuron.

This research was supported by Natural Sciences and Engineering Research Council of Canada Grant A0365 to G. V. Goddard. Present address: G. V. Goddard, Professor and Chairman, Department of Psychology, University of Otago, Dunedin, New Zealand.

250.19 FACILITATION OF AMYGDALOID KINDLING FOLLOWING PENTYLENETETRAZOL-INDUCED SEIZURES IN NEONATAL RAT PUPS. Mary E. Gilbert* and Donald P. Cain. (SPON: K.-P. Ossenkopp). Department of Psychology, University of Western Ontario, London, Ontario, CANADA, N6A 5C2.

The results of studies conducted in our laboratory have demonstrated that adult rats kindled by injection of pentylenetetrazol (PTZ) can be rendered more susceptible to seizures electrically kindled through the amygdala---the transfer facilitation effect (Cain, this conf.). The present study examined transfer facilitation of seizures induced very early in life to later kindling stimulation in adulthood. It is of theoretical interest and of clinical significance to discover if alterations in brain following seizure activity in the developing organism persist and influence the adult seizure susceptibility.

Single or multiple seizures were evoked by injection of PTZ in neonatal rat pups between 1 and 4 days of age. Following a 3-month growth period, these same animals, as adults, were implanted with bilateral electrodes in the basolateral amygdala. Kindling stimulation consisting of a 1-sec train of biphasic square-wave pulses at 60 Hz and at afterdischarge (AD) threshold was delivered once daily until a stage 5 generalized seizure occurred. Subjects pretreated with PTZ as neonates developed kindled seizures significantly faster than controls (means of 6.8 and 13.6 ADs respectively; $p < .001$). Seizure severity and the frequency of postictal discharge were also assessed during and after electrical kindling. The pretreated group displayed more severe seizures and a higher frequency of postictal seizure manifestations than the controls. No difference between the groups was observed in the AD threshold, but the generalized seizure threshold (minimum current intensity necessary to evoke a generalized seizure in a fully kindled animal) was significantly lower in the pretreated subjects (means of 48.6 μA and 118.0 μA respectively; $p < .001$).

These preliminary data suggest that seizure experience during the neonatal period can render subjects permanently more susceptible to electrically kindled seizures in adulthood. Similar findings in studies in which neonatal rats experienced hyperthermia-induced seizures (Gilbert, unpubl. data; Manetto, McCaughran & Schechter, Neurosci. Abst. 137.16, 1980) suggest that long term changes in the brain can result from early seizures induced by a variety of means.

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250.20 ^{35}S - METHIONINE INCORPORATION INTO SPINAL CORD PROTEIN. Louis Stodieck* and M. W. Luttges (SPON: D. Groswald). Department of Aerospace Engineering Sciences, University of Colorado, Boulder, CO 80309.

The effects of sciatic nerve stimulation and unilateral sciatic nerve crush on uptake and incorporation of L-^{35}S - Methionine in mouse spinal cord were studied. Stimulation was delivered to the sciatic nerve at a voltage high enough to elicit a cross spinal response recorded from the contralateral nerve. Half the mice received unilateral sciatic nerve crush six days prior to testing. ^{35}S - Methionine was administered by low lumbar interthecal injection. After a two hour stimulation-labeling period, the lumbar cord was removed for processing. Tissue distributions of radioactivity were obtained from crude exogenous pool, intracellular free pool, and protein fractions. The protein fraction was dissolved in sample buffer containing SDS and subsequently electrophoresed on 8.75 - 18.75% polyacrylamide gels. The stained gels showed readily resolvable protein bands ranging in molecular weight from 10,000 to 240,000. ^{35}S - Methionine was found in all bands but exhibited significant variations between individual bands, suggesting variations in turnover rate. In experimental animals, specific activity was determined in ten individual bands for comparison across experimental conditions. The bands varied both in estimated molecular weight and presumed subcellular origin.

Total recovered radioactivity was found to vary by more than two orders of magnitude across animals despite the constancy of evoked cross spinal responses in all preparations. Autoradiographic data indicating that most label was associated with spinal grey matter exhibited similar variability in levels as well as the localization of radioactivity. To circumvent such problems, the distribution of radioactivity between the exogenous pool, the endogenous free pool, and protein fraction was expressed as a percentage. Although spinal injections seem to have consequences for the amount of ^{35}S - Methionine made available to the spinal tissue, they appeared to have little effect on the uptake or incorporation of the label or upon the mechanisms supporting cross-cord responses.

Neither stimulation nor nerve crush influenced the distribution of recovered radioactivity. Similarly, the relative incorporation of label into specific proteins did not vary as a consequence of nerve damage or stimulation. Methionine may exhibit uptake and incorporation characteristics which produce a different estimate of protein turnover than is commonly observed.

250.21 REGIONAL BRAIN GLUCOSE METABOLISM AND PROTEIN SYNTHESIS FOLLOWING IBOTENIC ACID LESIONS OF THE STRIATUM. K. A. Frey* and B. W. Agranoff (SPON: M. Johnston). Neuroscience Laboratory, University of Michigan, Ann Arbor, MI 48109.

Intrastriatal injection of ibotenic acid (IBO), an excitotoxic conformationally restricted analog of glutamic acid, has been shown to result in a disappearance of striatal neurons without effects upon axons of passage or extrinsic nerve terminals (Schwartz et al., Exp. Brain Res. 37, 199-216, 1979). In the present study, we have examined the effects of striatal IBO lesions on regional cerebral glucose utilization and protein synthesis within the lesioned area as well as within distant brain structures. One week following unilateral striatal injection of 20 µg of IBO in a volume of 2 µl, rats were anesthetized with ether and a femoral artery and vein cannulated. After a 4 hr recovery period, animals received an intravenous bolus injection of either [^{14}C]2-deoxy-D-glucose (150 µCi/kg) or [1-^{14}C]L-leucine (300 µCi/kg), and timed arterial blood samples were obtained throughout a 45 min incorporation period. Brains were processed for quantitative autoradiography and regional rates of glucose metabolism (Sokoloff et al., J. Neurochem. 28, 897-916, 1977) or protein synthesis (Smith et al., Trans. Am. Soc. Neurochem. p. 94, 1980) determined for individual brain regions from the radioautograms and plasma precursor pool specific activities. Within the lesioned striatum there was a significant depression of both glucose utilization and protein synthesis, while ipsilateral brain regions receiving striatal projections (globus pallidus, entopeduncular nucleus, and substantia nigra) showed elevated glucose utilization and protein synthesis. The observed changes in glucose metabolic rates are consistent with a functional disinhibition of striatal projection sites following the removal of GABAergic input. The apparent increases in protein synthesis within these same regions may be related to the development of denervation supersensitivity which has been previously demonstrated by receptor binding techniques (Guidotti et al., Brain Res. 172, 566-571, 1979). This work was supported by Grant NS 15655. KAF is a trainee of NIH Grant I T32 GM07863.

250.22 RATS ARTIFICIALLY REARED WITH A BALANCED FREE-FATTY ACID DIET: A PRELIMINARY BEHAVIORAL, MORPHOLOGICAL, AND TELENCEPHALIC GOLGI EVALUATION. J. Diaz, G. Clark, F. Petracca*, and J. Schacher*. Dept. of Psychology, Univ. of Washington, Seattle, Wa. 98195.

The procedure of rearing rat pups exclusively by gastrostomy feedings and isolated from their mother and siblings has resulted in animals with somatic growth similar to their normally reared siblings but with significant differences in specific organ weights, including the brain. Behavioral differences between artificially reared animals (AR) and normally reared animals (NR) have also been demonstrated.

These behavioral and central differences between AR and NR animals were reduced when the formula fed to the AR animals was enriched with protein (Diaz, et al., Neurosci Abst, 6, 1980.) Evaluation of the standard diet fed to AR animals (Messer, et. al., J. Nutr., 98, 1969) reveals that in addition to low protein levels, the composition of the fats is inappropriate. The purpose of the present study is to evaluate artificial rearing with a formula enriched with protein and balanced amounts of short and long chained fatty acids.

4-day old female Long-Evans hooded rat pups were matched by weight and assigned to one of two groups: animals artificially reared and animals normally reared. All animals were weighed daily. On day 18, the animals were tested in an open field and then sacrificed. Peripheral organs were removed and weighed. The brain was removed, weighed, and prepared for a rapid Golgi according to the procedure of Scheibel. A microcomputer system was used to determine dendritic length and arborization from the neuron tracings.

The results indicate that somatic growth rates were not different for the AR and NR animals. However, peripheral organ weights were greater and brain weights were smaller for the AR animals compared to their NR siblings. The behavioral profile of the AR animals was also different than the NR animals. These differences between the AR and NR groups are similar to the differences observed when only protein was added to the diet of AR animals.

The analysis of the granule cells in the dentate gyrus indicated that there are no significant differences in dendritic length and dendritic arborization between the two groups. Analysis of cortical pyramidal cells is in progress and will be presented.

Despite significant decreases in whole brain weights in the AR animals, the quantitative and qualitative analysis of the Golgi material indicates that there are no gross neuronal abnormalities in animals that are artificially reared.

250.23 EFFECTS OF ENVIRONMENTAL ENRICHMENT ON RECOVERY OF LOCOMOTION FOLLOWING CORTICAL LESIONS IN RATS. J.M. Held, J. Gordon*, and A.M. Gentile. Teachers College, Columbia Univ., New York, NY 10027

Several factors influencing the rate and nature of motor recovery following damage to the motor cortex of rats have been identified; i.e., momentum, size, site, and symmetricalness of lesions (Gentile, et al., Beh. Biol., 1978, 22, 417-455; Gentile, et al., Soc. Neurosci. Abstr., 1980, 6, 650). The present study examined the role of environmental enrichment as a factor influencing recovery processes. Other investigators have demonstrated better recovery when immature animals were exposed postoperatively to an enriched environment (Will, et al., Physiol. and Beh., 1976, 16, 603-611). Extending these analyses, we evaluated the relative influence of pre- and postoperative enrichment on sparing and recovery of locomotor performance of mature rats after lesions of motor cortex.

Four groups of eight rats each were exposed to an enriched environment: 1) preoperatively, 2) postoperatively, 3) both intervals, 4) not at all. All animals within these four groups were administered bilateral lesions of sensorimotor cortex (18mm^2 each side). In addition, one sham operated group (eight rats) was exposed pre- and postoperatively to the enriched environment; a second control group (eight rats) had no exposure. The task on which all animals were trained preoperatively and tested postoperatively involved locomotion on a narrow elevated runway. The enriched environment was a 120cm x 120cm x 45cm wire mesh cage holding multiple apparatus for many gross motor activities but not for locomotion on narrow surfaces. Groups of rats were exposed to the enriched environment 2 hrs per day for 30 days during the immediate pre- and postoperative period. Postoperative testing began 31 days after surgery and continued until preoperative performance levels were achieved.

A combination of pre- and postoperative enrichment produced partial sparing, with rapid and full recovery; animals not receiving enrichment pre- or postoperatively were severely impaired and took considerable time to reattain criterion levels. Cinematographic analysis of high speed film data was used to examine movement patterns in order to elucidate processes underlying recovery. Both pre- and postoperative enrichment resulted in faster recovery on the locomotor task; however, it appeared that, of the two conditions, preoperative enrichment had a more potent influence on recovery. This finding suggests that the initial state of the system at the time of damage may be an important determinant of the extent of deficit and the rapidity of recovery.

250.24 DIFFERENCES IN THE CRIES OF DEAF AND HEARING KITTENS. C. Shipley*, J.S. Buchwald, E.C. Carterette* and J. Strecker*. (SPON: J.P. Segundo). Brain Research Institute, Mental Retardation Research Center, Departments of Physiology and Psychology, Univ. of Calif., Los Angeles, CA 90024.

Controversy presently exists as to whether the vocalizations of non-human mammals are formed in a purely reflexive fashion or are modulated by auditory feedback. Our approach to this issue has been to analyze andcompare the cries of normal and deaf animals during postnatal development.

We have studied the cries of 10 hearing-impaired kittens and those of 10 littermate controls. Experimental animals underwent bilateral destruction of the cochlea at 14 days of age. The results of the deafening procedure were assessed through recordings of the brainstem auditory evoked responses (ABRs). In the present series of animals, complete loss of ABRs was achieved in three cases; other animals had transmission losses varying from 30 to 70 db as measured by ABR thresholds.

The cries of the deaf or partially deaf kittens differ in several respects from those of normals. We are currently quantifying differences in the fine structure of the calls with digital filtering techniques. Preliminary spectrographic analysis of the calls suggests that the acoustic features of the cries of deaf animals (e.g., the fundamental frequency) are much less variable across calls than those of normal kittens. In several different behavioral contexts, the cries of deafened animals were also consistently louder and more numerous than those of littermate controls. Differences between deaf and normal kittens in the intensity of calls appears to reflect active modulation of loudness by normal animals. When control kittens were recorded during exposure to 80 db SPL white noise their calls were louder than during exposure to 60 db white noise or during recording without noise. These manipulations had no effect on deafened animals.

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251.1 PURKINJE CELL ACTIVITY IN VERMAL AND PARAVERMAL CEREBELLUM OF MONKEYS DURING TRAINED SACCADIC EYE MOVEMENTS. J.G. McElligott, Dept. of Pharmacol. Temple Univ. Sch. of Med., Phila., PA 19140, and E.L. Keller, The Smith-Kettlewell Inst. of Vis. Sci., San Francisco, CA 94115

Two hundred cells were recorded from the cerebellum of two awake head-restrained monkeys trained to make saccades to visual targets. Sixty one of these neurons were Purkinje cells. Comparisons of firing patterns were made for saccades of different amplitudes, directions, and absolute positions along the primary axes. All of these cells were constantly active and 50% of them had a multi-component related response (excitatory and/or inhibitory modulation of the background activity). These modulations occurred during a period from 20 msec before the initiation to 200 msec after the termination of the saccade. The presence of a particular component was dependent on the direction and/or the absolute position of the saccade. In general, smaller amplitude saccades had smaller responses. These responsive cells were located either in the posterior vermis or a non-contiguous paravermal region 4-5 mm from the midline. None of these cells responded to the presentation of the visual targets or an alerting auditory stimulus at the beginning of each trial. Controls for the influence of afferent inputs were (1) recordings made during spontaneous saccades in the dark, and (2) determining that there were no attempted head movements during the trained saccadic movements. The climbing fiber response could be discriminated well enough in 22 Purkinje cells to reveal that its occurrence was unrelated to the saccades or the presentation of the sensory stimuli.

The firing patterns of these cells are similar to those previously obtained in the cat (McElligott, Soc. Neurosci.,1979) with the exception that in the monkey they were not influenced by auditory/visual inputs. The multi-component nature of the response and its dependency on the absolute position of the saccade is in accord with the notion that individual cells (1)are influenced by several extra-ocular muscles and (2) that a cell may be correcting for the inherent non-linearities of the extra-ocular muscles. (Supported by a grant from N.S.F.)

251.2 MOSSY FIBER RESPONSES OF PURKINJE CELLS IN THE CEREBELLAR FLOCCULUS OF THE ALERT, PIGMENTED RAT DURING OPTOKINETIC AND VESTIBULAR STIMULATION. Robert H.I. Blanks and Wolfgang Precht. Depts. of Anatomy and Surgery (Otolaryngology), Univ. of Calif., Irvine, CA 92717 and Inst. for Brain Research, Univ. Zurich, Zurich, Switzerland.

The mossy fiber activity of floccular Purkinje cells (P-cell) was studied in the alert, paralyzed rat (DA-HAN) during optokinetic (OKS) and vestibular stimulation. Of a total of 98 P-cells, which were activated by rotatory stimulation of the horizontal semicircular canals (type I and type II P-cells), the vast majority (71/98) responded to constant velocity OKS (0.5^{o}-20^{o}sec) produced by means of a horizontal shadow projector system. The remaining P-cells responded only to vestibular stimulation (19/98) and were unresponsive to OKS.

P-cells which responded to horizontal canal stimulation were, in general, distributed in a longitudinal zone in the middle of the unifoliate rat flocculus. The OKS response of these units was generally bidirectional and synergistic with the horizontal canal input. During constant velocity OKS the discharge of a few P-cells rose exponentially and outlasted the stimulus by 10-15 sec and thus resemble OKS responses of vestibular nucleus neurons. However, the majority exhibited two components of the same polarity: a phasic component (time constant 0.3-1 sec) at the onset and termination of the stimulus and a smaller tonic component with a longer time course (time constant 3-5 sec). Both components showed peak sensitivities with OKS velocities of 1.5^{o}-2^{o}/sec which is slightly higher (i.e., an extended sensitivity range) than the ca. 1^{o}/sec determined for vestibular nucleus neurons in the same preparation (Cazin et al., Pflugers Arch. 384, 31-38, 1980).

Given the short time constant phasic-tonic nature and extended sensitivity range of P-cell-OKS responses, these cells are best suited for generating oculomotor pursuit signals rather than sustaining the slow rising components of optokinetic nystagmus or optokinetic afternystagmus.

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251.3 VISUAL RESPONSES OF EYE MOVEMENT RELATED NEURONS IN PRIMATE CENTRAL THALAMUS. M. Schlag-Rey and J. Schlag. Dept. of Anatomy and BRI, UCLA, Los Angeles, CA 90024.

The central thalamus of monkey (in and around the internal medullary lamina, IML) contains eye position and saccade-related neurons discharging in absence of visual input. The visual responsiveness of these and other neurons in close vicinity was studied in monkeys (Macaca nemestrina) trained to acquire, fixate and pursue a dim visual target (1.5^{o}) in various tasks.

1. Transient on- and off-responses (85-100 ms latency) were obtained from presaccadic neurons. Receptive fields were contralateral, large, with gradients of responsiveness declining around a restricted region of maximal sensitivity. This region was found at different degrees of eccentricity for different neurons and coincided with the movement field of these neurons. As previously observed in cats, this type of visual-oculomotor neuron discharged twice during trials where target acquisition was delayed beyond the normal time span of fixation reflexes (with no external event triggering the target saccade). In this case, the burst time-locked to stimulus onset often bore a striking resemblance to the burst time-locked to saccade onset. Some of these neurons gave signals purely retinotopically coded, others appeared influenced by eye or stimulus position.

2. A separate group of units, discharging tonically in relation to fixation and smooth pursuit, was found in the upper part of the IML. These units, mirror images of each other, were often found very close along the same microelectrode track. They had foveal or near foveal receptive fields and showed an exquisite resolution in modulating their firing rate as soon as the monkey relaxed its fixation with minute deviations of the gaze ($\pm$ 1 deg), suggesting that they may provide extremely sensitive error signals. The direction of gaze did not matter. The paroxysmal activation or suppression was never observed in darkness. The role of the stimulus in triggering or suppressing the firing was further attested by the constant delay with which the characteristic activity followed stimulus onset (100-150 ms latency) or the end of a targeting saccade. However, this activity (activation or suppression) could outlast the stimulus or even recur when the animal's gaze returned to the site where the stimulus had been, suggesting a role in intentional fixation.

These results bring evidence that the central thalamus is specifically involved in the voluntary control of the gaze. (Supported by USPHS grant EY 02305).

251.4 HEAD AND EYE MOVEMENTS EVOKED BY ELECTRICAL STIMULATION OF THALAMIC GAZE CENTER. H. Maldonado and J. Schlag. Dept. of Anatomy and BRI, UCLA, Los Angeles, CA 90024.

Electrical stimulation of the thalamic internal medullary lamina (IML) in awake, head-restrained cats evokes contraversive saccades. Unit recording in this region has disclosed the existence of cells discharging not only with eye movements (Ems), but also with head movements (Hms). Therefore, the effects of electrical stimulation of the IML were studied in head-free animals (i.e. able to rotate their head 60^{o} in the horizontal plane). Hms were recorded with a low torque potentiometer, Ems with implanted silver-silver chloride electrodes. Negative pulses of 0.2 ms at 250 Hz, 10 μA minimum current and in train of 50-500 ms duration were applied through tungsten microelectrodes, sometimes just after recording movement-related unit activity.

Three features were common to all eye-head movements evoked: (1) All evoked head rotations were contraversive and accompanied by ipsiversive compensatory eye movements; (2) If the eyes were initially deviated 7^{o} or more to the ipsilateral side, the first elicited movement always was a saccade to the center of the orbit or a few degrees upward contralaterally; (3) If the eyes were initially deviated 13^{o} or more contralaterally the stimulation had no effect or it first triggered a Hm.

Two types of eye-head movements patterns were distinguished according to the site of stimulation: (1) With low currents, saccades directed to a particulat site were obtained. The direction, amplitude and latency depended on initial eye position. At twice the threshold for evoking Ems, Hms occurred 25-50 ms after the initial saccade. This pattern was characteristic of the sites where units were identified as related to Ems. (2) The first movement evoked was a head rotation with a latency of 60-80 ms., irrespective of the stimulus intensity. Ems occurred later. This pattern was obtained by stimulating sites where Hms-related units had been recorded. These data give further support to the hypothesis that the IML is concerned with gaze control, not solely eye movements. (Supported by USPHS grant NS 04955; Dr. H. Maldonado was supported by CONACYT #16002, México).

251.5 A RETROGRADE HRP STUDY OF FRONTAL EYE FIELD INTERCONNECTIONS WITH THE MOTOR AND VESTIBULAR AREAS IN THE CAT.

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Classically the frontal eye field (FEF) has been considered to be the cortical site of voluntary eye movements. The work of Bizzi (1968), however showed that the FEF neurons became active after eye movements rendering this idea untenable. Stimulation and unit-recording have shown that eye movements and neck EMG activity are associated with FEF neuronal activity (Guitton and Mandl, 1978). With the object of uncovering the neural basis of the coordination of extraocular and neck muscle activity, we are investigating the connections between the FEF and motor cortex (MCx) using horseradish peroxidase (HRP) as a tracer.

In each brain of 12 cats, either two or three pressure injections of from 20 to 50 nl of 50% HRP (Sigma VI) in normal saline, were made over a 20 min. duration, bilaterally or unilaterally in the grey matter of the walls of the presylvian sulcus, the exact locations being confirmed later by the histology. After a survival time of two days each animal was anesthetized, perfused and 50μm (frozen) sections cut and then incubated according to the tetramethyl benzidine neurohistochemistry of Mesulam, (1976). Cortical areas were defined according to the architectonic criteria of Hassler and Muhs-Clement (1964).

The result of unilateral HRP injections into the FEF was observed to produce in the white matter a well defined fascicle of axons filled with the blue HRP reaction product. Medium-sized labelled pyramidal cell bodies (20-25μm dia),with their apices directed towards lamina I, were observed in the region of the cruciate sulcus. These neurons were found in lamina III and just outside the area containing the giant Betz cells. The projection was bilateral, unlike that of the labelled cell bodies found in the FEF after HRP injections in the ipsilateral pericruciate area. Clusters of irregularly shaped, labelled cell bodies were also found bilaterally in the coronal gyri. Unilaterally, dispersed (20-30μm dia) and small (12-20μm dia) labelled pyramidal-shaped cell bodies were observed in the anterior of the suprasylvian gyrus and buried in the ventral wall of the ansate sulcus a region that has been implicated in the reception of vestibular information by electrophysiological studies (Keminsky, 1951; Mickle and Ades, 1952 and Sana et al., 1970). From these observations it appears that interconnections do occur between the FEF and the MCx and also that the FEF receives input from a vestibular projection area of cortex. Such connections can be considered as candidates for part of the neuronal substrate underlying the coordination of eye and head movements.

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251.6 A RELATIONSHIP BETWEEN THE NIGROTECTAL AND CROSSED TECTORETICULAR PATHWAYS IN THE GREY SQUIRREL

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The superior colliculus projects to the paramedian reticular regions concerned with controlling gaze by means of a crossed descending pathway which originates from neurons in the intermediate grey layer (SGI). We have previously shown that the addition of saponin to injections of horseradish peroxidase (HRP) into the crossed pathway produces a homogeneous backfilling of neurons in SGI. The resultant "Golgi-like" appearance of their somas and dendritic fields allows them to be characterized and distinguished from other cell groups present in SGI. Analysis of the neurons which project to the contralateral reticular formation reveals an isodendritic population of cells which vary in the size and orientation of their dendritic fields. However, the dendritic fields of these neurons are confined to a band which occupies the deeper portion of SGI and, ventrolaterally, extends into the adjacent parts of the intermediate white (SAI) and deep grey (SGP) layers. Within SGI, this band corresponds to a sublamina that can be distinguished by its relatively dense cell population and by its modest number of myelinated fibers, which are oriented primarily in a rostral-caudal direction. One important input to SGI arises in substantia nigra pars reticulata. In order to characterize this input we have filled axons anterogradely by injecting HRP with saponin into substantia nigra. Following injections into the pars reticulata a dense plexus of homogeneously filled axons can be traced to the deep sublamina of SGI. These nigrotectal axons run for long distances within the sublamina. They branch occasionally and frequently exhibit enlargements along their course; but they seldom produce sprays of terminal boutons. The plexus of nigrotectal axons also crosses into SAI and SGP in a band which overlaps precisely the ventrolateral extension of the strip of backfilled cells. This close correlation between axonal arborizations and dendritic fields suggests that a special relationship exists between a major "motor" input to the superior colliculus and the cells which give rise to the crossed descending output to pontine gaze centers (supported by NS-09623).

251.7 SUBCORTICAL AFFERENTS TO THE MONKEY SUPERIOR COLLICULUS.

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The discharge of neurons in the intermediate layers of the superior colliculus precedes the occurrence of a saccadic eye movement, but the subcortical structures that contribute to this saccade-related activity remain unknown. Subcortical afferent connections to the superior colliculus in the Rhesus monkey were studied using the horseradish peroxidase (HRP) retrograde tracing method using a modification of the Mesulam TMB-reaction procedure. Injections were made in three awake monkeys using electrophysiological recording and stimulation to localize cells with eye movement-related activity. The large injections (0.4 ul 50% HRP, Sigma VI) in two monkeys were centered in the intermediate layers but also involved significant spread into the superficial and deep layers. In both cases numerous HRP-filled neurons were located ipsilaterally in the antero-lateral portion of the substantia nigra (pars reticulata), the parabigeminal nucleus, and in the contralateral intermediate and deep colliculus layers. In addition, both cases also contained a few labeled cells bilaterally, in the mesencephalic reticular formation and ipsilaterally, in the nucleus reticularis pontis oralis.

In addition to these structures that contained labeled cells, other structures contained cells in only one of the two cases. In one case, the injection site involved the antero-medial portion of the superior colliculus and spread into the nucleus of the posterior commissure. In this case, numerous HRP-filled cells were seen in the contralateral nucleus of the posterior commissure and in a region corresponding to the rostral interstitial nucleus of Cajal, below the fasciculus retroflexus and nucleus parafascicularis adjacent to the central gray. In the other case, with a lateral and posterior injection that spread into regions near the parabigeminal nucleus and inferior colliculus, HRP-filled cells were seen in the ventral lateral geniculate nucleus, zona incerta, and nucleus of the brachium of the inferior colliculus.

In a third monkey the injection was restricted to the superficial layers. In this case, HRP-labeled cells were not seen in either the substantia nigra or the parabigeminal nucleus.

Previous anatomical studies, in the cat, have found that numerous subcortical structures project to the superior colliculus. However, these preliminary results suggest that, in the monkey, the most prominent subcortical inputs to the colliculus arise from two midbrain structures: the substantia nigra (pars reticulata) and the parabigeminal nucleus.

251.8 ANATOMICAL CONNECTIONS OF A PORTION OF THE DORSOLATERAL MESENCEPHALIC RETICULAR FORMATION OF THE MONKEY ASSOCIATED WITH HORIZONTAL SACCADIC EYE MOVEMENTS.

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Anatomic connections of a region of the dorsolateral mesencephalic reticular formation (MRF) in monkeys were assessed with axonal transport techniques. This region, which roughly corresponds to nucleus cuneiformis in humans (Olszewski & Baxter, 1954), is believed to be related to generation of visually-guided and spontaneous saccadic eye movements to the contralateral side. Radioactive amino acids, ^{3}H-Leu and ^{3}H-Pro, were used to trace the efferent projections. ^{125}I wheat germ agglutinin (WGA) and HRP were used to demonstrate afferent pathways. Sites of injection were localized by recording activity of single units associated with saccadic eye movements or by electrically microstimulating the MRF to produce characteristic contralateral saccades. Efferent projections from this region go to both the ipsilateral and contralateral superior colliculus. Fibers cross to the contralateral side in the commissure of the superior colliculus. Only the deep layers of the superior colliculus contain the typical silver grain pattern indicative of axon terminals. Other structures receiving an afferent input from dorsolateral MRF include the intralaminar nuclei of the thalamus (MD pm, Olszewski, 1952), nucleus reticularis tegmenti pontis, and both medial and lateral regions of the pontine reticular formation including the raphe nuclei. Fiber projections to the contralateral pontine reticular formation cross the brainstem under rostral portions of the oculomotor nuclei. These findings in the monkey are in general agreement with those of Edwards and deOlmos (1975, 1976) in the cat.

Afferent projections to dorsolateral MRF labelled with HRP were demonstrated from discrete groups of cells in the deep layers of the ipsilateral superior colliculus. Evidence that uptake was at least in part due to incorporation of HRP by axon terminals comes from additional experiments in which ^{3}H amino acids were injected into the superior colliculus. This gave rise to a silver grain distribution typical of axon terminals in dorsolateral MRF. Double injections of HRP and WGA were made at different locations in the dorsolateral MRF of individual animals to determine if there was a differential projection of cells from superior colliculus to dorsolateral MRF.

The results show that dorsolateral MRF has the appropriate connections so that it could participate in producing saccadic eye movements.

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251.9 PROJECTIONS FROM THE VESTIBULAR TO ABDUCENS NUCLEI AS REVEALED BY SPIKE TRIGGERED AVERAGING. Charles A. Scudder* and Albert F. Fuchs. Dept. of Physiology and Biophysics, University of Washington Seattle, WA 98195

Projections of cells in the monkey vestibular nucleus (VN) onto abducens motoneurons were examined with the method of spike triggered averaging. Two EMG electrodes, designed to sample from many motor units, were chronically implanted in the left lateral rectus (LR) muscle. Recordings from vestibular neurons were obtained from two alert, behaving monkeys using standard chronic recording techniques. Extracellular action potentials were used to trigger a computer of average transients to obtain post spike averages of LR-EMG activity.

Cells were identified on the basis of their firing patterns while the monkey was either (1) smoothly pursuing a spot of light (2) suppressing the VOR, or (3) fixating a spot stationary in space while undergoing head oscillation. Of 130 cells recorded in the right (contralateral) VN, only Type I Tonic-Vestibular-Pause cells (TVP) (N=25) consistently and reliably yielded averages. These cells were characterized by increased firing for right head velocity, for left eye movements, and by a pause for right saccades. Of the remaining cells which yielded averages (N=9), the majority had some left eye movement sensitivity. Units with head velocity sensitivity but no eye movement sensitivity (N=30) consistently failed to yield averages, although very weak connections might not be revealed using this technique. Nearly all the averages were of the same polarity (i.e., negative) as EMG averages triggered from left (ipsilateral) abducens motorneuron spikes, but of longer latency (1.5 as opposed to 1.0 msec), and longer duration. We conclude that Type I-TVP cells make powerfull monosynaptic excitatory connections onto contralateral abducens motorneurons and constitute the dominant crossed input from the VN.

We had very little success in averaging EMG activity triggered from TVP or any other cells in the left (ipsilateral) VN. At present we are uncertain if these negative findings reflect weaker or fewer projections from the ipsilateral VN, or whether they reflect a limitation of the technique in detecting inhibitory connections. Left burst-tonics, which might be expected to excite left abducens neurons, also failed to yield averages.

251.10 EM DEMONSTRATION OF INTRACELLULAR HRP-LABELLED ASCENDING TRACT OF DEITERS NEURONS SYNAPSING WITH MEDIAL RECTUS MOTONEURONS. C.H. Markham, N. Furuya and I.J. Bak. Department of Neurology, UCLA School of Medicine, Los Angeles, CA 90024.

This presentation was designed to answer the question whether or not ascending tract of Deiters (ATD) unilateral termination axons are premotor neurons to ipsilateral medial rectus (MR) motoneurons. Horseradish peroxidase (HRP) was iontophoretically injected into ATD axons which were recorded in the MR motoneuron pool of the oculomotor nucleus. Criteria for the axons were:

1. They showed type I responses to horizontal rotation, monosynaptic responses on ipsilateral labyrinth, and no response on contralateral labyrinth or contralateral abducens nucleus stimulation.

2. Light microscopic examination showed the main stem axons to be in the ATD, and terminal boutons were in contact with ipsilateral identified MR motoneurons.

The electron micrographs showed the HRP-injected ATD axons have synapses on MR motoneurons which were identified by prior injection with HRP. ATD boutons made axosomatic and axodendritic synapses on MR motoneurons. The synapses showed symmetrical structure and contained numerous spherical synaptic vesicles. There were different appearances between HRP taken up retrogradely and transported to cell bodies and proximal dendrites and HRP injected directly into axons and transported anterogradely to nerve terminals. The former appeared as granules 50-500 nm in diameter while the latter appeared as fine electron dense particles precipitated in the axoplasm and sometimes adhering to vesicles and other organelles.

In conclusion, unilateral ATD neurons are premotor neurons to ipsilateral medial rectus motoneurons.

251.11 NEURONS OF THE NUCLEUS OF EDINGER-WESTPHAL ARE THE SOURCE OF ENKEPHALINERGIC AND SUBSTANCE P-CONTAINING TERMINALS IN THE AVIAN CILIARY GANGLION. Jonathan T. Erichsen, Anton Reiner, John B. Cabot and Harvey J. Karten. Dept. of Neurobiology and Behavior, SUNY, Stony Brook, New York 11794

Substance P-like and enkephalin-like immunoreactivity have been localized to preganglionic axons and terminal endings upon both ciliary and choroid neurons in the avian ciliary ganglion (Karten et al., Neurosci., submitted). Since the nucleus of Edinger-Westphal (EW) projects to the ciliary ganglion in birds (Narayanan & Narayanan, JCN 166:101, 1976), we investigated the possibility that EW is the source of these peptidergic terminals.

Using immunohistochemical techniques with antisera directed against either substance P (SP) or leucine-enkephalin (ENK), no SP-like or ENK-like immunoreactive cells were observed in EW of the normal pigeon. When the birds were pretreated with colchicine, however, both SP- and ENK-containing cells were found. In order to determine whether these cells are the source of the immunoreactive terminals in the ciliary ganglion, an electrode (#00 insect pins, epoxy-insulated to within 100μm of the tip) was localized to EW using stimulation and stereotaxic techniques. Electrodes were considered to be optimally centered within EW when short trains of cathodal, constant current pulses (.5msec pulse duration, 25-100μa, 100Hz, .25-1.0sec train duration) elicited both pupillary constriction and increases in the accommodative state of the eye. Subsequently, discrete unilateral lesions (1ma, DC anodal current, 30msec duration) were made. Following almost total destruction of EW on one side, nearly complete elimination of SP-like and ENK-like immunoreactivity was observed in the terminal endings around both the choroid and ciliary neurons in the ipsilateral ciliary ganglion.

These results indicate that the SP- and ENK-containing neurons in EW are the source of the SP-like and ENK-like immunoreactive terminal endings in the avian ciliary ganglion. Since the neurons of EW are known to be the source of cholinergic input to the ciliary ganglion (a parasympathetic ganglion), these results suggest that a peptide (SP or ENK) may coexist with acetylcholine in some preganglionic terminal endings within the ciliary ganglion. The possible coexistence of both a peptide and an established neurotransmitter in the same terminal ending implies that SP and ENK may play an ancillary role in neurotransmission in the ciliary ganglion. This research was supported by EY 07039 to J.T.E., NS 16857 to A.R., HL 24103 to J.B.C. and EY 02146 to H.J.K.

252.1 RELATION OF PUTAMEN NEURONAL DISCHARGE TO DIRECTION OF MOVEMENT OR PATTERN OF MUSCULAR ACTIVITY. M.D. Crutcher and M.R. DeLong, Depts. of Physiology and Neurology, Johns Hopkins Univ. Sch. Med., Baltimore, Md. 21205

Previous studies of the relation of neural activity to movement parameters have shown that neural activity within the basal ganglia is best correlated withdirection of movement (Georgopoulos and DeLong, Can. J. Neurol. Sci., 1979). The purpose of this study was to determine whether the activity of neurons in the putamen (the portion of the striatum receiving inputs from the motor and sensory cortices) is related to the direction of movement per se or to the underlying pattern of muscular activity.

Two rhesus monkeys were trained on a visuomotor tracking task. The arm was restrained to allow only flexion or extension movement at the elbow in the horizontal plane. Animals were required first to position and hold the arm within a center window indicated by a target light. After a variable hold time, different magnitudes of sustained flexor or extensor loads were randomly applied to the limb via a torque motor coupled to the manipulandum. After repositioning the handle within the center window and holding for a variable period against the load, the target light was instantaneously moved to the right or left. The animal was required to make a rapid elbow extension or flexion movement with assisting or opposing loads in order to realign the handle with the target light. This paradigm thus dissociated the direction of movement from the pattern of muscular activity required to perform the movement.

Most (128/134) cells in the arm area of the putamen responded to load application: 46% (59/128) of these showed short-latency "sensory" (25-50 msec) responses. 114 of 128 cells (89%) had different patterns of response to the two directions of load application. Only 46 of 138 cells (33%) showed tonic load effects as the animal maintained the arm stationary against the sustained loads and most of these static load effects were weak. During the step portion of the task 120 of 138 cells tested showed consistent changes in activity: of these 53% were primarily related to the direction of movement. The EMG's of the prime movers of the elbow, as well as more proximal muscles, showed a characteristic pattern of activity in the task. 13% (15/120) of cells in the putamen had a pattern of activity similar to that of muscles. 42 cells showed patterns of activity different from both the direction and muscle models. These results indicate that neuronal activity in the putamen is predominantly related to the direction of passive and/or active limb movement rather than to the pattern of muscular activity.

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252.2 ACTIVITY OF NEURONS IN THE PUTAMEN DURING MOVEMENT AND POSTURAL FIXATION OF THE ARM AGAINST EXTERNAL LOADS. Samuel L. Liles. Department of Physiology, Louisiana State University Medical Center, New Orleans, LA. 70119.

Monkeys were trained to grasp a manipulandum attached to a torque motor and move it by flexion or extension of the elbow. The animal initiated a trial by positioning the lever in a 1 cm-wide center zone (indicated by a white light) for a variable period of 2 to 4 sec. When a red or green stimulus light appeared, the animal was required to move the lever (extend or flex the elbow, respectively) into an adjacent 1 cm-wide zone 4 cm from the initial zone. A drop of fruit juice was delivered after the animal maintained this position for 1.5 - 2.5s. The animals were trained to perform this task under 5 static load conditions: zero load and 125 g or 250 g opposing flexion or extension. Additionally, a single torque pulse 20 msec in duration (same polarity as static load) was delivered to the lever 1-2s before the light stimulus in each trial. This stimulus elicited a 20 msec-latency discharge in the triceps or biceps muscle. After training, the animals were prepared for single unit recording after the technique of Evarts (1968).

The present sample consists of 28 task-related neurons which were adequately isolated for the required testing period (20-30 min, 32-64 trials per load condition). During the most favorable loading condition 57% of the cells exhibited a phasic burst of discharges during arm movement (mean burst frequency = 25.6 Hz, range 8.4 - 64.6 Hz) and a lower stable discharge rate (mean = 11.9 Hz, range 1.5 - 38.6 Hz) during postural fixation. The sensitivity of the load relation was significantly greater for phasic discharges during movement (mean slope = 3.03 impulses $\cdot s^{-1} \cdot N^{-1}$) than tonic discharges (mean slope = 1.22 impulses $\cdot s^{-1} \cdot N^{-1}$) during postural fixation. The onset latency of phasic activity preceded the onset of force increase during movement by an average of 68.7 msec ($p < 0.01$). In 26% of the sample, the discharge rate during postural fixation gradually increased during the holding periods of the task (ramp pattern). Some of these cells also showed a phasic discharge during movement. The remaining cells (13%) showed only tonic discharge activity (no phasic increases during movement). None of the above 28 cells responded to the torque pulse stimuli, although an additional 10 cells not related to the movement or positioning tasks did respond (latency = 60-100 ms). These data suggest that some neurons in the putamen code force signals generated by central programs during movement and to a lesser extent force exerted during postural fixation of the arm, but do not receive proprioceptive inputs. (Supported by N.I.H. Grant NS 15485).

252.3 RESPONSES OF STRIATAL NEURONS IN THE BEHAVING MONKEY: INFLUENCE OF DOPAMINE. E.T.Rolls, S.J.Thorpe*, D.I.Perrett*, M.Boytim*, F.A.W.Wilson* and I.Szabo*. Dept. Exptl. Psychol, Oxford University, Oxford, U.K.

To analyse the role of dopamine in striatal function, the normal responses of single neurons in different regions of the striatum of the behaving rhesus monkey were analysed, and the effect which dopamine has on these responses was determined. In the tail of the caudate nucleus and ventral putamen, which receive projections from the inferior temporal visual cortex (ITC), visual responses with latencies of 90-140ms were found. These responses were often related to physical characteristics of the stimuli such as orientation, color or size, but unlike responses in ITC, typically habituated rapidly if the stimuli were repeated. In a part of the putamen which receives from sensorimotor cortex, neurons were found with responses which preceded lick and arm movements. By contrast, in the head of the caudate nucleus, which receives from the prefrontal cortex, few units with these types of sensory or motor response were found. However, many units (27.1% in one sample of 394 neurons) responded when environmental events occurred which were cues for the initiation of behavioral responses, in for example a visual discrimination task to obtain food.

The influence of dopamine on these normal responses was determined by micro-iontophoretic application, which ensures that any effects obtained are not due to altered behavior produced by drug administration. In two monkeys, of 179 neurons influenced by dopamine, all showed a decrease and none an increase of spontaneous firing rate when dopamine was applied. In all the 40 cases in which it was possible to determine the effect of dopamine on the normal responses of striatal neurons, it was found that the dopamine reduced not only the spontaneous activity of the neuron, but also the magnitude of its response related to a cue or movement.

Two conclusions follow. First, neurons in different regions of the striatum have responses which reflect the activity in the region of cortex from which they receive. Second, dopamine, by reducing the magnitude of these striatal responses, must influence the transmission of this information from the cortex on to output structures of the striatum such as the globus pallidus.

252.4 PALLIDAL NEURONAL ACTIVITY AND THE CONTROL OF HEAD MOVEMENT IN CATS. J.S. Schneider, J.R. Morse and T.I.Lidsky, SUNY at Stony Brook, N. Y. The disruption of eating and drinking caused by globus pallidus (GP) lesions indicates a role for the GP in oro-ingestive behaviors. In order to understand the nature of this role, an assessment was made of the relationship between GP activity and some of the sensory and motor events which are involved in ingestion.

Units were recorded from behaving cats. Animals were partially restrained in a device which allowed horizontal rotational head turning as well as jaw and tongue movements. Von Frey hairs, blunt probes and an electromechanical device (that produced events of reproducible magnitude and duration) were used to present cutaneous oro-facial stimulation.

42% of GP units (160 sampled) were affected by oro-facial stimulation. Receptive fields were typically large and bilateral (49%) or contralateral (43%). The majority of receptive fields (43%) were from areas innervated by one branch of the trigeminal nerve (in every case the maxillary) though many fields were subserved by two (22%) or all three branches (35%).

Further analysis, directed at assessing the informational content of these sensory responses, indicated that GP units have an extremely limited ability to encode stimulus force. However, stimulus location was encoded. Stimulation within peri-oral zones was more likely to evoke a GP response than was stimulation applied further from the mouth. The majority of receptive fields (75%) had components which included peri-oral tissue. Half of the units which were affected by deflection of facial hair showed enhanced responding to stimuli moving in a particular direction. Of these units, 82% responded best to stimuli moving toward the mouth.

15% of GP cells (68 sampled) changed firing rate in relation to head movements. 37% of these movement-related cells changed firing rate prior to onset of neck muscle activity. Mean lead time was 433 msec though considerable variability was seen with successive movements (standard deviation = 392). The majority of these units (55%) fired best in relation to contralaterally directed head movements. The remaining neurons showed no directional preference. Other parameters of movement (e.g. velocity, magnitude) were not reflected in GP activity. None of the 86 neurons observed during ingestive activity were related to jaw, tongue or facial movements.

These data suggest that the GP's role in ingestive processes does not involve direct control of consummatory movements. Rather, the GP seems to be concerned with the head positioning movements which enable subsequent jaw and tongue movements.

252.5 SOMATOSENSORY PROCESSING IN THE ENTOPEDUNCULAR NUCLEUS OF CATS
J. R. Morse, J. S. Schneider and T. I. Lidsky, SUNY at Stony Brook, Stony Brook, N. Y. 11794.

As part of an ongoing series of experiments investigating the sensory-motor nature of basal ganglia function, neuronal responses to sensory stimuli were recorded from the entopeduncular nucleus in awake, restrained cats. Trigeminal sensory fields were explored since the basal ganglia have been implicated in oro-facial control. Reproducible punctate stimuli were applied to the face using an electromechanical stimulator fitted with calibrated Von Frey hairs. Moving stimuli were applied using a hand held probe. Of the entopeduncular units sampled to date, 21% had facial sensory fields most of which were contralateral or bilateral. These sensory fields were generally large and 77% of them included areas innervated by at least two trigeminal divisions. Both punctate and moving stimuli evoked responses from 55% of responding neurons. The remaining 45% were activated only by stroking and most of these were directionally sensitive, responding to stimuli moving toward the mouth. Cells responsive to punctate stimulation encoded force only to a very limited extent and responses within their receptive fields were heterogeneous. This kind of sensory information could be useful in controlling a variety of movements involved in prey killing and ingestion, including locating food objects and moving them toward the mouth.
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252.6 MOTOR AND SOMATIC SENSORY CORRELATES IN THE STRIATUM OF THE AWAKE RAT.
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The aims of this study were to determine the distribution, degree of specificity, and degree of convergence of motor and somatic sensory properties onto single striatal cells. Single units were recorded in the striatum of awake, behaving rats at locations which previous anatomical studies have shown to receive afferents from motor and/or somatosensory cortex. Free observation, film analysis and computer techniques were used to quantify motor and somatic sensory correlates. Cells in the quietly resting rat had very low spontaneous activity (up to 60 sec interspike intervals) but generally discharged during either spontaneous movement or orientation responses elicited by novel sensory stimuli.

Motor correlates were determined by using a computer to generate peri-event histograms (PEH's) of unit activity around limb, body and neck movements seen in frame-by-frame analysis of video recordings of rats during various behaviors. Nearly all striatal units examined were correlated with gross postural movements, but many could be more specifically correlated with a discrete movement such as head rotation, sniffing or movement of a single limb. The strongest correlate observed was with neck movements especially if they resulted in a change of orientation of the body axis in space. PEH's showed increases in unit activity up to 200 msec preceding the initiation of both ipsi- and contraversive turning. The firing was inhibited at the end of movement.

Cutaneous sensory receptive fields (RF's) were analyzed for these same cells by compilation of post-stimulus time histograms (PSTH's) to natural touch with a probe or to electrical stimulation through electrodes surgically implanted in the paws. Latencies of response to such stimulation ranged from 15 to 50 msec. Nearly 2/3 of the units had discernable cutaneous RF's. About half of these covered the whole body or contralateral half body and thus could be considered relatively "nonspecific". The rest were more specific, being restricted to the contralateral fore- or hindlimb. As yet no overall somatotopic organization of these correlates has been discerned. Striatal unit responses to peripheral stimulation were greater when the animal was aroused and oriented to the stimulus. Natural touch from a probe held by the experimenter was more effective in eliciting striatal unit responses than stimulation through implanted electrodes even when the later was sufficient to elicit limb flexion.

In summary, both motor and somatic sensory properties converged on single cells in all striatal areas tested. Our initial hypothesis is that the expression of both of these properties is predominantly seen during behaviors associated with reorientation of attention. Supported by NIAAA AA-0390 and the Biological Humanics Foundation.

252.7 ATTENUATION OF SENSORY EVOKED IPSP'S IN CAUDATE FOLLOWING BILATERAL ROSTRAL CORTICAL ABLATION.
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The purpose of this research was to study the effects of rostral cortical ablation on the sensory evoked postsynaptic potentials (PSP's) in the head of caudate nucleus (Cd). All cortex (Cx) rostral of the lateral ventricles was removed bilaterally by suction. Additionally, all Cx rostral of the ansate sulcus, extending medially to the midline and ventrolaterally to the anterior rhinal sulcus, was removed to a depth of 3-5mm. Mild electrical shocks to either forepaw and "clicks" served as somatosensory (SS) and auditory (Aud) stimuli, respectively. Intracellular recordings were made in three groups of cats: intact cats (N=25), 0-9 days postlesion cats (N=6) and 28-45 days postlesion cats (N=4). In intact cats, 97% of Aud and SS stimuli evoked intracellular responses in Cd neurons. Of these responses, 84% consisted of initial excitation (E) followed by inhibition (I), i.e. an E-I response. 100% of the responses had an I component. In the 0-9 days postlesion cats, only 68% of SS and 53% of Aud stimulation trials evoked responses. Only 36% of the responses were E-I and only 45% had an I component. In the 28-45 days postlesion cats, 86% of the SS and 78% of the Aud stimulation trials evoked PSP's. 72% of the responses were E-I's. 80% of the responses had an I component. Although these data suggest a partial recovery in responsiveness in the long-term survival animals, less than 5% of responses of this group had I's with amplitudes $\geq \bar{X}$ I amplitude of intact animals. In both the long- and short-term survival groups, the $\bar{X}$ I amplitude was significantly smaller ($p < 0.001$) than the $\bar{X}$ I amplitude of intact animals. In contrast to the I's, the initial E's of the responsive neurons were not greatly different for the three groups of animals. To test whether the attenuation of the postlesion sensory evoked I's might be due to non-specific changes in the CNS, the intralaminar thalamus (a direct, intact pathway to Cd) was stimulated. Postlesion, thalamic stimulation evoked E-I response sequences with amplitudes consistent with intact animals. Our data suggest that important components of Cd's responses to SS and Aud stimuli are mediated through rostral Cx. In particular, sensory stimulation was less effective in evoking I responses when rostral Cx was ablated.
Supported by HD 05958 and HD 07032.

252.8 METHYLPHENIDATE (MPH): (1) ABSENCE OF READINESS POTENTIAL IN PATIENTS WITH PARKINSON'S DISEASE AND IN PATIENTS FOLLOWING LONG-TERM MPH TREATMENT.
Boop, R.*, Garcia-Rill, E., Skinner, R. D., Dykman, R.* and Peters, J.* (Spon: J. Spyker-Cranmer), Depts. of Anatomy and Psychiatry, Univ. of Arkansas, Little Rock, AR 72205.

The Readiness Potential (RP) is a negative DC shift recorded from the scalp and beginning 600-800 ms prior to an uncued, voluntary movement. This wave may be a measure of the viability of the neurological substrates involved in the preparation for movement. Others have shown that patients with Parkinsonism have no appreciable RP. These patients also are known to lack striatal dopamine (DA) and exhibit deficits in the initiation of movement. We recorded the RP in a population of Parkinson's patients and confirmed the absence of a RP. An age-matched normal population was also studied which demonstrated a reduced amplitude of the RP, confirming an age-related decrease in the amplitude of the RP in the normal population. A small group of Parkinson's patients also were studied following thalamotomy for the treatment of tremor. The RP was not restored by the surgery although tremor was reduced or eliminated.

From another line of evidence, it has been shown that long-term depletion of striatal DA may occur following amphetamine administration. Our preliminary findings indicate that Methylphenidate (MPH) administration also may lead to a reduction of striatal DA in animals (this meeting). For these reasons we recorded the RP in a population of young adults (20-23 yrs) with a history of at least 5 years of MPH treatment for "hyperactivity". A group of age-matched normal individuals also was studied. Normal young adults had a peak amplitude of 5μv compared to 3μv for the older adult group (age-matched for Parkinson's patients). No member of the Parkinsonism or the MPH-treated group exhibited a RP. Some of the latter group were not currently under MPH treatment. It has yet to be determined if children with "hyperactivity" exhibit a RP. That is, whether the absence of RP is due to the condition of "hyperactivity" itself or whether it is due to its treatment with MPH.

These data, combined with our animal studies, indicate that MPH administration can lead to a reduction in striatal DA, and suggest that MPH treatment could compromise the neurological substrates involved in the generation of the RP. If this is the case, following long-term MPH treatment, these patients may be predisposed to a Parkinson-like syndrome.
Supported by USPHS grants NS-15359 and NS-16143.

252.9 METHYLPHENIDATE (MPH): (2) REDUCTION OF STRIATAL DOPAMINE AND COPPER CONCENTRATIONS FOLLOWING MPH TREATMENT IN CATS. Skinner, R. D., Garcia-Rill, E., DeLuca, D.* and Sorenson, J.*, Depts. of Anatomy, Biochemistry and Pharmacy, Univ. of Arkansas, Little Rock, AR 72205.

Recent findings from other laboratories have described long-term decreases in the concentration of striatal dopamine (DA) in the rat and cat following administration of amphetamine. Methylphenidate (MPH) is pharmacologically similar to amphetamine but its effects on striatal DA are unknown despite widespread clinical use. Intraperitoneal injections of MPH (5 mg/Kg) were administered to two groups of cats every two days. One group received a total of three injections and the other received a total of twelve injections. Behavioral effects were very similar to those reported following amphetamine administration. Two groups of control animals were given the appropriate number of vehicle injections. Two weeks after the last injection the animals were sacrificed in two ways.

For DA analysis, the cats were barbiturate anesthetized, the brains removed, the caudate nuclei dissected and frozen in liquid nitrogen within one minute. A radioenzymatic assay was used to determine DA concentration in control and MPH-treated groups.

For copper (Cu) analysis, anesthesia was induced with halothane, then a locally anesthetized, paralyzed preparation made before perfusing with metal-free saline and metal-free formalin. Thirteen different brain regions were dissected and the samples analyzed by atomic absorption spectrophotometry in control and MPH-treated groups.

Our results indicate that MPH induced a 30% decrease in striatal DA following each type of treatment. Striatal Cu was reduced by 25% and 40% after 3 and 12 injections, respectively. Other areas of the brain also suffered significant decreases in Cu (substantia nigra 25% and 40%, respectively; thalamus 30% and 50%, respectively; etc.).

These preliminary findings indicate that short-term, high-dose MPH-treatment can induce a reduction of DA and Cu in the striatum. Current studies will determine if long-term, low-dose MPH-treatment will have the same effects. Although the dose used in this study is several times the usual clinical dose, it is within the limits of MPH abuse.

When these findings are taken together with our results demonstrating an absence of Readiness Potential in patients with a history of long-term MPH treatment (this meeting), the implication exists that long-term MPH treatment or abuse could predispose subjects to a Parkinson-like syndrome.

Supported by USPHS grants NS-15359 and NS-16143.

252.10 NEUROCHEMICAL CORRELATES OF ESTROGEN INDUCED HYPER- AND HYPO-SENSITIVITY TO DOPAMINE. K. O. Perry*, J. C. Curtin and J. H. Gordon (SPON: C. M. Combs). Dept. of Pharmacol., The Chicago Med. School, N. Chicago, IL. 60064.

The administration of high doses (100ug/kg) of estradiol benzoate (EB) for 3 days in ovariectomized (OVX) rats results in a suppression of apomorphine induced stereotypy at 24 hrs after the last dose of EB. In subsequent tests (48, 72, 96 hrs after the last dose of EB) the animals are hypersensitive to the behavioral effects of apomorphine. The experiments reported here evaluate several neurochemical parameters in both the hyposensitive (24 hrs after lase dose of EB) and the hypersensitive(72 hrs after last dose of EB) animals.

The hypersensitive animals displayed several neurochemical alterations in dopamine function and/or activity. The tyrosine hydroxylase (TH) activity in striatal homogenates was decreased by 16 % in the hypersensitive animals. The TH activity in striatal synaptosomal preparations was decreased by 36 %. The ability of apomorphine to inhibit TH activity in striatal synaptosomal preparations was also increased, EC_{50} = 0.55 + 0.06 uM for the hypersensitive animals and 0.90+0.09uM for the control animals. Coincident with the decreases in TH activity was a decrease (28 %) in glutamic acid decarboxylase (GAD) activity in the substantia nigra (SN) of the hypersensitive animals. for

The hyposensitive animals were not different from contols in either the TH activity or the efficacy of apomorphine to inhibit TH activity in synaptosomes. However the GAD activity in the SN was significantly decreased in the hyposensitive animals. The behavioral response to apomorphine and preliminary binding data utilizing ^{3}H-spiroperidol as a ligand are indicative of a decreased postsynaptic sensitivity in the hyposensitive animals. The decrease in the postsynaptic efficacy of dopamine may be responsible for the decreased GAD activity seen in these animals (i.e. a decreased activity of dopamine at postsynaptic sites could result in a compensatory release of GABA inhibitory feedback). The hypersensitive animals appear to be hypersensitive postsynaptically, as reflected by the increased GAD activity in the SN (i.e. increased negative feedback to decrease dopamine release). This compensatory increase in the GABA feedback may be reflected by the decreased TH activity and/or the increased presynaptic sensitivity (as measured by apomorphine inhibition of synaptosomal TH activity). Thus it appears that the postsynaptic supersensitivity, induced by "withdrawal" from EB, may result in a compensatory presynaptic supersensitivity. This may account for the maintenance of the hypersenitivity seen in the OVX animal. Because an increase in presynaptic sensitivity would result in a decreased dopamine release, which would decrease any down regulation of pre- or post-synaptic receptors by endogenous dopamine. (These studies were supported in part by NIMH grant MH33991).

252.11 NEUROCHEMICAL CORRELATES OF HYPOPHYSECTOMY-INDUCED STRIATAL DOPAMINE SUPERSENTIVITY AND N. ACCUMBENS HYPOSENSTIVITY. J. H. Gordon and V. L. Radice*, Dept. of Pharmacol. The Chicago Med. School, N. Chicago, IL. 60064.

Seven to 9 days post-hypophysectomy (HYPOX) female rats are hyposensitive to the locomotor and hypersensitive to the stereotypy (i.e. sniffing, licking, gnawing) effects of apomorphine (APO). At one month post-HYPOX these animals were hypersensitive to both the locomotor and the stereotypy effects of APO.

Both the tyrosine hydroxylase (TH) activity in n. accumbens (ACB) and glutamic acid decarboxylase (GAD) activity in the ventral tegmental region (VTR) were significantly increased in the 7 day post-HYPOX animals relative to sham operated controls. At 29 days post-HYPOX both the TH activity in the ACB and the GAD activity in the VTR were decreased. Thus the hyposensitivity to the locomotor effects of APO is associated with an increase in TH activity and the hypersensitivity is associated with a decrease in TH activity in the ACB. The changes seen in GAD activity do not support the proposed inhibitory nature of GABA in the VTR, because with an increased TH activity the model would predict a corrisponding decrease in GAD activity, and this is not the case for the HYPOX animals.

The TH activity in the striatum (STR) and the GAD activity in the substantia nigra (SN) were decreased at both 7 and 29 days post-HYPOX This decreased TH activity appeared to be associated with the increased sensitivity to the stereotypy effects of APO. Coincident with the decreased STR TH activity and increased APO efficacy was an increased ^{3}H-spiroperidol binding to STR membranes (38% increase in B_{max} with no change in affinity).

The behavioral and neurochemical data support the hypothesis that following HYPOX female rats will develop a transient decrease in ACB dopamine sensitivity while the STR dopamine sensitivity is increased at this time. Very few experimental manipulations offer the advantages contained in the HYPOX animal (i.e. simultaneous mesolimbic hyposensitivity and STR hypersensitivity), thus the HYPOX animal may prove beneficial in studing the roles of these two dopaminergic systems in modulating various behaviors. (These studies were supported in part by NIMH grant MH33991).

252.12 HYPOPHYSECTOMY INDUCED STRIATAL DOPAMINE SUPERSENITIVITY IN MALE RATS: ANTAGONISM BY PROLACTIN, EXACERBATION BY CHRONIC HALOPERIDOL. B. I. Diamond and J. H. Gordon Dept. Anesthes., Mt. Sinai Hosp., Chicago, Il. and Dept. Pharmacol. The Chicago Med. School, N. Chicago, IL.

Recent reports (Hruska et al., Eur. J. Pharm. 65:455-456, 1980) have suggested that hypophysectomy (HYPOX) in male rats would antagonize the development of striatal dopamine supersensitivity, induced by chronic administration and withdrawal of haloperidol (Haldol). Conversely we have reported (Perry et al., Soc. Neurosci. Abstrs. 759, 1980) that HYPOX produced striatal dopamine supersensitivity in female rats, which could be enhanced by chronic administration of Haldol. These two reports would appear to indicate a profound sexually dimorphic response to HYPOX, to test for this possibility we have repeated our studies on apomorphine-induced stereotypy in HYPOX male rats.

HYPOX or sham operated (SHAM) male rats (180-200 gm) were purchased from Hormone Assay Labs, Chicago. Groups of 8 animals were treated daily, for 16 days, with 1.0 mg/kg of Haldol, 1.0 mg/kg Haldol ± 1.0 mg/kg prolactin, or saline. Animals were then tested with a threshold dose of apomorphine (0.25 mg/kg, i.p.) and the resulting sterotypy scored on the 8th day of withdrawal. During the behavioral test the SHAM animals, treated with saline, displayed an increase in locomotor activity with very few observations of stereotyped sniffing (score = 1.0±0.1). The HYPOX animals treated with saline displayed a significant increase in stereotyped sniffing (score = 1.7±.2). Chronic Haldol treatment and withdrawal in the HYPOX animals resulted in a significant increase in gnawing relative to all other test groups (score = 3.7+.2). The HYPOX animals that received both Haldol and prolactin displayed a decrease in gnawing behavior, relative to the Haldol only animals (score = 2.6±0.2).

Because of the ability of prolactin to attenuate the development of the Haldol-induced striatal dopamine supersensitivity, the experiment was repeated using prolactin injections as the only variable The behavior tests (3 days after the last injection of prolactin or saline) were indicative of a HYPOX-induced striatal dopamine supersensitivity, while the prolactin treatment resulted in a significant decrease in stereotypy.

The data from these studies indicate that: 1. HYPOX in male male results in striatal dopamine supersensitivty; 2. That this supersensitivity can be antagonized by prolactin administration; 3. Chronic Haldol does not required the presence of the pituitary to induce striatal supersensitivity to dopamine, however the presence of a pituitary secretion, perhaps prolactin, can modulate striatal dopamine sensitivity. (These studies were supported in part by NIMH grant MH33991).

252.13 ACETAMINOPHEN METABOLISM IN CAT CAUDATE NUCLEUS MEASURED BY IN VIVO ELECTROCHEMISTRY. Curt R. Freed* and Bryan Yamamoto* (SPON: P. Molinoff). Departments of Medicine and Pharmacology, U. Colorado School of Medicine, Denver, Colorado 80262.

The in vivo electrochemical detector has been shown to be useful for measuring catecholamine release from caudate nucleus of rat and cat. We have demonstrated that acetaminophen can be used as an internal standard for in vivo electrode calibration because it is an electrochemically active compound that can be administered systemically, that penetrates into brain, and that can be seen as an electrochemical peak distinct from dopamine (Morgan and Freed, Soc. Neurosci. 6, 141, 1980). We have now seen evidence of acetaminophen metabolite formation in cat brain. Mongrel cats 3-4 kg were anesthetized with ketamine and a tracheostomy performed. Animals were then maintained on halothane/N_2O/O_2 anesthesia. A carbon paste electrode 250 μ was inserted into left caudate at stereotaxic position 15 mm anterior, 5 mm lateral, and 15 mm vertical relative to the interaural line. Data were recorded with cyclic voltammetry using a Bioanalytical Systems DCV-4 and output was processed by semidifferentiation. Under these conditions, dopamine oxidized at 0.2-0.3 volts relative to an Ag/AgCl electrode also implanted in brain. After signal stabilization, acetaminophen 75 mg/kg i.p. was injected. The expected peak at 0.4 volts corresponding to acetaminophen rose rapidly, reached a peak value within 30 min, and then slowly fell with a $t_{½}$ of 160 min and 587 min in two different animals. Shortly after acetaminophen injection and peak development, a second peak appeared at an oxidation potential of 0.1-0.2 volts, lower than the catechol peak. This peak rose slowly, reaching a maximum at 120 min and 250 min in the two animals described above. The magnitude of this signal was 40 to 50% of the acetaminophen peak. Several pieces of evidence suggest this is a metabolite of acetaminophen produced in brain. First, the acetaminophen administered was chemically pure so that the early oxidizing peak was not due to contaminated drug. Second, the apparent metabolite peak appeared after acetaminophen. During the drug decay phase of acetaminophen from brain, the second peak reached a constant fraction of the decreasing acetaminophen peak (27%) suggesting that the rate of synthesis and rate of elimination of the metabolite were in equilibrium with the falling acetaminophen concentration. Attempts to isolate and identify this apparent metabolite of acetaminophen are ongoing.

252.14 ESTRADIOL SUPPRESSES THEN ENHANCES INTRACAUDATE DOPAMINE-INDUCED CONTRALATERAL DEVIATION. J.N. Joyce, R.L. Smith*, and C. Van Hartesveldt. Psychology Department, University of Florida, Gainesville, Florida 32611.

Recent research has shown that estrogen can affect behaviors elicited by drugs that act on dopaminergic systems in the brain; however, it has not been clear whether estrogen enhances the effect of dopamine at the receptor, or blocks it. Problems in interpretation of research in this area include first, the fact that dopamine (DA) agonists or antagonists have been administered systemically, and estrogen might influence the rate of their metabolism; and second, that the effects of these drugs were measured at varying times after estrogen treatment. In order to resolve these points of controversy, we tested the effects of estradiol priming on the contralateral postural deviation elicited by unilateral intracaudate injection of DA at 2 and 6 days after hormone treatment.

Male Long-Evans hooded rats weighing 300-350 g were implanted bilaterally with 21 ga stainless steel guide cannulae directed at the dorsal anterior caudate nucleus (CPU). Through 27 ga injection cannulae extending 3 mm below the guide cannulae, rats were unilaterally administered first a vehicle solution consisting of sucrose in 0.1 M phosphate buffer; and four hours later, dopamine (DA, 25 μg in 0.25 μl buffer solution). Immediately after each intracaudate injection, rats were observed for 30 min in a circular chamber. Duration of time spent in ipsilateral or contralateral postural deviation was recorded. Then, either estradiol benzoate (EB, 150 μg/animal) or its vehicle (peanut oil, 0.5 cc/kg) was administered subcutaneously, and the behavioral effects of both buffered vehicle and DA injection into dorsal CPU were again measured as above 6 days later. In another group of animals, testing was carried out both 2 and 6 days after EB or peanut oil treatment.

Consistent with previous research, unilateral injection of DA into dorsal CPU induced significantly greater durations of contralateral deviation than the vehicle. Two days after EB treatment, DA injection into the CPU elicited less contralateral deviation, and 6 days later, more contralateral deviation, than in the pre-treatment test. Animals treated with sesame oil showed no changes in DA-elicited postural deviation either after 2 or 6 days; EB had no effect on postural deviation elicited by intracaudate injection of the phosphate buffer.

Both these results and those of Gordon (1980) are consistent with the interpretation that estrogen blocks the action of DA immediately, but as it leaves the system DA receptors increase in number in the CPU, resulting in increased behavioral sensitivity to DA agonists.

252.15 RAPID AVOIDANCE BEHAVIOR DEFICITS AND MOVEMENT INITIATION IN RATS AFTER 6-HYDROXYDOPAMINE MICROINJECTIONS IN SUBSTANTIA NIGRA. W. W. Spirduso,[1] R.E. Wilcox,[2] T.J. Schallert,[3] P. Gilliam,[1] D. Vaughn,[2] and M. Upchurch.[3]*Depts. of PHE,[1] Pharm.[2] and Psy.[3] Univ. of Texas, Austin, TX 78712.

Deficits in movement initiation and conditioned avoidance behavior are known to occur following bilateral 6-hydroxydopamine (6-OHDA) induced damage to the nigrostriatal dopamine pathways. In the present study unilateral microinjections of 6-OHDA (or a sham vehicle) were placed in the medial forebrain bundle, and the results from unilaterally produced escape and avoidance latencies, neurological tests, rotation tests, and unilateral striatal [^{3}H]-dopamine uptake, [^{3}H]-spiroperidol, and [^{3}H]-apomorphine receptor binding were analyzed in terms of being ipsilateral or contralateral to the lesion. These results were also compared to the behavior of control animals.

One week post-operatively, escape and avoidance movement initiation capacity was determined by measuring, over a seven day acquisition schedule, the latency (msec) to release a lever which terminated shock. A special jacket restrained either the left or right forelimb, providing simple reactive capacity latencies for each paw independently. Ipsilateral and contralateral neurological tests included tests to assess sensorimotor deficits (e.g. neglect), lateral hopping/bracing reactions, and spontaneous/apomorphine-induced movement asymmetries (e.g. rotations, slant grid performance).

The results were that unilateral 6-OHDA microinjections induced DA system damage that was associated with sensorimotor deficits, movement asymmetries, and drug-induced rotation. Movement initiation latencies, percent successful avoidance, and avoidance acquisition curves were also severely affected by the unilateral lesions.

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252.16 SYNTHESIS OF GABA IN CAUDATE-PUTAMEN AND SUBSTANTIA NIGRA IN VIVO: ALTERATIONS INDUCED BY DESTRUCTION OF NIGROSTRIATAL DOPAMINE NEURONS. M. Casu* and K. Gale (SPON: K. Dretchen). Department of Pharmacology, Georgetown University, Schools of Medicine and Dentistry, Washington, D.C. 20007.

The synthesis of GABA in vivo in caudate-putamen (CP) and substantia nigra (SN) was evaluated by examining the rate of accumulation of GABA after irreversible inhibition of GABA-transaminase (GABA-T) by gamma-vinyl-GABA (GVG). GVG was microinjected directly into SN (5ug in 0.5ul saline over 5 min) or into CP (20ug in 1.0ul saline over 10 min), and the rats were killed 1.5 and 3.0 hr after GVG. We have previously demonstrated that after intracerebral microinjection of GVG, GABA-T is rapidly inhibited (less than 5% remaining at 10 min) and GABA levels increase linearly for at least 3 hr; a significant proportion of this GABA elevation was found to be associated with nerve terminals. In rats which had received unilateral 6-hydroxydopamine (6-OHDA) lesions in the medial forebrain bundle 4 weeks before, tyrosine hydroxylase was <5% of control and the rate of accumulation of GABA was significantly greater in the CP from the 6-OHDA lesioned hemisphere than in control CP (0.40 nmol/mg prot/min vs. 0.26 nmol/mg prot/min). On the other hand, the rate of accumulation of GABA in the SN of the lesioned hemisphere was significantly reduced as compared to controls (0.41 nmol/mg prot/min vs. 0.67 nmol/mg prot/min). Since steady-state levels of GABA in SN and CP were not significantly changed by the 6-OHDA lesions, these data suggest that the alterations in rate of GABA accumulation reflect a change in GABA turnover. It appears that destruction of dopamine projections causes an increase in GABA turnover in CP and a decrease in GABA turnover in SN. This is consistent with a model in which nigrostriatal dopamine pathways exert an inhibitory influence on striatal GABA interneurons, and a disinhibitory influence on striatonigral GABA projections.

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252.17 AMPHETAMINE-INDUCED TURNING BEHAVIOR AND IN VIVO ELECTROCHEMICAL RECORDING FROM CAUDATE IN RATS WITH 6-HYDROXYDOPAMINE LESIONS OF SUBSTANTIA NIGRA. Michael E. Morgan* and Curt R. Freed*, (SPON: S. Roper). Depts. Med. and Pharm., U. Colo. Sch. Med., Denver,CO.

Rats injected with 6-hydroxydopamine (6-OHDA) unilaterally in substantia nigra are known to have dopamine (DA) and DOPAC depletion in caudate on the same side. Systemic administration of amphetamine (AMPHET) will cause animals to turn toward the side of the lesion, presumably as a result of the decreased DA release from that side. To evaluate the dynamic relationship between catechol release and turning behavior, we have studied 6-OHDA lesioned rats with electrochemical recording. Male Sprague-Dawley rats 150-200 gm had substantia nigra lesions made stereotaxically. After a recovery period of 2 to 4 weeks, animals had carbon paste 250 μ electrodes implanted bilaterally in caudate. An Ag/AgCl and a stainless steel electrode were also placed in brain. After overnight recovery, animals were placed in a circular drum and a 30 min recording of basal catechol release was made. Measurement was with a Bioanalytical Systems DCV-4 cyclic voltammetry apparatus with electrochemical output processed by semidifferention. Detectors on each side of brain were scanned alternately every 5 min over the range 0.0-0.8 volts with the peak at 0.2-0.3 volts defined as the catechol peak. Turns were monitored by an observer. After basal measurements, animals were injected with 1.5 mg/kg i.p. d-AMPHET and turns and catechol release assessed for 150 min thereafter. At the end of the experiment, relative electrode sensitivity was measured by injection of 75 mg/kg i.p. acetaminophen. Animals were sacrificed and caudate DA and DOPAC measured by HPLC with electrochemical detection. Results show that all animals had at least 80% DA depletion and 4/7 had greater than 95% depletion. Only 4/7 animals turned to the side of the lesion despite the marked DA depletion. In the animals turning appropriately, turning appeared within 5 min of AMPHET injection, reached maximum intensity at 20-30 min and then declined over a period of 90 min. By contrast, the electrochemical detector response on the unlesioned side rose and then plateaued by 90 min post-injection. Little fall-off in signal occurred over 150 min of observation. Turning intensity appears, therefore, to be related to the rate of change in the electrochemical signal observed in the caudate rather than its absolute magnitude. Electrochemical signals on lesioned and unlesioned sides were compared. Although tissue assays demonstrated 80% DA depletion and 60% DOPAC depletion, output from the lesioned side was never less than 50% of the unlesioned side. Higher than expected release from the lesioned side may be due to accelerated DA turnover in remaining neurons and to comeasurement of oxidizable substances like ascorbic acid.

252.18 EFFECTS ON DOPAMINE-MEDIATED CIRCLING BEHAVIOR OF GABA-ERGIC ACTIVITY AND NEUROTENSIN IN NUCLEUS ACCUMBENS. S.L. Hartgraves[4] and P.H. Kelly. Dept. Physiology and Biophysics, University of Southern California, Los Angeles, CA 90033.

Previous studies suggest that dopamine activity in the nucleus accumbens facilitates the amphetamine- and apomorphine- induced circling of rats with unilateral 6-hydroxydopamine (6OHDA)-induced lesions of the nigrostriatal pathway (Kelly and Moore, 1976). Since GABA-ergic mechanisms in the nucleus accumbens have been reported to inhibit dopamine-mediated locomotor activity, but to increase dopamine-mediated stereotyped behavior (Pycock & Horton, 1976; Scheel-Kruger, Cools & Van Wel, 1977) the present studies investigate the effects of GABA-ergic mechanisms in nucleus accumbens on dopamine-mediated circling. Neurotensin in the nucleus accumbens also antagonizes certain behavioral effects of amphetamine (Nemeroff, 1980), and has also been studied here.

Rats received unilateral 6OHDA (8μg of base) injections into the tail of the caudate nucleus. As assayed by the high-affinity uptake of 3H-dopamine into crude synaptosomal preparations of caudate nucleus and nucleus accumbens these injections destroy 70-80% of dopaminergic terminals in the ipsilateral caudate nucleus, without markedly damaging mesolimbic dopaminergic terminals. Bilateral microinjections of GABA (125-500μg in 1μl) into the nucleus accumbens via chronically-implanted cannulae resulted in a dose-dependent inhibition of the ipsiversive circling provoked by d-amphetamine (5mg/kg s.c.). Similar injections of muscimol (8-200 ng) into the nucleus accumbens also reduced amphetamine-induced circling. A dose of muscimol (40 ng) which markedly reduced amphetamine-induced circling less severely reduced (by 45%) the contraversive circling produced by apomorphine (1 mg/kg. s.c.). Bilateral injection of neurotensin (2μg in 1μl) into the nucleus accumbens did not significantly affect amphetamine-induced circling. These studies suggest that increased GABA-ergic activity in the nucleus accumbens exerts an inhibitory influence on circling provoked by asymmetry of nigrostriatal dopaminergic activity. At least in the dose used here neurotensin has no significant effect on this behavior.
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252.19 INTRASTRIATAL INJECTIONS OF KAINIC ACID (KA) DISRUPT THE BLOOD BRAIN BARRIER (BBB) AND CAUSE CEREBRAL EDEMA. Peter H. Cooper[1], Larry L. Butcher[1,2] and Donald Novin[1,2]. Department of Psychology[1] and Brain Research Institute[2], University of California, Los Angeles, CA 90024.

A large literature exists demonstrating that lesions which disrupt the BBB allow for extravasation of serum proteins and for the development of cerebral edema (e.g., Olsson & Hossman, Acta Neuropath. 16:103, 1970). Horseradish Peroxidase (HRP, Sigma Type II) is one of the protein tracers commonly used to assess the extent of damage to the BBB. Because KA has been used widely in attempts to destroy cell bodies without affecting fibers of passage, the present study was undertaken to determine whether KA causes extravasation of HRP. At various times after unilateral intrastriatal injection of KA, HRP (50 mg/kg) was administered intravenously to conscious unrestrained rats through indwelling jugular cannulae. The brains were subsequently examined histologically for BBB involvement.

Two days after the injection of KA there was massive leakage of HRP into the striatum. In all animals, the tracer engulfed the fascicles of corticofugal fibers traversing this structure. In some animals there was spread of the tracer to the overlying corpus callosum and the cerebral cortex. BBB breakdown also occurred in several other sites in which the distribution of HRP was not continuous with the HRP which was extravasated into the striatum. These sites included the lateral septal nucleus, portions of the hippocampus, entorhinal and pyriform cortices as well as dorsal medial thalamic structures. However, BBB breakdown did not occur consistently in all animals at all of these sites. It is possible that this extrastriatal damage is due to the epileptogenic action of KA (e.g., Ben-Ari, et al., Brain Res., 191:79, 1980). In all structures, the amount of extravasated HRP decreased over the next several weeks suggesting repair of the BBB.

The extravasation of HRP is evidence that BBB breakdown and edema result from KA administration. Because cerebral edema may functionally impair fibers of passage, the present observations should be considered when interpreting the effects of KA on behavior and brain biochemistry. Conversely, because BBB breakdown may be involved in the regeneration of axons (Kierman and Contestabile, Acta Neuropath., 51:39, 1980), these findings may also have implications for understanding the cause of the sprouting of axons in the hippocampal formation after certain types of brain damage. (Supported by USPHS Grant NS10928 to LLB.)

252.20 REGIONAL CEREBRAL BLOOD FLOW AND BLOOD-BRAIN BARRIER IN RATS WITH SUBSTANTIA NIGRA LESIONS: EFFECTS OF APOMORPHINE AND AMPHETAMINE. D.K. Zucker and G.F. Wooten; Depts. Neurology and Pharmacol., Wash. Univ. Sch. Med., St. Louis, MO 63110

Regional cerebral glucose utilization (GU) was increased in ipsilateral globus pallidus (GP) following unilateral substantia nigra (SN) lesions with 6-hydroxydopamine (J. Neurosci. 1:285-291, 1981). Further, administration of apomorphine (Apo) 0.5mg/kg resulted in increased GU most prominently in ipsilateral striatum (ST), substantia nigra pars reticulata(SNPR), and entopeduncularis (EP). In contrast amphetamine administration (2.5mg/kg) produced relative increases in GU in contralateral ST, EP and SNPR (Neurosci. Abstr. 6:124.2, 1980).

To determine if these focal, asymmetric changes in GU were accompanied by changes in regional cerebral blood flow, we studied the distribution of ^{14}C-iodoantipyrine (IAP) autoradiographically in the same experimental paradigm. Also, to ascertain if the focal changes in GU might be accompanied by alteration of the blood brain barrier (BBB), the cerebral distribution of ^{14}C-aminoisobutyric acid (AIB) and trypan blue dye was determined in nigral-lesioned animals after administration of Apo and Amph. In rats with unilateral lesions of SN, IAP accumulation was increased by 24% in ipsilateral GP compared to contralateral. No abnormal accumulation of AIB or trypan blue was noted. Apo 0.5mg/kg S.C. resulted in increased ipsilateral IAP accumulation relative to contralateral in ST (31%), EP (18%), GP (30%) and SNPR (41%) (P at least < 0.025); while Amph 2.5mg/kg S.C. resulted in an increase in IAP accumulation only in contralateral SN (28%) (P < 0.01). Neither Apo nor Amph administration affected the normal distribution of AIB or trypan blue. Both Apo and Amph treated groups had significant increases in cerebral blood flow throughout both hemispheres compared to untreated controls.

These results suggest that measurement of regional cerebral GU is a more sensitive and specific index of regional brain metabolic activity than is regional cerebral blood flow as assayed by IAP distribution. Further, focal changes in basal ganglia GU after SN lesion and treatment with Apo and Amph do not appear to be the consequence of an alterred blood-brain barrier as estimated by cerebral distribution of AIB and trypan blue.

253.1 IMPORTANCE OF THE IMMUNOLOGICAL STATUS OF AMYOTROPHIC LATERAL SCLEROSIS PATIENTS. Fred Westall and Charles Jablecki*. Autoimmune and Neoplastic Disease Lab., Salk Institute for Biol. Studies, San Diego, CA 92138 and Department of Neurosciences, Univ. Calif., San Diego, San Diego, CA 92093.

The immunological status of 63 amyotrophic lateral sclerosis (ALS) patients has been assessed over a three year period. Seven of these patients possessed exceptional high serum IgA. As a group these patients had low serum IgG. All seven patients had slowly progressive clinical courses. Another group of six patients with subnormal values of serum IgA were examined. The serum IgG of these patients were also low. Clinically these patients had an intermediate rate of clinical progression. No correlation between IgA and IgM concentrations were found in either subpopulation of ALS.

The percentages of various subclasses of T and B lymphocytes were estimated. The average percentage for Tμ lymphocytes in ALS patients is 20% while the average for normals is 38% ($p<.0002$). This difference was found in all ALS patients and correlates neither with the rate of clinical progression nor with the level of neurologic disability of the patient. Suppressor T cell levels are elevated in ALS patients (38% for ALS compared to 31% for normals) and the levels correlate directly with the rate of clinical progression of the disease. This data suggests that some ALS patients have an immune disorder which predisposes to development of ALS and/or that the rate of clinical progression may be related to immunologic parameters.

253.2 ABNORMAL GANGLIOSIDE PATTERNS IN AMYOTROPHIC LATERAL SCLEROSIS BRAIN. Maurice M. Rapport, William Brunner*, Hyman Donnenfeld*, and Harry Bartfeld*. Division of Neuroscience, N.Y. State Psychiatric Institute, New York, N.Y. 10032 and ALS Center, St. Vincent's Hospital, New York, N.Y. 10011.

A ganglioside pattern comprising 12 ganglioside species (GM1, GM2, GM3, GM4, GD1a, GD1b, GD2, GD3, GT1a, GT1b, GQ1b, Gx) has been determined in cortex from 4 regions of post-mortem brain (middle frontal gyrus, middle temporal gyrus, motor, parahippocampal gyrus) representing 16 cases of ALS and 11 non-ALS controls. The ALS group (11 M/5 F, 63±16 years) were diagnosed on the basis of a combination of upper and lower motor neuron findings. The control group (6 M/5 F, 65±21 years) was comprised of 6 non-neurological cases and 5 with neurological involvment (2 multiple sclerosis, 1 multiple infarct, 1 infarct of brain stem, 1 progressive multifocal leukoencephalopathy). The ganglioside pattern in ALS showed marked abnormalities: three species were low (GD1b, GT1b, GQ1b) and two were high (GM2, GD3), with the differences highly significant ($p< .01$) for some regions. For example, the values for frontal cortex (as % of total sialic acid determined by high performance TLC and densitometry) were 7.98 vs. 17.60 (GD1b), 8.74 vs. 17.91 (GT1b), 1.11 vs. 3.16 (GQ1b), 8.25 vs. 2.01 (GM2), and 16.19 vs. 7.40 (GD3). Several ganglioside species did not show marked alterations: GM1, GD2, and GT1a were normal; GD1a was slightly low ($p<.05$). Other ganglioside species (GM3, GM4, Gx) were not detectable or present only in traces in 6 or more of the ALS cases and in most of the control group. Abnormal patterns were similar in frontal cortex, motor cortex and temporal cortex, but much less pronounced in cortex of parahippocampal gyrus. These findings indicate that the disease process in ALS is not limited to motor neurons. The low values of GD1b, GT1b, and GQ1b indicate a metabolic disturbance in the 1b biosynthetic sequence (GD2→GD1b→GT1b→GQ1b) that is not apparent in the 1a biosynthetic sequence (GM1→GD1a→GT1a). Since the value of GD2 is normal, a partial metabolic block is indicated in the conversion of GD2 to GD1b. Since the total ganglioside sialic acid in these 4 cortical regions in ALS was only slightly decreased from that in the controls (7± 5%), it is unlikely that the abnormal pattern results from cellular loss. We conclude that in ALS there may be an abnormality in neuronal and/or glial cell membranes and that this abnormality may be due to a metabolic disturbance in ganglioside biosynthesis.

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253.3 ALTERATIONS IN THE CENTRAL CATECHOLAMINE SYSTEMS OF THE RAT MUTANT DYSTONIC (dt). Joan F. Lorden, Gary A. Oltmans, Mitchell Beales,* Henry J. Baker,* Nancy Cox,* Steven U. Walkley, and Mary Martin.* Depts. of Psych. and Comp. Med., Univ. of Alabama in Birmingham, Birmingham, AL 35294; Dept. Pharmacol., Chicago Med. Sch., No. Chicago, IL 60064; and Dept. Neurosci., Albert Einstein Coll. Med., Bronx, N.Y. 10461.

An animal model of human torsion dystonia was discovered in Sprague-Dawley rats in 1978 by Baker and Cox. At about 10 days of age, affected pups display clinical signs which include a stiff paddling gait and falling to the side. As the disorder progresses, mutants display a lack of coordinated movement, rolling left or right, self-clasping of limbs, hyperflexion and rigidity. Pedigree analyses indicate that both sexes are affected and that the ratio of diseased to normal pups is 1:4. Histopathological examination of the brain and spinal cord of dt rats has not revealed any morphological changes at the light microscopic level.

Neurochemical and pharmacological observations were made on pups 16-25 days of age provided with supplemental hand feeding. Litter size was also reduced to improve the nutritional status of mutants. Cerebellar norepinephrine (NE) levels were elevated by 42% in mutants in comparison with normal littermates. This increase occurred in the absence of a reliable difference in tissue weights. No significant differences in NE levels were found in cortex or hippocampus. Decreased β-adrenergic receptor numbers were also noted in cerebellar tissue of dt rats. A small increase in dopamine (DA) receptor number but no difference in DA levels was found in the striatum of dt rats. Changes in receptor binding appeared selective, as no differences between normal and dt rats were noted in quinunclidinyl benzilate binding in the striatum or spiroperidol binding in the nucleus accumbens.

The effects of intraperitoneal injections of haloperidol (.5-10 mg/kg), amphetamine (5 mg/kg), apomorphine (1-3 mg/kg) and scopolamine (1 mg/kg) were examined. No drug, including doses of haloperidol which produced profound catalepsy in normal rats, inhibited all dystonic movements. Apomorphine decreased the incidence of torticollis and scopolamine, of paw clasps in dt rats. The frequency of stereotyped movements such as gnawing, licking and sniffing in response to apomorphine was markedly reduced in comparison with controls. Since these behaviors are seen in dt rats in response to appropriate food stimuli, this difference probably reflects an insensitivity to the drug.

The differences observed between normal and dt rats suggest biochemical abnormalities in the motor systems of the mutants. Further study will be needed to relate the specific changes observed to dystonic movements.

253.4 INDUCTION OF AN EXPERIMENTAL NEUROPATHY IN THE MONKEY BY PASSIVE TRANSFER OF HUMAN GUILLAIN-BARRE SYNDROME (GBS) WITH IMMUNOGLOBULINS. K. Heininger+, H.-G. Ross, K.V. Toyka+, S. Cleveland+, U.A. Besinger+, A.P. Anzil+, Neurolog. Klinik and Neurophysiol. Inst., Univ. Düsseldorf; Neurolog. Klinik, Techn. Univ. München; Max-Planck-Inst. f. Psychiatrie, München; Federal Republic of Germany

Recently, the possible role of humoral factors in the pathogenesis of the GBS-type neuropathy has been proposed on the indirect evidence that patients with the disease may benefit from plasma exchange treatment. Using plasma from a patient with chronic GBS who had responded dramatically and reproducibly to plasma exchange (Toyka et al., Ann. Neurol. 8: 205, 1980), we have studied the potential pathogenetic role of immunoglobulins (Ig) by adapting the passive transfer model of myasthenia gravis (Toyka et al., Science 190: 397, 1975) to the marmoset monkey. The animals were injected i.m. with multiple doses of crude or purified IgG from the patient or from healthy human controls for 3 weeks. Serial measurements of motor nerve conduction velocities were carried out at the sciatic and tail nerves using standard techniques. In the 2 monkeys treated with GBS-IgG nerve conduction velocity gradually slowed from 60 m/s to 35 m/s; none of the controls showed slowing of nerve conduction velocity. In subsequent acute experiments under nembutal anesthesia, ventral and dorsal roots of the lumbar spinal cord were cut following laminectomy, and the afferent and efferent conduction velocities and refractory periods were determined. This procedure revealed a predominant involvement of efferent fibers. Reconstruction of the "fiber spectrum" according to Smith (J. neurol. Sci. 48: 191, 1980) also confirmed a greater effect of the GBS-IgG on α motor fibers. Preliminary light and electronmicroscopic analysis of excised nerve tissue revealed only mild lesions.

Our results suggest that typical features of the human neuropathy may be reproduced by the passive transfer of human IgG to monkeys.

253.5 SENSORY PROCESSING DEFICIT IN AN INHERITED MOVEMENT DISORDER: DECREASED VISUAL CONTRAST SENSITIVITY IN DYSTONIA MUSCULORUM DEFORMANS. Joy Hirsch* and Donald Kay Riker (SPON:W.F. Riker, Jr.). Depts. Opthal. & Visual Sci. & Pharmacology, Yale Univ. Sch. Med., New Haven, CT 06510 & West Haven V.A. Med. Ctr., West Haven, CT 06516.

Movement disorders occupy an important position in neurology since they exemplify how discrete dysfunction of human neurotransmitter systems can alter behavior. Sensory deficits are not widely recognized pathognomic features of movement disorders since, if they occur, they may be subtle and superfluous to diagnosis. For example, Parkinson's disease is distinguished by the triad of tremor, rigidity, and akinesia; nevertheless, latent sensory signs are manifest findings in many patients (Snider et al, Neurology 26:423, 1976), and increased latencies in visual evoked potentials have been reported (Bodis-Wollner & Yahr, Brain 101:661, 1978). Nigrocollicular pathways are suspected in visual and oculomotor deficits in basal ganglia disease. Dystonia musculorum deformans (DMD) is an inherited disorder marked by powerful, slow contractions of muscles of the extremities, trunk, and neck. Sensory (including visual) deficits have not previously been reported in DMD.

Visual sensitivity to contrast of spatial frequency gratings (alternating vertical light and dark bars) of six patients with DMD was compared to that of a well parent or sibling. All observers were free of eye disease and were corrected for normal vision. Comparisons of threshold data across observers was achieved by use of a signal detection theory parameter (d') to correct for differences in response bias. In each paired comparison the affected family member showed significant loss of contrast sensitivity (approximately 0.25 log unit) over the range of spatial frequencies (bar sizes) tested.

Categorization of inherited neurologic disorder simply by overt clinical signs may not direct attention to the true extent of biochemical lesions. Neurologists suspect that DMD is a basal ganglia disorder. These data suggest that, in addition to motor output, sensory processing may also be affected. (Supported by The Dystonia Medical Research Foundation, Beverly Hills, CA; NEI grants EY00785 and EY00167-01; and Research to Prevent Blindness).

253.6 PLASMA ACETYLCHOLINESTERASE ACTIVITY - A POTENTIAL MARKER OF 2,5-HEXANEDIOL NEUROTOXICITY. Barbara F. Bass* and Alan M. Goldberg. Division of Toxicology, Dept. of Environmental Health Sciences, The Johns Hopkins University, Baltimore, MD 21205.

Neuromuscular disorders such as muscular dystrophy (Lyles, J.M., et al. J. Neurochem. 34:978, 1980) and amyotrophic lateral sclerosis (Festoff, B.W., et al. Neurochem. Abstr., #83, 1980) have been associated with increases in plasma acetylcholinesterase (AChE) activity. Muscle AChE activity is also known to undergo changes in response to neuromuscular damage. The present study was undertaken to examine whether a known neurotoxic agent, 2,5-hexanediol, would produce alterations in plasma or muscle cholinesterase activity and if so, would these changes preceed the development of overt toxicity. Female Sprague-Dawley rats were exposed to 0.5% 2,5-hexanediol via their drinking water for up to 9 weeks at which time treatment was terminated and the animals were given tap water. At weeks 2,4,6,8,10, 12 and 15, control and treated rats were killed and plasma and muscle samples obtained. Plasma was assayed for both acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) activities. ^{14}C -ACh was used as the substrate in both assays with BW284C51 and iso-OMPA used as selective inhibitors of AChE and BChE respectively. By week 6, 12% of the animals exhibited signs of the neuropathy and by week 8, all treated animals had developed peripheral motor disturbances. Once exposure to the hexacarbon was terminated, recovery slowly occurred over the next several months. Plasma AChE activity was found to differ significantly between control and treated groups ($P < .01$). At week 4, plasma AChE activity in the treated group was elevated. This upward trend continued, reaching significance at week 10 ($P < .01$). This was one week after exposure to 2,5-hexanediol had been stopped. By week 12, AChE activity had returned to control levels. It should be noted that AChE activity increased in the experimental group prior to any overt manifestations of a neuropathy. No changes were observed in plasma BChE activity over the 15 weeks. In muscle no significant differences were seen in total AChE activity per muscle. However, total protein content per muscle decreased as compared to controls after week 8 and remained so throughout the rest of the experiment (week 15). Therefore, the activity of AChE per mg protein showed an increase during this time period.

In conclusion, the present study is further evidence that plasma AChE activity is a potential marker of neuromuscular disorders, whether chemically induced or naturally occurring, and that it may be useful as an early indicator of the hexacarbon induced neuropathy.

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253.7 PRESENCE OF "SLOW" MYOSIN IN FAST-TWITCH MUSCLES OF THE DYSTROPHIC MOUSE. D.J. Parry* (Spon: K.C. Marshall). Dept. of Physiology, Univ. Ottawa, Ottawa Ont. Canada K1N 9A9

The fast-twitch anterior tibialis (AT) and extensor digitorum longus (EDL) muscles of normal mice contain few, if any, type I fibres as demonstrated by myofibrillar ATPase histochemistry. This has been confirmed by showing the almost complete absence of fibres which react with anti-myosin raised against myosin extracted from the slow-twitch cat soleus. (N.B. The anti-myosin was prepared by Dr. Anthea Rowlerson). AT and EDL of the dystrophic mouse, C57 BL dy^{2J}/dy^{2J} aged 6-8 months exhibit significantly slower isometric twitches than their control counterparts. Time to peak tension (CT) and half relaxation time (1/2 RT) were both significantly prolonged. In addition EDL also shows post-tetanic and cooling potentiation effects characteristic of slow-twitch muscle. With ATPase histochemistry a large number of fibres with intermediate staining intensity are seen in dystrophic hind-limb muscles. Using the "sandwich" technique for myosin immunohistochemistry it may be seen that a much higher proportion of fibres in both AT and EDL of dystrophic mice contain "slow" myosin. Many of the fibres containing "slow" myosin were atrophic. Forearm muscles did not show such a pronounced increase in "slow" myosin content. The isometric twitch of extensor carpi radialis longus (ECRL) was prolonged, but this was almost entirely due to an increase in half-relaxation time. 1/2 RT in dy^{2J} ECRL was 21.1±3.5 msec compared with control values of 10.4±2.5 msec. CT in the dystrophic forearm muscle was 10.9±0.8 msec and in control mice was 9.0±0.6 msec. It is well known that the hind limb muscles of dystrophic mice undergo spontaneous twitching, a phenomenon not observed in fore-limb muscles. It is suggested that synthesis of "slow" myosin in dystrophic hind limb muscles may be a result of the altered activity pattern and not of the dystrophic process per se.

Supported by Muscular Dystrophy Association of Canada.

253.8 ALTERATIONS IN SKELETAL MUSCLE AChE AND PLASMA CPK OF HENS WITH TOCP-INDUCED DELAYED NEUROTOXICITY. C. Michael Cisson[1] and Barry W. Wilson, Department of Avian Sciences, University of California, Davis, California 95616.

Degenerative changes in chicken skeletal muscle following surgical denervation have been previously studied in this laboratory. Most notably, acetylcholinesterase (AChE, E.C. 3.1.1.7) activity increased eight times that of normal innervated muscle, sarcoplasmic enzyme activity appeared outside the region of the motor endplate, and the 20S AChE molecular form virtually disappeared. In addition, plasma creatine phosphokinase (CPK, E.C. 2.7.3.2) significantly increased following denervation. Axons within the sciatic nerve of hens with a delayed neurotoxicity induced by TOCP (tri-ortho-cresyl phosphate) are known to become swollen and undergo fragmentation, and motor control of the legs is lost. The muscles of hens treated with TOCP (500 mg/kg s.c.) were observed for degenerative changes following the development of this neuropathy. Mean muscle weight and fiber diameter of the M. gastrocnemius, a muscle innervated by a branch of the sciatic nerve, were significantly decreased. The muscle fibers were also rounded, and high AChE activity appeared in the muscle fiber sarcoplasm in regions where there were no apparent motor endplates Biochemical studies showed the concentration of AChE and nonspecific cholinesterase (ChE, E.C. 3.1.1.8) activities were twice that of control, and sedimentation of the AChE molecular forms on sucrose density gradients showed a 57% decrease in the 20S form. Plasma CPK activity was four times normal after the onset of delayed neurotoxicity, and there was a concurrent 56% decrease in the CPK activity of the M. gastrocnemius. Degenerative changes were not observed in muscles of birds treated with parathion (O,O-diethyl-p-nitrophenyl phosphorothioate), the negative control for these studies, which suggested the alterations were directly related to delayed neurotoxicity. (Supported by NIEHS grant ES 00202 and a grant from the University of California cooperative Extension Western Regional Pesticide Impact Assessment Program.)

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253.9 EFFECTS OF PRECENTRAL CORTICAL LESIONS ON GLUCOSE UTILIZATION IN THE DEEP CEREBELLAR AND RELATED NUCLEI IN THE MONKEY. S. Gilman, G. Dauth, K. Frey*and J. Penney, Department of Neurology, The University of Michigan, Ann Arbor, MI 48109

In humans and infrahuman primates damage to precentral cortex results in a contralateral hemiparesis, initially hypotonic, and later hypertonic. The neurological events following such a lesion are largely unknown. In particular it is not known which structures are important in the development of hypertonia.

The primate model with unilateral 4 & 6 cortical lesions and the [^{14}C]-2-deoxyglucose ([^{14}C-2-DG]) quantitative autoradiographic technique were used to evaluate changes in motor system structures following the lesions. The [^{14}C]-2-DG technique as described by Sokoloff et al. (1977) was used to determine rates of glucose utilization. One group of animals was sacrificed 1 week after surgery during the hypotonic phase and another group at 8 weeks when the animals were clearly hypertonic.

An analysis of glucose utilization rates reveals that there are asymmetries in glucose utilization in the red nuclei, pontine nuclei and inferior olivary nuclei both at 1 and 8 weeks after the lesion. The structures ipsilateral to the lesion had lower glucose utilization rates than those contralateral to the lesion. The asymmetries in these structures were similar at both 1 & 8 weeks. Analysis of the deep cerebellar and vestibular nuclei revealed that there were no consistent left-right asymmetries in glucose utilization at either 1 or 8 weeks after a unilateral 4 & 6 lesion.

These findings suggest that the red, pontine and olivary nuclei may be important in hemiparesis but not important for the development of hypertonia. The lack of asymmetries in the deep cerebellar and vestibular nuclei suggest that alteration of neural activity in these structures may not be of primary importance for the hemiparesis which follows cerebral injury.

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253.10 ACUTE EFFECTS OF THE NEUROTOXIN KAINIC ACID ON NEURONS OF THE PIGEON BASAL GANGLIA: BEHAVIORAL, ELECTROPHYSIOLOGICAL, LIGHT AND ELECTRON MICROSCOPIC OBSERVATIONS. G. K. Rieke and D. E. Bowers, Jr.* Department of Anatomy, Texas A&M Univ. College of Med., College Station, Tx 77843.

The paleostriatal complex (PC) of the pigeon is the probable avian analogue of the mammalian caudatoputamen and globus pallidus. Intracerebral injection of kainic acid (KA, 0.00053µg-1µg) into the PC resulted in marked postural problems, significantly elevated discharge rates of KA sensitive neurons, and morphological changes in neurons and neuropil. Birds anesthetized with ether and intracerebrally injected with 1µg KA/µL demonstrated postural disturbances of the head and neck. The head and neck were twisted to the side of injection and the head was characteristically inverted. There was resistance to passive movement of the head and neck away from the side of rotation. The bird also circled toward the side of injection. Kainic acid sensitive neurons, particularly the large neurons of the paleostriatum primitivum, responded to KA with elevated discharge rates (3-12 times the base line rate). The discharge rates of individual units treated with aliquots of KA (0.2µL, 1µg KA/µL) remained elevated for 1-2 hrs, after which the treated unit suddenly ceased firing. Units treated with low concentrations of KA (0.00053µg/µL) responded with elevated discharge rates that were sustained for longer periods of time, while discharge rates were not significantly elevated by treatment with aliquots of 0.9% NaCl vehicle alone. Morphological changes induced by KA appeared to involve initially the neuropil, as dilated dendritic profiles were a common feature 1 hr after the injection of 1µg KA. The morphological changes became more intense and by 4 hrs both dendrites and soma showed marked pathology. There appeared to be a relationship between tissue response and the dose of the toxin. The larger the dose the more rapid the destruction of neurons. In addition, there was a dose-survival time correlation as KA is a persistent toxin and small doses ultimately have the same destructive effect on neurons over a longer period of time. The close correlation between cessation of cell discharge, metabolic alterations and morphological changes suggests that KA sensitive neurons do not recover from the influence of the toxin. The mechanisms by which KA destroys neurons are not clearly understood; however, our observations support, in part, the excitotoxin hypothesis as one means for the toxicity of KA. The pigeon may be a useful model to study both movement disorders and the actions of KA or KA-like substances on sensitive neurons that appear to play a role in the regulation of movement and maintenance of posture. Supported by Office of Univ. Res., Grant #15707.

253.11 WHICH IS THE MAIN SUBSTANTIA NIGRA RETICULATA (SNR) EFFERENT INVOLVED IN TURNING BEHAVIOUR? M. Garcia-Munoz*, P. Patino*, and A. Zainos*. (SPON: R. Salceda). Centro de Investigaciones en Fisiología Celular, Universidad Nacional Autónoma de México, Apartado Postal 70-600, México 20, D.F., México.

The administration of dopaminergic (DA) agonists to rats with unilateral destruction of striatal DA nerve terminals induces circling behaviour. This alteration in the motor system has been used to test different drugs interacting with the DA receptor and has led to a tendency to explain turning only in terms of the degree of receptor activation. Little is known about the structures involved. Recent studies propose that the activation of a gabaergic system projecting from SNR may be involved as an output system for nigro-striatal motor responses. Gabaergic agonists induce turning, and the administration of GABA-receptor blockers ipsilateral to a 6-OHDA lesion antagonizes the turning produced by DA agonists. Efferents from SNR are to thalamus (VMT), superior colliculus (SC) and the reticular formation of the ponds (RFP). To clarify the possible participation of these areas in turning we made kainic acid lesions (1.25 ug/0.25ul). Rats with unilateral lesions of VMT turned 160.7+75 times/30 min (apomorphine 2.5 mg/kg). This same lesion performed 10 days after a 6-OHDA injection in the medial forebrain bundle reduced the number of turns by 60% if compared against turning before the TVM lesion and by 81% if compared against a 6-OHDA lesioned group operated once but tested twice as the double lesioned animals, (apomorphine 0.25 mg/kg). Rats with a bilateral lesion in VMT after a 6-OHDA injection also reduced the number of turns by 66% compared against themselves and by 79% compared against the 6-OHDA lesioned group. A unilateral lesion of RFP produced 59+37 turns/30 min and a unilateral lesion of SC produced 28.5 turns+17 turns/30, (apomorphine 2.5 mg/kg). Animals with lesions in these areas after a previous 6-OHDA injection did not alter significantly their turning. Bilateral TVM lesions decrease the number of turns providing the lesion is symetric and made with kainic acid. It is possible that some fibers lesioned electrolitically may also participate. We propose that the main efferent from SNR mediating this behaviour produced by striatal DA nerve terminal degeneration is to VMT.

253.12 THE EFFECTS OF STIMULATION OF THE DORSAL SPINAL CORD ON THE STRETCH REFLEX IN THE DECEREBRATE CAT. C.E. Chapman* and M. Wiesendanger (SPON: V.B. Brooks). Institut de Physiologie, Universite de Fribourg, CH-1700 Fribourg, Switzerland.

It has been reported that stimulation of the dorsal spinal cord may, by an unknown mechanism, produce a dramatic and long-lasting reduction in spasticity. The effects of dorsal cord stimulation (DCS) on stretch reflex activity in triceps brachii and triceps surae were investigated in 10 intercollicular decerebrate cats and 2 decorticate cats. During stimulation (0.2 ms, 50 Hz, 80-150 µA for 1-20 min. at the C_1 level) the tonic stretch reflex (TSR) and the phasic, monosynaptic reflex (MSR) of triceps brachii were inhibited in both the decerebrate and the decorticate cat. After cessation of stimulation, the MSR always recovered quickly whereas the TSR often remained depressed for up to 5-20 min. Stimulation at more caudal levels (T_2) had weaker inhibitory effects on the triceps brachii TSR. DCS (T_8 level) had similar, but less marked, inhibitory effects on the triceps surae TSR, in the decerebrate cat. The corresponding MSR was either weakly inhibited or unchanged. Dorsal column section (T_{10}) exaggerated the effects of stimulation rostral to the lesion. Stimulation caudal to the lesion failed to elicit any inhibition, suggesting that the inhibitory effects were mediated by dorsal column afferent activation of more rostral structures.

Thus, the present results indicate that DCS produces a marked inhibition of stretch reflex activity. In forebrain-lesioned cats, the inhibitory effects did not, however, outlast the period of stimulation for hours as found in spastic man.

254.1 SODIUM ION EFFECTS ON THE RESPIRATION PROPERTIES OF ASTROCYTES FROM PRIMARY CULTURE. James E. Olson and David Holtzman. Depts. of Pediatrics and Neurology, Stanford University School of Medicine, Stanford, CA 94305.

In a previous study, we reported that oligomycin-insensitive, or phosphorylation-independent, respiration increases with increasing NaCl concentration in cerebral astrocytes from primary culture (Olson and Holtzman, Trans. Soc. Neurosci. 186.1, 1980). This increase did not occur in sucrose media of the same osmolarity. We have investigated further the ionic dependence of and effects of metabolic inhibitors on astrocyte respiration in vitro.

Primary cultures of astrocytes were prepared from 3 day old rat cerebral cortex. Respiration was measured polarographically as previously described (Olson and Holtzman, J Neurosci Res. 5(6):497, 1980). Phosphorylation-independent respiration was measured using a maximally inhibiting concentration of oligomycin (5 μM). This respiratory rate increased as the osmolarity increased from 274 to 1096 mOsm with NaCl or Na acetate but not with choline Cl or sucrose as the osmotic agent. Amiloride, known to block passive movement of Na^+ across the plasma membrane of other cell types, produced a dose-dependent decrease in phosphorylation-independent respiration in astrocytes suspended in the 1096 mOsm NaCl medium. A 43% inhibition was obtained with 1.5 mM amiloride. No effect of amiloride was observed on the phosphorylation-independent respiration of cells suspended in 1096 mOsm sucrose medium.

Ouabain (1 mM), an inhibitor of Na-K ATPase, was found to inhibit the basal respiration of astrocytes by 31% and 27% in 274 and 1096 mOsm NaCl medium respectively. Addition of amiloride (1 mM) to ouabain-inhibited cells produced no change in the respiratory rate in 274 mOsm NaCl, but further inhibited respiration in cells suspended in 1096 mOsm NaCl by 13%. This same concentration of amiloride added to cells without the prior addition of ouabain produced a 23% inhibition at each osmolarity.

Measurements of the volume of astrocytes in media of increased osmolarity were the same with NaCl or sucrose as the osmotic agent. In hypo-osmotic solutions, cells swelled more in NaCl compared to sucrose solutions.

These results suggest that, in the astrocyte, there exists a respiratory component, responsive to extracellular Na^+, which is not coupled to oxidative phosphorylation. This respiratory component may be important in the astrocyte's proposed function as a buffer of extracellular ion and water activities.

This study was supported by NIH grants ES 02571 and NS 16256 and a Stanford University Dean's Post-doctoral Fellowship.

254.2 OXIDO-REDUCTION OF FLAVINE ADENINE DINUCLEOTIDE IN THE RESPIRATORY CHAIN OF NEURON SOMAS OF FROG DORSAL ROOT GANGLION. Carlos Rodríguez-Estrada. Cátedra de Fisiología. I.M.E. Fac. Medicina, U.C.V., Caracas, Venezuela.

It has been reported (Rodríguez-Estrada Soc. Neuroscience Abst.#300,p91,1979) that an increase of carbon dioxide partial pressure produced a decrease of reduced nicotinamide adenine dinucleotide, i.e. oxidation of respiratory chain in neuron somas of dorsal root ganglion in vitro preparation. This change could be due to a new equilibrium constant pH dependent (E. Racker J.Biol.Chem. 184:313,1950). It has been accepted that reduced nicotinamide adenine dinucleotide in the neuron somas is located in their mitochondrias and that this hydrogen carrier is a link in the respiratory chain between substrate donor and flavoproteins of the respiratory chain. Therefore, an oxidation of NADH could be achieved in either direction (toward flavoproteins or substrate) by lowering the pH with carbon dioxide but in fully reduced state of NAD the flavine adenine dinucleotide also is fully reduced. Hence the hydrogen acceptor is the substrate and oxidation of $FADH_2$ also could be observed. Dorsal root ganglion preparation were used with a temperature controled at 25°C, alternating fluorometric determinations of NADH and FAD were done. NADH determinations were performed as previously describe. FAD fluorescence emission was recorded with 5200 angstroms interference filter. Light excitation of 4350 angstroms was used. The oxido-reduction level determined in O_2 atmosphere was taken as reference level. When replacing the O_2 for N_2 there is an increase of $FADH_2$. In fully reduced state replacing this N_2 atmosphere for one containing N_2 97.5% and CO_2 2.5% there is a decreased of the reduction level, both NADH and $FADH_2$, i.e. an oxidation was observed. After incubating the preparation with Amytal (5-30 minutes) the reduction in N_2 atmosphere was not seen, and the oxidation with N_2-CO_2 mixture was not observed. The Amytal produced a partial block, the oxidation in the N_2-CO_2 mixture was also observed, but a smaller reduction than was expected. The results indicated that the oxidation recorded by the increase of carbon dioxide is a chemical reaction change only occurring in the respiratory chain.

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254.3 INFLUENCE OF CEREBRAL POTASSIUM EFFLUX ON CYTOCHROME a,a_3 REDOX STATE DURING ISCHEMIA IN THE RAT. T.J. Sick, R.B. Duckrow, J.C. LaManna and M. Rosenthal. Dept. of Neurol., Univ. of Miami Sch. of Med., Miami, FL. 33101.

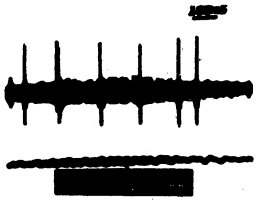
Cerebral ischemia is accompanied by an increase in the ratio of reduced/oxidized cytochrome c oxidase (cyt a,a_3) in brain. The initial period of ischemia is characterized by fast reduction of cyt a,a_3. This is followed by a period when cyt a,a_3 reaches a plateau or even becomes transiently oxidised, and finally by a period of further reduction of cyt a,a_3. The multiphasic nature of this response was evaluated, in the present study, by comparing the timecourse of changes in cerebral oxygen availability and extracellular potassium ion activity (K^+ o). The redox state of cyt a,a_3, tissue oxygen tension and K^+ o were monitored continuously in male and female Wistar rats made ischemic by coagulation of the vertebral arteries and subsequent occlusion of the carotid arteries. Cyt a,a_3 was monitored by dual wavelength spectrophotometry while tissue oxygen tension and extracellular potassium ion activity were monitored respectively with oxygen and potassium sensitive microelectrodes.

Carotid ligation resulted in decreased brain oxygen tension, reduction of cyt a,a_3, elevation of K^+ o from 2-4 mM to 6-8 mM and ECoG silence. During this period, cyt a,a_3 reached a plateau and in many cases then became transiently oxidized. This period often was characterized by partial reoxygenation of the brain. K^+ o eventually underwent rapid elevation to levels in excess of 50 mM. This rise in K^+ o was accompanied by further reduction of cyt a,a_3. Release of the carotid clamps resulted in rapid re-oxygenation of the brain, re-oxidation of cyt a,a_3, a later return of K^+ o to control levels and sometime thereafter a resumption of EcoG activity. Recovery was dependent upon the duration of cerebral ischemia which was varied from 5 to 30 min.

The multiphasic changes in cyt a,a_3, tissue oxygen tension, K^+ o and ECoG during initial phases of ischemia may reflect attempted compensation in brain to overcome the imbalance between energy production and energy utilization when blood flow is limited. Such conpensation, reflected by slowed reduction or partial re-oxidation of cyt a,a_3, may reflect partial tissue re-oxygenation, decreased energy utilization reflected by ECoG depression, and/or limitation of substrate availability. (Supported in part by NIH NS14325, NS14319, NS00399, NS06300, and the AHA of Greater Miami).

254.4 MICROREGIONAL PO_2 AND ACTION POTENTIAL MEASUREMENT IN THE GERBIL CORTEX. R.M. Martin and J.H. Halsey. Dept. of Neurology and the Neurosciences Program, University of Alabama in Birmingham, Birmingham, AL 35294.

Simultaneous PO_2 and action potential recordings were made from a recessed oxygen microelectrode (tip diameter = 1 μM, current = 10^{-11} amp) utilizing a feedback current amplifier that maintained a constant polarization voltage (see Kunke et al., J. Appl. Physiol., 32:436, 1972). This capacity afforded an opportunity to analyze the spike frequency for selected neuron populations within the gerbil cerebral cortex during normoxia and hypoxia (nitrogen breathing) to observe the disappearance of spikes with falling PO_2 and reappearance upon reoxygenation. We observed a number of responses to this brief hypoxic insult ranging from total recovery with no apparent permanent effects to a massive discharge of spikes followed by no recovery of activity. This technique was used to approach a basic question. Namely, can it be demonstrated that as a group neurons normally exposed to a high resting PO_2 lose function sooner and/or regain it later than neurons which exist at much lower PO_2. If this selective vulnerability can be shown, further experiments are planned to attempt to determine if this vulnerability derives from a higher oxygen demand. Supported in part by NS 08802 and NS 07123.



254.5 CEREBRAL OXYGENATION DURING RECURRENT SEIZURES: ROLE OF CHANGES IN ARTERIAL PO_2. N. Kreisman, H. Follis*, A. Light*, M. Rosenthal, J. LaManna and T. Sick. Dept. of Physiol., Tulane U. Sch. Med., New Orleans, LA 70112 and Depts. of Neurology and Physiol. Biophys., U. of Miami Sch. Med., Miami, FL 33101.

Changes in systemic physiology secondary to recurrent seizures can have profound effects on cerebral O_2 supply and may be involved in the development of neuronal damage following status epilepticus. We have reported that initial seizures in a series elicited in rats with pentylenetetrazol (PTZ) were accompanied by increases in cerebral PO_2 and oxidation of cytochrome a,a_3 (cerebral O_2 sufficiency). Subsequent seizures, however, were accompanied by decreases in cerebral PO_2 and shifts of cytochrome a,a_3 toward reduction (cerebral O_2 insufficiency) (Kreisman et al, Brain Res., in press). This transition was accompanied by failure of arterial blood pressure (aBP) or cerebral blood volume (cBV) to increase or by hypotension, separately or in combination, in association with succeeding seizures. In some cases, prolonged increases in aBP occurred immediately preceding transition.

Two hypotheses were tested in the present study. First, that cerebral O_2 insufficiency during later seizures may be the result of decreases in arterial PO_2 (PaO_2). Second, that increasing the fraction of inspired O_2 (FiO_2) can prevent the transition from occurring.

Rats were anesthetized, paralyzed and artificially ventilated with 30% O_2/70% N_2. Measurements were made of cerebral PO_2, cytochrome a,a_3 redox levels and cBV from cerebral cortex during PTZ or bicuculline seizures. Decreases in mean PaO_2 from 124.8± 9.6 to 60.5±5.0 occurred in association with the prolonged increases in aBP. This hypoxemia was accompanied by a decrease in cerebral PO_2, reduction of cytochrome a,a_3 and an increase in cBV. Respiring other rats continuously with increased FiO_2 delayed, but did not prevent, the onset of transition. Raising FiO_2 from 30% to 100% after transition temporarily reversed seizure-induced cerebral PO_2 decreases and cytochrome a,a_3 reductive shifts back to the normal cerebral PO_2 increases and cytochrome a,a_3 oxidative shifts observed prior to transition. Conversely, transition could be precipitated on occasion by lowering FiO_2 from 30% to 21%.

The results indicate that systemic hypoxemia is one potential factor causing cerebral O_2 insufficiency during recurrent seizures. They suggest also that increasing FiO_2 may have some benefit in temporarily ameliorating this cerebral O_2 insufficiency.

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254.6 DIRECT ELECTRICAL STIMULATION OF THE CEREBRAL CORTEX SUGGESTS THAT VASCULAR AS WELL AS METABOLIC FACTORS DETERMINE THE OXIDATIVE RESPONSE. R.B. Duckrow, J.C. LaManna and M. Rosenthal. Department of Neurology, University of Miami School of Medicine, Miami, Fl. 33101.

Direct electrical stimulation of the cerebral cortex produces an increase in extracellular potassium activity and transient oxidations of all respiratory enzymes, including cytochrome a,a_3. The magnitude of these transient oxidations is proportional to the magnitude of the potassium increase and the corresponding shift of the cortical steady potential (SP shift). To test the hypothesis that vascular mechanisms might play a role in the response of cytochrome a,a_3 to increases in cortical "work" induced by direct stimulation, the amplitude of the transient oxidative response of cytochrome a,a_3 was measured by dual-wavelength spectrophotometry after electrical stimulation delivered at various pulse widths and frequencies. Male Wistar rats were anesthetized with pentobarbital, paralyzed with curare, and mechanically respired. Arterial blood pressure, arterial blood gas tensions, arterial pH, and rectal temperature were monitored and maintained within normal limits. After left parietal craniectomy, the intact dura in the center of the optical field was stimulated by a bipolar stainless steel electrode with 0.5 mm tip separation. Monophasic square wave pulses were delivered in stimulus trains of 2 sec duration. Pulse width was varied from 0.05 to 5 msec and pulse frequency varied from 3 to 300 Hz. The evoked SP shift was measured with a single Ag-AgCl wire 1 mm from the stimulating electrodes with an indifferent reference. Stimulus voltage was varied to obtain a range of SP shifts not greater than 4 mV. The slope of the linear relation between the magnitude of the SP shift and the evoked cytochrome a,a_3 oxidation was used as an index of cytochrome a,a_3 oxidation for a constant metabolic demand. The amplitude of the transient oxidation of cytochrome a,a_3 for a given SP shift was maximal for a pulse duration of 0.5 msec and a stimulus frequency of 50 Hz. The myogenic and neurogenic components of vascular responses to direct stimulation vary with stimulus pulse width, frequency, and duration. If the cytochrome response were solely determined by the metabolic demand imposed by increased extracellular potassium, one would expect invariance of the slope of this response over the range of stimuli presented. The variance observed in this study suggests that there is a vascular contribution to the transient oxidative response of cytochrome a,a_3 to direct cortical stimulation, and that there may be a relative surplus of oxygen supplied to cortex under the "active" condition imposed by this stimulation. (Supported by NIH NS14325, NS14319, NS00399 and the AHA of Greater Miami.)

254.7 CALCIUM-DEPENDENT INCREASE IN AXONAL ATP DURING REPETITIVE ACTION POTENTIAL DISCHARGE. D.O. Smith and C.T. Gibson* Department of Physiology, University of Wisconsin, Madison, WI 53706.

Levels of ATP were measured in the terminal region of the excitor axon innervating the opener muscle of the crayfish walking leg. In each experiment, about 16 preparations were placed in a well-oxygenated physiologic saline at 23°C. In half of the preparations, the axon was stimulated at 50 impulses/s while the other half served as unstimulated bath controls. The tissue was quickly frozen in liquid nitrogen and then lyophilized at ≤ -80°C to prevent hydrolysis of ATP. Axon collaterals, weighing less than 1.0 µg dry weight, were dissected free and subsequently assayed for ATP content using the luciferin-luciferase method.

Following 1 min of stimulation, the average (± S.E.; n=8) ATP content rose from 10.7 ± 0.7 nmol/mg dry wt to 21.8 ± 1.3 nmol/mg dry wt. This increase is significant at the 0.05 level. Smaller increases in ATP levels were observed following longer stimulation times. After ≥ 10 min of stimulation, the ATP levels were not significantly higher than in the bath controls.

The increase in ATP was directly related to the concentration of extracellular Ca^{2+}. When $[Ca^{2+}]_e$ was raised to 40 mM (3x normal), ATP levels increased to 32.4 nmol/mg dry wt following 1 min of stimulation at 50 impulses/s; this is 2.5 times the amount of ATP in the corresponding bath controls. However, when $[Ca^{2+}]_e$ was 0.01 mM, there was no change in ATP level following stimulation. Addition of 10 mM $CoCl_2$, which blocks Ca^{2+} currents in this preparation, also prevented a rise in ATP.

It is doubtful that the increased ATP is due entirely to enhanced mitochondrial activity. ATP was still found to increase during stimulation in the presence of 10^{-4}M atractyloside, which should block the transport of ATP to the cytosol via the ATP-ADP antiport system. In related experiments, addition of ruthenium red (10 µM) led to an increase of ATP levels to 15.5 ± 1.5 and 13.9 ± 3.6 nmol/mg dry wt in the stimulated and the nonstimulated tissue, respectively. After incubating the tissue in the Ca^{2+} ionophore A23187 (50 µg/ml), the ATP level dropped from 11.1 ± 1.7 to 8.9 ± 1.3 nmol/mg dry wt in the nonstimulated and the stimulated axons, respectively. The Ca^{2+}-dependent rise in ATP may be due to a change in intracellular pH during action potential activity which causes a shift in the arginine kinase reaction $ArgP + ADP + H^+ \rightleftharpoons ATP + Arg$.

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254.8 THE EFFECT OF ACUTE OR REPEATED ADMINISTRATION OF DESMETHYLIMIPRAMINE ON THE REGIONAL CEREBRAL METABOLIC RATE OF GLUCOSE. J.C. Gerber, III, J. Choki*, M. Reivich and D.J. Brunswick*. Depts. of Psychiatry and Neurology, University of Pennsylvania, School of Medicine, and V.A. Hospital, Philadelphia, Pa. 19104.

It has become clear that the acute pharmacological effects of tricyclic antidepressants (TCA's) can be very different from the effects they produce after repeated administration. To determine whether differences in the functional activity of brain neurons occur after the administration TCA's we treated rats with desmethylimipramine (DMI) either acutely (10mg/kg, 1 hour before experiment) or repeatedly (10mg/kg twice a day for 7 days) and measured glucose utilization in 30 regions of rat brain with the quantitative ^{14}C 2-deoxyglucose technique. This technique is a potentially powerful method for studying the effects of centrally acting drugs in vivo and since little is established on the mechanism of antidepressant action of TCA's this method was applied to determine the effect of these drugs on local cerebral energy metabolism.

Neither an acute dose nor repeated administration of DMI had a statistically significant effect on the metabolic rate of glucose in any of the 30 brain regions studied. These findings indicate that a single dose or 7 days of dosing with DMI may not result in a significant functional alteration in basal CNS activity in rats. Administration of DMI for longer than 7 days may be necessary to achieve significant changes in glucose utilization. Alternatively, use of the ^3H 2-deoxyglucose technique, which would enable changes in glucose utilization on a cellular level to be measured, may shed more light on this matter. (Supported by funds from MH-14654 and NS-10939-09)

254.9 BEHAVIORAL CONTROL OF ERYTHROCYTE GLYCOLYSIS. R. Jevning*. Dept. of Medicine, Univ. of Calif., Irvine, Irvine, CA. 92717.

Several behavioral states, including states of stress and anxiety, have been correlated with altered blood lactate concentrations; however, the underlying cause of the blood lactate changes is little understood. The mental technique known as "Transcendental Meditation" (TM) is associated with acute decline (within 30 minutes) of blood lactate concentrations, and therefore can serve as an ideal behavioral tool for in depth investigation of the changes in metabolism underlying a behavioral state and in particular of the mechanism and cellular origin of the noted change of blood lactate metabolism.

We now report rapid decline of erythrocyte metabolism during TM. Blood samples were drawn every 15 minutes during 45 minutes of this behavior and for 30 minutes after cessation of practice in 25 normal adults who had been regularly eliciting this state twice daily for at least 7 years.

Blood samples were incubated at 37 and 25 and rate of lactate generation measured. Subsequent measurement was made of rate of glycolysis in blood samples from which white cells were removed. Both sets showed rapid decrease (30%) of glycolytic rate in the samples drawn during the practice period. Therefore, since blood plasma has no glycolytic capacity, the decline of blood glycolytic rate is attributed to erythrocytes.

Since concomitant measurement of blood pH, pCO_2, pO_2, and hematocrit showed no change of these parameters, the effect is not an epiphenomenon of respiratory changes.

This acute effect of behavior on red cell glycolysis constitutes a unique and possibly important finding for many fields of research in behavior, since this cell is believed to typify the membrane, the metabolism and the metabolic control of more advanced tissue such as nerve and muscle. Also the factor(s) responsible may affect metabolism in general since oxygen delivery to tissues is affected by status of red blood cell metabolism.

254.10 BINDING AND RELEASE OF ZINC IN HOMOGENATES AND SLICES OF RAT HIPPOCAMPUS. N.F. Harris and I.L. Crawford. Depts. of Pharmacology and Neurology, UTHSCD and VAMC, Dallas, Texas.

The high concentration of zinc (Zn) in the hippocampus relative to other brain regions may be attributed in part to the presence of the metal in mossy fiber terminals. Zinc in the paleocortex is present in several compartments: free ions, and bound with varying affinities to protein and metalloenzymes. Some of this Zn may be available for release in response to certain stimuli. A series of experiments were designed to determine the extent of binding and release of Zn in the hippocampus. Flame atomic absorption (AA) was used to assay Zn. Although flameless AA is more sensitive and requires a smaller sample, the technique was more variable and gave erroneous absolute values.

For determination of relative quantities of free and bound Zn, whole hippocampi were dissected from brains after decapitation. Homogenates of tissue (10% w/v) were made in deionized double-distilled water buffered to pH 7.4 with sodium bicarbonate. Samples were spun at 7000 x g and filtered through regenerated cellulose membranes with 0.2 µm pores. The final supernatant not retained by the membrane contained 48% of total Zn. Zinc concentrations measured in homogenates dialyzed (molecular wt. cutoff was 12,000 daltons) 18 hrs against "Zn-free" artificial cerebrospinal fluid (CSF) showed only a 16% decline from initial homogenate values (17.1 $\pm$ 1.2 µg/g wet wt. $\pm$ SEM).

In another set of experiments slices of hippocampus (500 µm) were depolarized with potassium (K) in artificial CSF. No significant changes of endogenous Zn were noted between fresh slices (18.5 $\pm$ 0.7), and those incubated in 6 mM K (17.8 $\pm$ 1.6) and 60 mM K (16.5 $\pm$ 0.8). Problems in reporting values in brain slices on a wet wt. basis may be due to tissue swelling. Values for Zn in hippocampus varied depending on the basis used to express concentration. A comparison of slice wts. gave the following results: dry wt. = 12% of wet wt; ashed wt. = 8% of wet wt.

Binding studies suggest Zn exists in several pools: chelated or structurally bound to large molecules; associated with smaller molecules or colloids which can pass through membrane pores, but are not dialyzable; and freely dialyzable Zn. In slices, release of a free fraction of endogenous Zn was not detectable by AA methods.

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254.11 THE EFFECT OF ANDROGEN RECEPTORS AND ITS INTERACTION WITH HEAVY METALS ON THE METABOLISM OF MOTOR NEURONS IN TISSUE CULTURE. R.C. Yu, and B.G.W. Arnason. Department of Neurology and Brain Research Institute, University of Chicago, Chicago, Ill. 60637.

Androgen is preferentially taken up by motor neurons both those of the cranial nerves and those of the spinal cord. Little androgen is found in the nuclei of sensory nerves. Sensory neurons have been demonstrated to take up estrogen predominantly (Biochem 7:1163). The topographic distribution of androgen receptors in motor neurons differs between regions and coincides with those neurons involved or spared in patients with amyotrophic lateral sclerosis(ALS)(Ann.Neurol. 4:245). Notable in this regard is the fact that motor neurons of the III,IV,VI and X cranial nerves takes up little androgen and are spared in ALS. The pathological finding of Bunina body inclusions in the Perikarya of motor neurons is consistent with a disorder in protein metabolism in ALS. Epidemiological data has suggested that heavy metals may be associated with the development of motor neuron disease, although the point is controversial.

We used organotypic cultures developed from 14-day-old rat embryonic spinal cord to examine the role of androgen receptors and heavy metals in relation to motor neuron metabolism as reflected by the enzymatic activity of choline acetyltranferase (CAT). Heavy metals Zn^{++}, Hg^{++}, Pb^{++} dissolved in water and cyproterone(α-MPD), a potent anti-androgen-receptor drug in ethanol as supplements to regular tissue culture medium at final concentrations of 10^{-5}M. Water or ethanol alone was added in medium for control. Selected well developed spinal cord cultures were incubated with one of these media for one week before being homogenized and assayed blind for CAT activity. Cultures incubated with Hg^{++} showed an effect of general toxicity as reflected by a substantial reduction of total protein content below the level of the control group. In each of the experimental groups, CAT was calculated based on the total amount of protein measured (CPM/mg). There was a significant decrease of CAT activity both in cultures treated with α-MPD ($P<0.05$) and with lead ($P<0.01$) as compared with the controls. Zinc in contrast had no effect.

Certain bivalent metals are known to alter the androgen receptor complex and prevent it from entering into the nucleus. These results lead us to conclude that intrinsic androgen receptors may play an important role in regulating cellular metabolism which dictates motor neuron survival.

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254.12 KINETIC CONSTANTS OF BLOOD-BRAIN BARRIER NEUTRAL AMINO ACID TRANSPORT: COMPARISON OF NEWBORN RABBIT AND ADULT RAT. L.J. Mietus* and W.M. Pardridge (SPON: A. Yuwiler). UCLA School of Medicine, Los Angeles, CA 90024.

Amino acid availability in brain regulates a number of pathways of cerebral amino acid metabolism (Fernstrom and Wurtman, 1977). Amino acid supply in brain is a function of (i) plasma amino acid levels and (ii) the K_m, V_{max}, and K_D of blood-brain barrier (BBB) transport. The newborn period is associated with a level of plasma aminoacidemia that would nearly fully saturate the neutral amino acid carrier in the adult rat. Therefore, the present studies were designed to determine the kinetics of neutral amino acid transport through the BBB of the newborn rabbit using a nonlinear regression analysis of brain uptake index (BUI) data obtained with the carotid injection technique.

Amino acid	K_m (mM)		V_m (nmol $min^{-1}g^{-1}$)		K_D (ml $min^{-1}g^{-1}$)	
	Rat	Rab	Rat	Rab	Rat	Rab
Phenylalanine	0.12±0.02	0.41±0.15	36± 7	101±53	0.015	0.027
Leucine	0.12±0.01	0.41±0.06	30± 3	56±13	0.019	0.032
Tyrosine	0.15±0.01	0.52±0.13	42± 5	64±25	0.016	0.050
Tryptophan	0.19±0.01	0.52±0.08	35± 3	75±19	0.002	0.019
Isoleucine	0.29±0.05	0.69±0.21	53±12	70±30	0.005	0.042
Methionine	0.20±0.08	0.93±0.42	31±15	94±58	0.022	0.031
Histidine	0.27±0.03	1.43±0.07	37± 6	101±18	0.011	0.040
Valine	0.54±0.06	1.29±0.33	40± 6	96±36	0.003	0.016

A 67% solution of newborn rabbit serum resulted in a 45% inhibition of tryptophan transport, and bound 43 $\pm$ 3% of serum tryptophan in vitro at 37°C. However, dialyzed newborn rabbit serum or commercial defatted bovine albumin, which actively bound tryptophan in vitro with a dissociation constant (K_D) of 138 $\pm$ 7 µM, exerted no inhibition of tryptophan transport in vivo. The extraction of alanine, 16 $\pm$ 1%, and glutamine, 19 $\pm$ 2%, by the newborn rabbit brain was higher than the adult rat. However, alanine or glutamine transport was not inhibited by 25 mM N-methylaminoisobutyric acid, a model amino acid specific for the alanine preferring or A-system. Conclusions: (i) K_m and V_{max} values obtained for the adult rat with a nonlinear regression analysis are not statistically different from our previously reported values obtained with a linear transformation. (ii) Newborn BBB transport of amino acids is characterized by a lower affinity and higher capacity relative to the adult rat and this change in the newborn accomodates the hyperaminoacidemia of the neonatal period. (iii) Albumin of newborn rabbit serum binds tryptophan in vitro, but exerts no inhibition of tryptophan transport in vivo, owing to the high capacity of tryptophan binding by the brain capillary neutral amino acid carrier.

254.13 A METHOD FOR THE MEASUREMENT OF LOCAL BRAIN PROTEIN SYNTHESIS.
B. E. Dwyer*, P. Donatoni* and C. G. Wasterlain* (SPON: R. Nishimura). Epilepsy Research Laboratory, V.A.M.C. Sepulveda, CA 91343 and the Department of Neurology and the Brain Research Institute, UCLA, Los Angeles, CA.

We have developed a method for measuring local brain protein synthesis. It combines the intraperitoneal injection of a large amount of L-(1-^{14}C) amino acid of low specific activity (Dunlop et al. J. Neurochem., 24:337, 1975)and the use of an internal standard with quantitative autoradiography. Four day old rats were injected with L-(1-^{14}C)valine (150mM) (10μmole/g; 0.5μCi/μmole)and were decapitated at varying time intervals up to two hours. Some brains were frozen in cryo-embedding media, cut into 20μm sections and exposed with ^{14}C standards to single layer X-ray film for autoradiography. In some animals,one half the forebrain was acid precipitated and delipidated and the rate of valine incorporation into protein was measured directly. The other half forebrain was mechanically dispersed to form a homogeneous brain "mix". The "mix" was frozen, cut in 20 μm sections and used for autoradiography. Film darkening was related to the rate of ^{14}C-valine incorporation measured directly in the other half of the same brain. Stable ^{14}C standards were calibrated with the brain "mix" and included with every exposure. Film darkening in various brain regions was related to rates of amino acid incorporation via the standard brain "mix" curve. Rates of protein synthesis (% per hour) can be calculated for individual brain regions if two other parameters are known: 1) a factor relating the regional protein concentration to that of the brain "mix" standard and 2) the regional valine content of the acid precipitated and delipidated protein extract. Both must be calculated for each experimental condition. Smith et al. (Trans. Amer. Soc. Neurochem., 11:94, 1980) described a method for measuring local brain protein synthesis in adult rat after i.v. injection of L-(1-^{14}C) leucine. Our method avoids the stress of surgery and anesthesia. It is advantages for small animals where arterial and venous catheterization is impractical and in behavioral experiments where immobilization is undesireable. Since flooding amounts of amino acid are used, the problem of estimating the specific activity of the precursor pool under pathological conditions is minimised. The use of an internal standard should correct for problems such as self absorption of radiation, which could vary in pathological tissue.

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255.1 THE CORTICAL LOCALIZATION AND CHARACTERIZATION OF SEROTONERGIC FIBERS IN THE PRIMATE BRAIN. E.C.Azmitia, P.J.Gannon*, C.Clewans*, Dept. of Anatomy, Mt.Sinai Sch. of Med., N.Y., N.Y. 10029.

Serotonergic fibers were reported to be uniformly distributed in the cortex of humans and monkeys. However, boutons labeled by high-affinity reuptake of ^{3}H-5HT shows a varied cortical distribution.

The brains of Macaca fascicularis(2.5-3Kgm female) were quickly removed for analysis. These were prepared either for synaptosomal uptake measurements of radioautographic localization of ^{3}H-5HT uptake into slices (Azmitia & Marovitz, J.Histochem Cytochem, 28:636, 1980). Kinetic studies showed that subcortical and cortical regions had similar uptake mechanisms (Km 1.5x10^{-7}M; (^{3}H-5HT)=0.15-2.00x10^{-7}M, NEN). Comparisons between regions were made at 5x10^{-8}M ^{3}H-5HT. The dorsal raphe (650 pmole/gm/8 min), Amygdala (445±125, n=5), Hypothalamus (470±131, n=4) were among the highest, and the lateral thalamus (142±12, n=3), and the lateral geniculate (82±36, n=3) among the lowest in uptake. The cortical distribution was highest in temporal lobe and lowest in the frontal lobe. The highest uptake occurred in the Sup and Inf Ctx (342±65, n=4; 373±107, n=40, Enthorhinal Ctx (360±95, n=4) and the calcarine Ctx (224±5, n=2) while the sup frontal ctx (165±3, n=2), nonstriate occipital ctx (103±50, n=4) and Precentral ctx (98±36, n=4) were the lowest. The uptake in the hippocampus (214±75, n=7) was highest in the dentate gyrus (288±107, n=4) and lowest in the CA fields (119±48, n=7). Studies to measure the distribution of tryptophan hydroxylase activity are now in progress.

Radioautography showed a dense accumulation of silver grains at high-affinity uptake sites. These were identified as neuronal boutons at the EM level. The density in subcortical areas paralleled the uptake measurements, and the pattern was strikingly similar to that seen in subprimates. In the cortex these sites were seen in all layers, but were usually heaviest in layer IV. In cingulate cortex a heavy band was seen in layer V and in the calcarine cortex the sites occurred in the upper part of layer IV. These findings suggest the serotonergic fibers may have a special relationship with granule cells.

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255.2 THE ONTOGENY OF CENTRAL SEROTONERGIC SYSTEMS IN THE CHICK EMBRYO: AN IMMUNOCYTOCHEMICAL STUDY. J.A. Wallace, M.T. Libber* and J.M. Lauder. Department of Anatomy, Univ. of North Carolina, School of Medicine, Chapel Hill, NC, 27514.

Although mammalian species have frequently been used in morphological studies of monoaminergic neuronal development in the central nervous system, no previous reports have dealt with the ontogeny of monoamine containing neuronal systems in the chick embryo. Because of the advantages of using the chick embryo as an experimental model for developmental studies, we have begun an investigation of the initial phases of the embryogenesis of central serotonergic systems in this species, employing immunocytochemical techniques. Antibodies against serotonin (5-HT) have been used to detect 5-HT -containing cells in serially sectioned paraffin-embedded material utilizing the peroxidase anti-peroxidase staining method. Preliminary results have been obtained from embryos taken at 3,4,5,7 and 9 days of incubation.

Small bilateral groups of 5-HT -containing cells were first observed at the mesencephalic-metencephalic border on day 4 (E4). These lightly stained cells were located adjacent to the floor plate of the neural tube, at the outer boundary of the ventricular zone and contained little if any staining in neuronal processes. By E5, however, a marked increase was observed not only in the number of cells stained, but also in the elaboration and projection of their processes. Large continuous groups of cells were found caudally from the rostral border of the metencephalon extending through myelencephalic regions into the upper cervical spinal cord. Migration and reaggregation of various components of the serotonergic system were already apparent at E5, as well as extensive ascending and descending fiber bundles. Large, intensely stained processes were observed entering the diencephalon at E5, which became more numerous and highly branched throughout the diencephalon at E7, and penetrated ventral portions of the telencephalon by E9.

One especially significant finding was the observation of a small group of cells situated bilaterally in the posterior wall of the presumptive hypothalamus, which appeared to transiently stain for 5-HT, beginning on E5, but could no longer be detected on E7 or E9, although this region was heavily innervated by 5-HT axons at these ages.

These initial studies form the basis for an extensive ontogenic analysis of the serotonergic systems in the chick embryo.

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255.3 IMMUNOCYTOCHEMICAL LOCALIZATION OF SEROTONIN CONTAINING CELL BODIES AND FIBERS IN THE RAT HYPOTHALAMUS. M.Frankfurt*, J.M.Lauder, V.G.Daniels*, E.C. Azmitia, Dept.of Anatomy, Mt.Sinai Sch.Med.NY, NY 10029.

The serotonergic innervation of the rat hypothalamus has been studied by radioautographic, biochemical and immunocytochemical techniques. In this report the rat hypothalamus was studied using a specific antiserum to serotonin. Sections of hypothalamus (50um) were cut on a vibratome and incubated with serotonin antiserum (1/2000) or serotonin antiserum preabsorbed with 10^{-3}M serotonin (18h at 4 C). After incubation with the primary antiserum sections were processed by an immunoperoxidase technique.

Moderate to heavy staining of fibers was seen throughout the entire hypothalamus. In normal, pargyline (200mg/Kg) pretreated and pargyline (200mg/Kg) and L-tryptophan (200mg/Kg) pretreated rats the heaviest staining of fibers was seen in the lateral hypothalamus, the suprachiasmatic nucleus and the periventricular areas. Fibers were seen in the median eminence in both the inner and outer layers, however no labelling of tanycytes was observed. In rats that had received colchicine pretreatment (50 or 100ug) fibers were seen in the arcuate, dorsomedial and ventromedial area.

In addition to the fiber staining a group of neuronal cell bodies immunostained for serotonin in the dorsomedial hypothalamus of rats pretreated with both pargyline and L-tryptophan. These cells were located adjacent to the third ventricle, primarily in the dorsomedial nucleus. The cells measured approximately 9um and extended over a rostro-caudal distance of 900um. No serotonin containing cell bodies were detected in any other areas of the hypothalamus.

These cells corresponded to those described by (Beaudet and Descarries, Brain Research, 160:231-243, 1979). Studies involving lesions of the dorsomedial hypothalamus with 5,7 dihydroxytryptamine, are currently in progress to further establish whether these cells constitute an additional serotonergic cell group, B-10. (Sponsored E.C.Hwang)

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255.4 THE RAPHE NUCLEI OF THE RAT BRAIN STEM, A COMBINED CYTOARCHITECTONIC AND IMMUNOHISTOCHEMICAL STUDY. H.W.M. Steinbusch and R. Nieuwenhuys[x], Dept. of Anatomy and Embryology, Univ. of Nijmegen, Nijmegen, The Netherlands.

Specific antibodies to serotonin(5-HT) were applied to visualize immunohistochemically cell bodies with their dendrites in 50 and 100 µm thick vibratome sections of the rat brain. The aim of this study was to elucidate the localization of 5-HT immunoreactive neurons within and beyond the raphe nuclei. To this end 1) the seven raphe nuclei were subjected to a detailed cytoarchitectonic analysis, 2) the 5-HT positive cell bodies present in the brain stem were plotted and 3) the dendritic pattern of the 5-HT containing neurons were investigated. The cytoarchitectonic analysis was based on a) counterstaining the 50 µm thick vibratome sections mentioned above with cresyl violet to delineate each raphe nucleus and b) Nissl- or Klüver-Barrera stained paraffin material to analyse the cell types. This survey revealed that within the confines of each raphe nucleus two to four cell types are present: small, medium, large and very large. No specific cytoarchitectonic similarities were found among the various raphe nuclei. Cells in all of the four categories may contain 5-HT, but most of the serotoninergic elements belong to the categories medium-sized and large. The cell-type small, present in the four rhombencephalic raphe nuclei, did not contain serotonin. The category very large was only present in the nucleus raphe dorsalis. The investigation of the dendritic pattern of the 5-HT positive neurons showed that they have in general three to five dendrites with relatively few branches. The length and orientation of these processes vary among the different nuclei. The 5-HT immunoreactive cells are not equally distributed within the raphe nuclei and moreover, they form, except for the nucleus raphe dorsalis, only a minority of the total cell population in these centers. Studies in our own and in other laboratories have revealed that in the raphe nuclei, apart from serotoninergic cells, elements containing eight other neuroactive principles occur (see table, in which our own results are indicated with an x).

	raphe obscurus	raphe pallidus	raphe magnus	raphe pontis	raphe dorsalis	centr. superior	lin. oralis
5-HT*	+	+	+	+	+	+	+
DA					+	+	+
NA*					+		
GABA/GAD*					+		
LEU-ENK		+	+		+		
MET-ENK*		+	+		+		
Sub-P*	+	+	+		+		
VIP					+		
CCK					+		+

255.5 SEROTONIN AND THE ULTRASTRUCTURAL ORGANIZATION OF THE RAPHE NUCLEI IN THE MONKEY: AN IMMUNOHISTOCHEMICAL STUDY. N. C. de Lanerolle, S. Kapadia* and C. LaMotte, Sections of Neurosurgery, Neuroanatomy and Gross Anatomy, Yale Univ. Sch. of Med. New Haven, CT 06510.

The distribution of serotonin in the brainstem of Macaca fascicularis was examined with an antibody to a 5HT-BSA conjugate, and the indirect antibody peroxidase-antiperoxidase method of Sternberger. Perikarya with serotonin-like immunoreactivity were located in the nuclei raphe dorsalis, centralis superior, raphe magnus, pallidus and obscurus, the lateral reticular formation, and the lateral reticular nucleus. Electron microscopic examination of the nu. centralis superior and raphe magnus areas revealed the presence of large, medium and small neuronal perikarya, and a neuropil exhibiting a variety of synaptic formations. The immunoreactive perikarya were of medium size (15-30 microns). They are characterized by spherical or oval shaped cell-bodies, which are devoid of somatic spines. They contain a spherical and indented nucleus, and the cytoplasm has a variety of organells. The rough, granular endoplasmic reticulum appears as clumps and laminar stacks. Within the area were also immunoreactive terminal like profiles of two types-- those containing dense-core vesicles in addition to small round vesicles, and those having only small round vesicles. They formed predominantly asymmetrical type junctions. The immunoreactive terminals formed junctions onto both immunoreactive soma and dendrites, as well as onto the soma of small unlabelled perikarya and unlabelled dendrites of a variety of sizes. Unlabelled terminal profiles (those containing dense core vesicles, and those with round agranular vesicles alone) also formed junctions with immunoreactive soma and dendrites. Occasionally, small unlabelled cells formed junctions onto immunoreactive soma, and immunoreactive terminals formed junctions with unlabelled terminals. Glial cells in close aposition to immunoreactive soma were also observed. Further, immunoreactive myelinated axons were seen within the neuropil of these areas.

The observed ultrastructural features of serotonin containing processes provides the probable morphological basis for physiological observations such as autoregulatory modulation of serotonin neurons, correlative firing of serotonin and non-serotonin neurons, and short latency activation of spinal neurons upon raphe stimulation. (Supported by NIH grant NS13335)

255.6 COLLATERALIZATION OF SINGLE NORADRENERGIC NEURONS TO CEREBRAL CORTEX AND SPINAL CORD. K.N. Westlund, R.M. Bowker*, M.G. Ziegler, and J.D. Coulter. Depts. Psychiat. and Physiol. and Marine Biomedical Institute, Univ. of Texas Medical Branch, Galveston, TX. 77550, and Dept. of Medicine, Univ. of California Med. Center, San Diego, CA. 92103.

Individual neurons in the nucleus locus coeruleus have been suggested by a number of studies to have divergent axons which project to two or more different regions of the brain. Until only recently, suitable techniques were not available for demonstrating simultaneously both the collateralizations and chemical identification of neurons in the brain. In this study cells have been labeled from cerebral cortex and spinal cord utilizing retrograde axonal transport techniques and immunocytochemistry with an antibody to dopamine-β-hydroxylase (DβH). In anesthetized rats, HRP was injected into the cortex while antiserum to DβH was injected into the spinal cord. While both HRP and DβH antiserum are known to be retrogradely transported to the cell body, the DβH antiserum is specifically taken up and retrogradely transported by noradrenergic neurons (Silver and Jacobowitz, Brain Res., 1979). Following a 24-48 hour survival period, animals were routinely perfused with 3.8% paraformaldehyde. Frozen tissue sections were first reacted for the retrogradely transported HRP using $CoCl_2$ and diaminobenzidine as the chromagen. The sections were then processed immunocytochemically using rabbit anti-goat IgG and goat peroxidase-antiperoxidase to visualize the cells of origins of spinally projecting noradrenergic neurons. Cells retrogradely labeled with HRP contained black punctate reaction product within a relatively clear to grey tinted cytoplasm. Neurons projecting to forebrain structures were located dorsally in the nucleus locus coeruleus. Locus coeruleus neurons specifically labeled with DβH antiserum from the spinal cord contained small brown granules against the light brown staining of the cytoplasm and proximal dendrites. Noradrenergic spinally projecting cells were located ventrally and caudally in the locus coeruleus in agreement with other studies. In addition to cells labeled with one or the other of the retrograde markers, a population of cells was identified which had black granules within a light brown stained cytoplasm, indicating that these cells contained both HRP and DβH immunoreactivity. Using this technique, it is possible to simultaneously visualize chemically specified, descending noradrenergic neurons and neurons projecting to other areas of the brain. We have also clearly demonstrated that single noradrenergic neurons collateralize and project to widely divergent areas of the brain and spinal cord.

(Supported by NIH Grants NS 12481 and NS 11255).

255.7 THE ONTOGENY OF NE AND THALAMIC PROJECTIONS TO MURINE NEOCORTEX: A SPATIAL SUBSTRATE FOR SEQUENTIAL INNERVATION. Barry E. Kosofsky* and Mark E. Molliver. Depts. of Cell Biology & Anatomy and Neuroscience, J.H.U. Sch. of Med., Baltimore, Md. 21205.

Early in development, monoamine (MA) neurons in the brainstem project widely to the cerebral cortex; there are conflicting reports as to whether they exert a trophic influence upon the maturation of cortical circuitry. We have studied - in fetal mouse - the development of cortical MA afferents in order to characterize the early temporal and spatial relationships of these axons of brainstem origin with respect to other (e.g., thalamic) projections to cortex and to the cellular compartments of immature cortex which they reach. In the mouse telencephalon at E16, five zones constitute the full thickness of the pallium: (1) a proliferative zone nearest the ventricular surface; (2) a migratory intermediate zone that later becomes the subcortical white matter; (3) a subplate zone that is composed of the most mature neurons; (4) the cell dense cortical plate; (5) The molecular layer, a cell-sparse superficial zone.

Noradrenergic (NE) axons in fetal cortex were visualized by histofluorescence enhanced by intrauterine administration of timed fetuses with α-methyl-norepinephrine. Cerebra from littermates were prepared for Nissl and axon stains as well as for serotonin immunocytochemistry (in conjunction with H. Lidov). The NE axons establish an organized and characteristic pattern, well seen at E16: thin, varicose fibers form a tangentially continuous, bilaminar plexus above and below the cortical plate, most dense rostrally with more caudal sites displaying progressively decreased axon density. Fiber bundles reaching the ventral limit of the telencephalon were seen to divide and encase the developing cortical plate. Fluorescent axons, extending longitudinally through the marginal and subplate zones, are not visualized within the cortical plate or in the intermediate zone of the pallium. At these fetal stages, thalamo-cortical axons reach only as far as the intermediate zone, hence restricting their influence upon those most immature neurons migrating outward to the cortical plate. The early presence of brainstem fibers in the subplate zone along with relatively mature neurons, combined with the previous report that this zone is the major site of early cortical synaptogenesis (Kostovic & Molliver, Anat. Rec. 178 (1974) 395), suggest that MA-cortical interactions occur in this location. The spatial segregation of thalamic and MA axons to the intermediate and subplate zones, respectively, provides an anatomic substrate in the form of a spatial template for temporally sequential interaction of these afferents with cortical neurons. (Support NIH Grant NS08153 and GM07309).

255.8 DIFFERENTIAL REGULATION OF TYROSINE HYDROXYLASE IN INDIVIDUAL NORADRENERGIC AND DOPAMINERGIC NEURONS OF RAT BRAIN AS DEMONSTRATED BY COMPUTER ASSISTED QUANTITATIVE IMMUNOCYTOCHEMISTRY. R.H. Benno, L.W. Tucker*, T.H. Joh and D.J. Reis. Lab. of Neurobiology, Cornell Univ. Med. Coll., New York, NY 10021

We used a computer assisted quantitative immunocytochemical technique to examine at the cellular level the differential regulation of tyrosine hydroxylase (TH) in individual noradrenergic (NE) neurons of the locus ceruleus (LC) and dopaminergic (DA) neurons of the substantia nigra (SN). Three groups of six rats were studied. One received reserpine (10 mg/kg, 3d survival), a drug which increases the amount and activity of TH in LC but not SN homogenates by increasing accumulation of enzyme protein (induction) (JPET:193:775, 1975). A second received oxotremorine (dose 1.5 mg/kg), a cholinergic agonist which increases activity but not amount of TH in homogenates of LC but not in SN (JPET:200 :523,1977). The third group was untreated. Sagittal sections of 5u were taken through LC and SN and immunostained for TH using reaction conditions necessary to produce linearity of the staining intensity. Density was measured by a video based image analysis system and expressed as units of average optical density (A.O.D. x 10^{-3} ± SEM), a measure of concentration of TH per unit of tissue. Two regions of LC were examined: the central core with neurons with less TH than in the rim (including subceruleus). These were compared with neurons in SN. Cellular analysis was made at 40X; regional analysis (for neurons and neuropil) at 6.3X. The results of the cellular analysis of 1168 neurons are shown in the Table.

	LOCUS CERULEUS			SUBSTANTIA NIGRA		
	Core	Rim	Rim/Core	SN	SN/Core	SN/Rim
Control	108±9.4	140±7.3	1.30**	133±11.4	1.23*	0.95 NS
Oxotrem	111±9.0	137±8.7	1.23*	141±12.2	1.27*	1.03 NS
Ox/Cont	1.03 NS	0.98 NS	-	1.06 NS	-	-
Reserp	133±7.8	160±5.7	1.20**	132±9.4	0.99 NS	0.83**
Res/Cont	1.23*	1.17*	-	0.99 NS	-	-

*=P<.05 **=P<.01 NS= Not Significant

Reserpine but not oxotremorine increased TH for the entire LC 1.8 fold (P<.01). We conclude: (a) The amount of TH/neuron is similar in the LC rim and SN, thus some NE and DA neurons contain equal amounts of TH; (b) Oxotremorine fails to increase the immunoreactivity of TH; (c) Reserpine increases TH in all classes of LC neurons but not in any cells in the SN , the increase being primarily in neuropil. The close parallelism between the biochemical and cytochemical results demonstrates that quantitative cytochemistry can be used to detect changes in neurotransmitter enzyme protein at the cellular level.

255.9 LAMINAR AND REGIONAL DISTRIBUTION OF NOREPINEPHRINE FIBERS IN SQUIRREL MONKEY NEOCORTEX AS REVEALED BY IMMUNOHISTOCHEMISTRY. S. L. Foote, J. H. Morrison, F. E. Bloom and D. O'Connor*. Salk Institute and (*) UCSD School of Medicine, La Jolla, CA 92037.

The purpose of these studies was to analyze the laminar and regional distribution of norepinephrine(NE) fibers in primate neocortex and to determine the major intracortical trajectories utilized by these fibers as they extend into and permeate various cortical regions. We have utilized an antibody to human dopamine-beta-hydroxylase(DBH), visualized by peroxidase immunohistochemistry, to provide detailed, permanent, bright-field visualization of NE fibers in squirrel monkey neocortex.

Adult squirrel monkeys were perfused with cold phosphate buffer followed by cold 4% paraformaldehyde in phosphate buffer. The brain was then removed, cut into blocks, and post-fixed for 1.5 hours. The blocks were then placed in an 18% sucrose solution overnight. Sections were cut on either a cryostat or sliding microtome and incubated either freely floating or slide-mounted. The primary antibody was raised in rabbit and directed against DBH purified from human pheochromocytoma cells. Sections were incubated overnight in a 1:2000 solution of anti-DBH, washed, then incubated in peroxidase-conjugated goat anti-rabbit IgG and treated with diaminobenzidine in order to visualize the antibody complex.

The laminar pattern of NE cortical innervation in the squirrel monkey is similar to that described previously for the rat (Morrison, et al.,JCN,1978): NE fibers in layers I and VI are predominantly tangential to the cortical surface while fibers in layers II and III are typically radial. The pattern of NE innervation in layers IV and V is more heterogeneous, being characterized by: a) long tangential fibers, b) short, tortuous axon segments of diverse orientations, and c) occassional pericellular swirls. This laminar pattern was similar in several cytoarchitectonic regions. The effects of cortical and subcortical lesions on NE cortical innervation are being studied in order to determine the trajectories of NE fibers as they enter and distribute themselves over the cortical mantle. In preliminary experiments, aspiration lesions within the frontal lobe produced a dramatic reduction in the density of NE innervation in distant cortical regions caudal to the lesion. This result suggests that in primate, as in rat (Morrison,et al.,Neuroscience,1981), a large group of NE fibers enters the neocortex at the frontal pole and proceeds caudally through the deep layers of dorsolateral cortex. Supported by USPHS Grants NS 16209, AA 03504, and AA 07273.

255.10 PEPTIDE-MONOAMINE INTERACTIONS AT THE LEVEL OF BRAIN STEM CATECHOLAMINE GROUPS. J.R. Sladek, Jr. and J. Schöler*. Dept. Anatomy and Center for Brain Research, Univ. Rochester Sch. Med. & Dent., Rochester, NY 14642.

Brain stem catecholamine (CA) groups which comprise the ventral noradrenergic pathway contribute a dense innervation pattern to hypothalamic neurosecretory neurons of the paraventricular (PVN) and supraoptic (SON) nuclei. Simultaneous analysis of monoamine histofluorescence and peptide immunohistochemistry indicates that the noradrenergic innervation patterns favor vasopressin over oxytocin target neurons by about a 3.5:1 ratio in rat (Sladek et al., Peptides 1, supp.1:141, 1980); a similar pattern, although as yet unquantified, is seen in monkey. The PVN is known to be the site of origin of descending projections which reach caudal brain stem and spinal cord. Many of these fibers reach the A2 region of the medulla (Nilaver et al., Neuroendo.30: 150, 1980) which raises the possibility of a reciprocal-type innervation existing between vasopressin and/or oxytocin fibers and CA-containing perikarya. The present study addressed this concept in 3 month old, male Fischer 344 rats by the application of a combined histofluorescence-immunohistochemical approach. Analyses were made from sections which had been prepared for formaldehyde-induced fluorescence of monoamines. Tissue samples were freeze-dried, treated with formaldehyde vapor at 80°C, paraffin embedded, and serially sectioned. Catecholamine perikarya were identified in brain stem groups A1, A2, A5 and A7 with fluorescence microscopy. Adjacent sections were stained for rat neurophysin (courtesy of Alan Robinson) and were analyzed in concert with histofluorescence sections in a comparator bridge microscope. Neurophysin-positive fibers were found within each CA group; moreover neurophysin fibers were seen in apposition to several CA-containing perikarya in each group. This phenomenon was especially prominent in the A1 group of the lateral reticular formation wherein the intensely fluorescent, multipolar, CA neurons often appeared rimmed with neurophysin-positive fibers along their perikaryon and proximal dendrites. Pertinent to this is the recent observation (Sawchenko & Swanson, Anat. Rec. 199: 225A, 1981) that the A1 group contributes the main source of CA fibers to the PVN. Together, these findings suggest a possible morphological link between hormone-specific neurosecretory neurons of the hypothalamus and CA neurons of the brain stem which could serve as a reciprocal feedback circuit for the mediation and/or monitoring of neuroendocrine activity. Supported by USPHS Grants NS 15816 and AG 00847 to JRS.

256.1 INTERACTION BETWEEN SEROTONERGIC AND BETA ADRENERGIC RECEPTORS IN THE NICTITATING MEMBRANE OF THE CAT. E. Adler-Graschinsky * (SPON:
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It has been proposed that presynaptic serotonergic and β-adrenergic receptors of the rat cerebral cortex participate, through a common site of action, in a positive feedback regulation of norepinephrine (NE) release by nerve stimulation (Adler-Graschinsky and Martínez, Adv. Biosci. 18: 299, 1979). The aim of this communication has been to study whether such interaction was also present at the level of the peripheral neuroeffector synapses.

In the experimental model selected, the isolated nictitating membrane of the cat, two consecutive frequency-response curves to nerve stimulation were applied. The frequency of stimulation that produced 50% of the maximal contractile response (EF 50), expressed as the log (100 x EF 50) was 2.15 ± 0.12 in the first and 2.01 ± 0.08 in the second control curve (n = 6).

The frequency-response curve to nerve stimulation was shifted to the left in the presence of 0.1 μM serotonin. The log (100 x EF 50) was 2.08 ± 0.07 for the first control curve and 1.82 ± 0.06, $p < 0.025$, for the second curve in the presence of serotonin . This displacement was prevented both by β-adrenoceptor antagonists (0.1 μM propranolol) and by serotonergic antagonists (0.1 μM methysergide, 0.1 μM pizotifen and 0.1 μM morphine).

The effect of serotonin is not likely to be related to changes in the sensitivity to the neurotransmitter released by the stimulation since it did not modify the concentration-response curve to exogenous NE (pD_2=5.31 ± 0.05 in a first control curve and 5.16 ± 0.07, n=6, in a second curve in the presence of 0.1 μM serotonin). With the exception of morphine, that produced a slight shift to the right in the concentration-response curve to NE, the sensitivity to the exogenous neurotransmitter was not modified by the antagonists employed.

The present results suggest that the potentiation of the responses to nerve stimulation produced by serotonin may result from an increase in the amount of NE released by the stimulation. In addition, the data presented are compatible with the hypothesis that an interaction between presynaptic serotonergic and β-adrenergic receptors, similar to that described for the central nervous system,is also present at the level of the peripheral sympathetic fibers.

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256.2 IONTOPHORETICALLY APPLIED BENZODIAZEPINES INHIBIT LOCUS COERULEUS UNIT ACTIVITY. Howard K. Strahlendorf and Jean C. Strahlendorf. Departments of Medical and Surgical Neurology and Physiology, Texas Tech University Health Sciences Center, Lubbock, Texas, 79430.

Benzodiazepines (BDZ) represent the most widely used class of anxiolytics. These compounds also possess skeletal muscle relaxant effects, anticonvulsant properties and are useful in suppressing motor and autonomic symptoms of withdrawal from opiates and ethanol. BDZ presumably enhance the synaptic efficacy of GABA at central nervous system synapses. Neurons of the locus coeruleus (LC) have membrane receptors for GABA and iontophoretically applied GABA profoundly inhibits LC unit firing. The LC by virtue of its efferent connections to the spinal cord, cerebellum, cerebral cortex and limbic system may represent an important substrate for many BDZ actions e.g., anxiolytic, sedative, skeletal muscle relaxant. We are studying the effects of BDZ on LC neurons applied by microiontophoresis.

Chloral hydrate anesthetized male rats were used in all experiments. Conventional 5 or 7 barrel micropipettes containing 2M NaCl saturated with fast green in the center recording barrel were employed to record single LC unit activity. Drug barrels contained 0.2M GABA, 0.1M flurazepam di-HCl (FLU), 0.1M chlordiazepoxide HCl (CDP), 0.1M glycine (all in distilled water) and 0.01M clonidine HCl in 165mM NaCl. The current balance barrel contained 4M NaCl. LC neurons were identified by characteristic electrophysiologic criteria at the time of recording and histologically at the end of each experiment. Both CDP and FLU markedly suppressed LC firing when applied iontophoretically in pulses 30 sec. long at currents ranging from 5nA to 30nA. Higher currents often produced membrane effects. Percent inhibition of discharge rate ranged from 5% to 82% (n=20 cells) and appeared to be dose related for a given set of neurons tested with the same pipette. FLU was more active than CDP. With a 40 sec. off time between ejection pulses no cumulative effects were seen. Occasionally (n=3 cells), tolerance appeared to repeated test applications and a rebound excitation also occurred. Interaction with GABA-elicited inhibition will also be presented. These data demonstrate that BDZ can profoundly inhibit LC unit activity and provide preliminary evidence that the LC may be an important site for some BDZ actions.

256.3 DOPAMINERGIC MEDIATION OF EFFECTS OF APOMORPHINE ON MIDBRAIN RAPHE NEURONS. E.H-Y. Lee* , and M.A. Geyer. Dept. of Neurosciences and Psychiatry, Univ. of California at San Diego, Sch. of Med., La Jolla, CA 92093.

In an effort to assess the functional inter-relationships between monoaminergic systems in brain, we have used quantitative cytofluorimetry to measure changes in cellular serotonin (5-HT) produced by the dopaminergic agonist apomorphine. A measure of fluorescence fading using a computerized microspectrofluorimeter enables us to discriminate changes in 5-HT from changes in catecholamines and to discriminate intracellular from extracellular amines.(Geyer et al.,1978.J. Pharm. Expl. Ther. 207 :650-667) Thirty minutes after intraperitoneal injections of saline or various doses of apomorphine, male rats(125-150gms) were sacrificed, the midbrain raphe area was removed, freeze-dried, treated with formaldehyde, and embedded in paraffin. Microscopic measures were made in both the dorsal and median raphe nuclei in 8 micron sections. The results showed that 0.1,1,3 and 10 mg/kg apomorphine all increased intracellular 5-HT content in the dorsal raphe significantly without affecting the median raphe. On the other hand, 20 mg/kg apomorphine significantly decreased intracellular 5-HT level in both nuclei. Thus, this high dose of apomorphine may cause a non-specific pharmacological action. Extraperikaryal 5-HT levels were also elevated in the dorsal raphe by 3,10 and 20 mg/kg apomorphine, suggesting some alteration in 5-HT release and/or turnover rate within this nucleus. The dorsal raphe nucleus can be divided into the following five subdivisions: the dorsomedial, dorsolateral,ventrolateral and ventromedial, which is further divided into the dorsal and ventral parts. Regional mapping experiments revealed that apomorphine slightly increased intracellular 5-HT level in each of these subdivisions but this effect was most pronounced in the dorsal part of the ventromedial subdivision.

To examine the time-course of apomorphine's effect, additional animals were sacrificed at various times (10 min to 80 min) after 0.1 mg/kg apomorphine. Apomorphine increased the intracellular 5-HT fading measure in the dorsal raphe as soon as 20 min after injection, with the maximal effect occurring at 1 hr.

Haloperidol, a dopaminergic antagonist, decreased 5-HT levels in cells of the dorsal raphe at a dose of 0.8 mg/kg. A lower dose of haloperidol (0.4 mg/kg), which had no significant effect by itself, completely blocked the effect of 1 mg/kg apomorphine in the dorsal raphe. This result supports the hypothesis that the effects of apomorphine on 5-HT neurons are secondary to dopamine receptor stimulation. Further studies are in progress to clarify the changes in catecholamines within the raphe nuclei and to determine the anatomical pathway and mechanism responsible.

256.4 NE AND 5-HT LESIONS DIFFERENTIALLY ALTER BASAL ACTIVITY AND RESPONSIVENESS TO SENSORY STIMULI OF TYPE A AND B SUBSTANTIA NIGRA DA CELLS. L.A. Chiodo, A.R. Caggiula and S.M. Antelman*. Psychobiology Program, Depts. of Psychology and Psychiatry, Univ. of Pittsburgh, Pittsburgh, PA 15260.

We have recently demonstrated the existence of two functional subclasses of dopamine (DA) cells in the zona compacta of the substantia nigra (Chiodo et al., Brain Res. 189:544, 1980): type A cells which increase, and type B cells which decrease their discharge rate in response to sensory stimuli. Since other work has shown that both NE and 5-HT systems also modulate DA cell activity, in the present study we examined the influence of NE and 5-HT lesions, both alone and in combination, on both basal firing and the responsiveness of A and B cells to sensory stimuli.

The basal discharge rate of verified DA cells and their response to mild tail pressure and light flashes were determined in male rats which were either unoperated or given one of the following lesions 3-4 weeks beforehand: 1) 5-HT; an intraventricular injection of 5,7-DHT (225 ug free base) 30 min. after DMI (30 mg/kg i.p.), 2) NE; intraventricular 6-OHDA (250 ug free base) 30 min. after buproprion hydrochloride (50 mg/kg i.p.), 3) combined 5-HT and NE lesions, 4) sham lesions.

Basal discharge rates of type A and B neurons were significantly increased by NE lesions (151% and 197% of control respectively) and by combined NE and 5-HT lesions (140% and 165%). On the other hand, selective 5-HT destruction increased the activity of type B cells (159%) but had no effect on type A neurons.

Similar to our previous reports, tail pressure increased type A activity (135% of baseline) and decreased that of B cells (72%) in controls. Light flash increased the activity of both cells (130%). The responsiveness of type A and B neurons to sensory stimuli was totally abolished by 5-HT lesions and by combined 5-HT and NE lesions. By contrast, selective NE lesions significantly enhanced the effects of sensory stimuli on type A cells while having no influence on the type B response.

Our results indicate that the responsiveness of nigral DA neurons to sensory stimuli is critically dependent on the integrity of 5-HT but not of NE-containing neurons. However NE does appear to exert an influence, at least for the type A cell. Further, this study provides no evidence that either 5-HT or NE is responsible for the divergent effects of sensory stimuli on A and B cells. These data, together with additional observations that A and B neurons respond identically to the iontophoretic application of GABA and that kanic acid lesions of striatonigral feedback loops also fail to abolish A/B differences, suggest the possibility that the organization of local intranigral circuitry may underlie these effects.

256.5 SEROTONIN'S POTENTIATION OF ACETYLCHOLINE INDUCED CONTRACTIONS IN THE LEECH PENILE ENVERSION MUSCLE. E. K. McGlade*, W. J. Higgins* and B. Zipser. (SPON: C. R. Creveling). Dept. of Zoology, Univ. of Maryland, College Park, Maryland 20742.

Cholinergic and serotinergic neurons are found in the vicinity of the leech (Heamopis marmorata) penile enversion muscle (LPEM). Contractures of the LPEM induced by either acetylcholine (ACh) or electric field stimulation are potentiated by low doses of serotonin (5HT; threshold = 5×10^{-9}M). In addition 5HT accelerates relaxation. However, 5HT by itself at any concentration does not measurably alter the resting tension. Other biogenic amines investigated (norepinephrine, epinephrine, octopamine, and dopamine) only nonspecifically (threshold = 10^{-4} or 10^{-3}M) alter the resting tension of the ACh induced response.

The current investigation will ultimately determine the mechanism of 5HT potentiation of the ACh and electric field stimulated contractions. This information will characterize the role of 5HT in regulation of LPEM contractility and perhaps clarify the modulatory role which amines play in intercellular communication.

While several conventional cholinergic blocking agents (d-tubocurarine, hexamethonium, or atropine) fail to inhibit the ACh response; however, benzoquinonium (Bzq) is a potent ACh antagonist in this system ($I_{50} = 10^{-7}$M). In experiments utilizing Bzq, 5HT continued to potentiate the contractions elicited by electric field stimulation and accelerated relaxation. These data suggest that 5HT acts on the muscle but do not exclude the possibility that 5HT also acts presynaptically at the cholinergic motor neuron.

Future work will investigate the actions of 5HT on the electrical properties of the LPEM.

256.6 SOCIAL ISOLATION IN RATS ALTERS FIRING RATES OF SINGLE DOPAMINERGIC AND SEROTONERGIC NEURONS AND AFFECTS THEIR RESPONSIVENESS TO CLONIDINE. G. R. Christoph*, R. J. Leonzio*, L. G. Davis and M. D. Dibner. Central Research and Development Department, E. I. du Pont de Nemours and Co., Wilmington, DE 19898.

Conventional single-neuron recording techniques were used to record the spontaneous firing rate of presumed dopamine-containing neurons in the substantia nigra pars compacta and serotonin-containing neurons in the dorsal raphe nucleus of chloral hydrate anesthetized rats. For one to eight days prior to the recording session, the rats were housed 2-3/cage (GROUP) or 1/cage (SINGLE). Presumed dopamine-containing neurons fired significantly slower in GROUP rats (3.6 ± 0.35 spikes/sec, N=17) than in SINGLE rats (5.0 ± 0.33 spikes/sec, N=10). Administration of clonidine (0.01-0.04 mg/kg, i.v.) significantly increased the firing rate in GROUP rats (to 4.9 spikes/sec), whereas clonidine had no effect in SINGLE rats. When SINGLE rats were converted to the GROUP condition for two days the electrophysiological and clonidine responses of nigral cells were the same as those for rats that only had GROUP housing. Unlike dopaminergic cells, presumed serotonin-containing neurons in the dorsal raphe nucleus fired significantly faster in GROUP rats (1.01 ± 0.11 spikes/sec, N=10) than in SINGLE rats (0.66 ± 0.05 spikes/sec, N=17). Clonidine administration reduced the firing rate of serotonergic neurons for both SINGLE and GROUP rats but was more effective in SINGLE rats ($ID_{50} = 0.003$ mg/kg, i.v.) than in GROUP rats ($ID_{50} = 0.006$ mg/kg, i.v.). Manipulation of ambient temperature of SINGLE rats (37°C, 3 hr) prior to recording dopaminergic and serotonergic neurons produced a pattern of results (baseline rates and clonidine effects) similar to that observed for normal GROUP-housed rats. Another physiological measure, systolic blood pressure, was significantly higher in GROUP rats (131 mm Hg) than in SINGLE rats (118 mm Hg). Scatchard analysis of receptor binding experiments with α_2 (^{3}H-p-aminoclonidine) and α_1 (^{3}H-WB4101) ligands in rat cerebral cortex and thalamus showed no detectable receptor differences between GROUP and SINGLE rats. The present work demonstrates that relatively brief manipulations of a rat's environment alters monoaminergic neuronal activity, responsiveness to clonidine, and blood pressure. These physiological changes apparently are not due to changes in α-adrenergic receptor properties.

256.7 DOPAMINE CAN MODULATE THE INHIBITORY EFFECTS OF GABA ON SUBSTANTIA NIGRA PARS RETICULATA NEURONS. B.L. Waszczak and J.R. Walters. NINCDS, NIH, Bethesda, MD 20205.

The presence of dopamine (DA) in dendrites of substantia nigra (SN) pars compacta DA neurons raises the possibility that dendritic release of the transmitter might exert local effects on adjacent non-dopaminergic SN pars reticulata neurons. These cells, which project largely to motor-related areas such as the thalamus and superior colliculus, are markedly sensitive to inhibition by iontophoresed GABA and likely to receive a GABAergic input. It was of interest to assess whether DA could influence non-dopaminergic nigral output pathways either by 1) directly altering reticulata cell firing, or 2) modifying the effects of GABA on these cells.

Extracellular, single unit activity of pars reticulata neurons was monitored in chloral hydrate-anesthetized male rats, 250-300g. Attempts were made to antidromically activate the cells from the ventromedial nucleus of the thalamus (VMT). For each cell, repeated 30 sec iontophoretic pulses of GABA (0.001 M in 0.2 M NaCl) and glycine (GLY; 0.1 M), separated by 30 sec baseline periods, were applied before and during simultaneous iontophoresis of DA (0.2 M, 10 nA ejection current). Ejection currents were selected for GABA and GLY which could inhibit firing by more than 50%, but not totally. Under these condtions, iontophoresed DA caused increases in reticulata cell firing to an average of 27% over baseline ($n=20$, $p<.001$). In addition to the increase in rate, DA consistently and markedly attenuated the inhibitory responses of cells to GABA. The degree of this attenuation was not correlated with nor dependent upon the increase in firing. Both the absolute inhibition, in numbers of spikes, as well as the percent inhibition by GABA were significantly reduced by applied DA ($n=18$, $p<.001$). In contrast to its attenuation of GABA responses, DA did not consistently alter responses of cells to iontophoresed GLY ($n=15$).

To assess the specificity of these actions of DA on reticulata neurons, studies were conducted in which an equimolar solution of norepineprhine (NE; 10 nA ejection current) was substituted for DA. While NE caused similar average increases in firing to 30% over baseline ($n=16$, $p<.01$), it did not consistently or significantly alter, over all cells tested, the absolute amount of inhibition elicited either by GABA ($n=15$) or GLY ($n=12$). In both NE and DA experiments, approximately 60% of reticulata cells tested could be antidromically activated from the ipsilateral VMT.

These results indicate that DA, released from dendrites within the nigra, could potentially modulate the actions of GABA on SN pars reticulata cells projecting to motor areas, including the VMT. The increased firing elicited by DA alone may reflect either a direct excitatory action and/or its ability to modulate inhibitory influences provided by GABAergic inputs these cells receive.

256.8 PROLACTIN STIMULATES DOPAMINE RELEASE FROM RAT STRIATAL TISSUE SUPERFUSED IN VITRO. Yiu-Fai Chen* and V. D. Ramirez. Department of Physiology and Biophysics, University of Illinois, Urbana Illinois 61801 (SPON: E. J. Roy)

Spontaneous and induced release of dopamine from fragments of male rat striatal tissue superfused in vitro was examined in the present experiments. Adult male Holtzman rats were killed by decapitation at 1000-1100 h (during a 0500-1900 h light schecule). Striatal tissues were quickly dissected and placed in ice cold Krebs Ringer Phosphate buffer (pH 7.4) containing glucose(10 mM), bovine serum albumin(0.1%), ascorbic acid(0.1%), and pargyline (3.5×10^{-4}M). This same buffer was also used for superfusion of the tissue. The equivalent of 2 rat striatal fragments were used in each experimental chamber. The superfusion chamber was positioned in a constant 37° C water bath. The volume held in the chamber was 500 ul. The medium was pumped through the chamber at a 100 ul/min flow rate. Following a 40 minute stabilization period, effluent samples were collected on ice in 0.1 N $HClO_4$(final concentration) at 4 minute intervals. Dopamine was determined by a specific radioenzymatic assay with a sensitivity of 31 to 62 pg. The enzymatic reaction for this assay involved transfering a tritium labeled methyl group from S-adenosyl-L-methionine to the catecholamines. The methylated products were extracted and finally separated on Dowex-50-W-X-4 cation exchange columns. The spontaneous release of dopamine from striatal tissue was relatively stable. The basal release rate over a 76 minutes period (19 intervals) was 22.5 ± 1.2 pg/mg/min. Augmented release in response to a depolarizing pulse injection of 60 mM K^+ verified tissue viability at the end of superfusion.

In experimental chambers, prolactin infused for 24 minutes significantly increased dopamine release from pre-infusion basal levels of 17.9 ± 1.6 to 34.0 ± 4.1 pg/mg/min during infusion ($n = 4$ experiments). Within 8 - 12 minutes after the removal of prolactin the release of dopamine returned to basal levels (18.1 ± 2.9 pg/mg/min). Infusion with heat-denatured prolactin (boiled for 30 minutes) had no effect on dopamine release (24.3 ± 4.1 pg/mg/min, $n = 4$ experiments). Furthermore, thyrotropin-releasing-hormone (TRH, 10 ng/ml infused for 24 minutes) had no effect on dopamine release from striatal tissue (16.7 ± 1.7 pg/mg/min, $n = 4$ experiments). These results demonstrate that prolactin has a stimulatory effect on dopamine release from striatal fragments of male rats in vitro. The absence of a response to heat-denatured prolactin and TRH demonstrates the specificity of this response and suggests that the basal ganglia may constitute a target tissue for prolactin. (Supported by a NIH grant HD 14625 to VDR.)

256.9 AMPHETAMINE INCREASES ACETYLCHOLINE RELEASE FROM THE PHRENIC NERVE: EVIDENCE FOR A NORADRENERGIC-MEDIATED RESPONSE. R. Michael Snider and Michael C. Gerald, Division of Pharmacology, College of Pharmacy, The Ohio State University, Columbus, Ohio 43210.

(+)-Amphetamine (AMPH) increases physical performance and endurance in humans and animals. A similar stimulatory effect has been demonstrated in the isolated mammalian nerve-skeletal muscle preparation, suggesting the increase in performance may be due, in part, to a peripheral component. Experiments were conducted to examine the mechanism by which AMPH stimulates neuromuscular function employed the rat phrenic nerve-diaphragm preparation. Using indirect methods, the results of previous studies in this laboratory suggest that AMPH may be capable of increasing acetylcholine (ACh) release from motor nerves: (1) AMPH increased MEPP frequency and EPP amplitude (Snider and Gerald, Pharmacologist 22:165, 1980); and (2) in the incannulated diaphragm AMPH enhanced contractions elicited by nerve stimulation but not by ACh injection (Gerald and Snider, Fed. Proc. 40:278, 1981).

In the present studies ACh release from the vascular perfused rat phrenic nerve-diaphragm preparation was directly measured radioenzymatically (Bierkamper and Goldberg, JEPT 6:40, 1978). AMPH (30-300 μM) produced a 45-478% increase in ACh release. To investigate the mechanism responsible for this stimulatory effect, rats were pretreated with reserpine (5 mg/kg, 18 hr) or α-methyl-p-tyrosine (α-MT) (250 mg/kg, 18 hr) and the AMPH-induced release of ACh measured. AMPH (100 μM) alone produced a 315% increase in ACh release. α-MT pretreatment completely blocked this stimulatory effect, whereas reserpine pretreatment decreased the AMPH-induced enhancement of ACh release to 108%. These results, suggesting catecholamine involvement in AMPH-induced ACh release, are supported by the result that norepinephrine (5×10^{-6}M) also increased ACh release (99%).

AMPH (135-270 μM) produced a significant 10-30% increase in nerve-stimulated muscle contractions. This stimulatory effect was completely blocked by tissue preincubation with phentolamine (3×10^{-5}M). Similarly, phentolamine attenuated the AMPH-induced (100 μM) increase in ACh release to 30%.

These studies demonstrate that AMPH increases ACh release. Results presented are consistent with the hypothesis that this effect is mediated by catecholamines which in turn stimulate ACh release via a presynaptic alpha-adrenergic mechanism. (Supported by NIDA grant DA-01477).

256.10 CORTICAL MODULATION OF STRIATAL CHOLINERGIC ACTIVITY. J.R. Simon. Inst. of Psych. Res., Depts. of Psychiat. & Biochem., Indiana Univ. Sch. of Med., Indianapolis, IN 46223.

The striatum receives a substantial innervation from the cerebral cortex, and this is believed to be, in large part, glutamatergic in nature. Thus, destruction of the frontal cortex results in decreased uptake and content of glutamate in the striatum. The interactions of this cortico-striatal system with other neuronal systems in the striatum is of considerable interest. This study focuses on the possibility of a glutamatergic-cholinergic interaction. To examine potential alterations in striatal cholinergic activity, use was made of the ability of the high affinity choline uptake (HAChU) system to change in response to varying states of activity of cholinergic neurons. In the present study, the effect of selective ablation of the frontal cortex on striatal HAChU was investigated. Two weeks after cortical ablation, the levels of glutamate were decreased approximately 30% in synaptosomes prepared from the ipsilateral striatum. In these same tissue samples, glutamate uptake and HAChU were also decreased by approximately 40% and 50%, respectively, whereas GABA uptake was unaffected. In acute experiments (1 hr. post cortical ablation), HAChU was again found to be significantly reduced, while in the same samples, glutamate and GABA uptake were unchanged relative to sham operated animals. This observation of decreased choline uptake 1 hr. after decortication suggests the existence of an excitatory (perhaps glutamatergic) influence of cortical origin on striatal cholinergic neurons. To further investigate this possibility, 10 nmoles of kainic acid (KA) was locally injected into the striatum 1 hr. after removal of the ipsilateral frontal cortex, and the animals were sacrificed 2 minutes after injection. This was done in an attempt to restore the excitatory input to the striatum which was presumably removed by the cortical ablation. KA injection into the striatum of control animals (acute; 2 min) resulted in a significant increase (30%) in HAChU compared to uptake in the contralateral striatum. KA injection into the striatum of decorticate animals also resulted in increased HAChU of similar magnitude (25%) compared to the contralateral striatum. When compared to uptake in the striatum from the lesioned side, this increase amounted to approximately 70% owing to the significantly reduced uptake due to the lesion. Thus, KA injection into the striatum of decorticate animals appeared to reverse the effect of the lesion on HAChU. These observations suggest that cortical afferents to the striatum, which may use glutamate as the transmitter, influence the activity of striatal cholinergic neurons. Whether this cortical modulation of cholinergic activity is mediated via direct or indirect interactions remains to be examined. (PHS NS 15951).

256.11 FFFECT OF MORPHINE ON HIGH-AFFINITY CHOLINE UPTAKE IN THREE REGIONS OF THE RAT BRAIN. M. L. Arceneaux* and S. F. Atweh (SPON: R. J. Dinerstein). Dept. of Neurology, University of Chicago, Chicago, IL 60637.

Sodium-dependent High-Affinity choline uptake (HACU) in synaptosomal preparations is associated with cholinergic terminals in rat brain. Recent evidence suggests that HACU may be the rate limiting step in the synthesis of acetylcholine (Ach). Changes in the rate of HACU correlate well with cholinergic activity in rat brain (Atweh et. al., Life Sci, 17:1535, 1975), thus making it feasible to use synaptosomal HACU as a measure of Ach turnover. Morphine inhibits the release of Ach in the guinea-pig intestine and rat occipital cortex. Opiate receptors and endogenous opioids are also enriched in certain brain regions which are rich in cholinergic markers. We investigated the effect of morphine on HACU in three brain regions where interactions between opiate receptors and Ach may take place.

Rats were injected with varying doses of morphine or saline and sacrificed 30 minutes later by decapitation. Brains were quickly removed and dissected in cold saline. Crude synaptosomal fractions of brain homogenate were prepared from frontal cortex, hippocampus and striatum. HACU was measured by incubating the synaptosoms with ^{3}H-Choline in normal and Na-free Kreb's. HACU showed a 35% decrease in Vmax in hippocami of morphine treated rats as compared to controls ($P < 0.05$), but not in striatum or cortex. This decrease was dose dependent, but statistically significant only at high sedating doses of morphine (30-50 mg/kg). Naloxone by itself did not affect hippocampal HACU, but reversed the morphine effect. In vitro morphine did not affect HACU in all brain regions tested. Preincubation of synaptosomes in high-K^+ (62meq) solution resulted in a Ca^{++}-dependent stimulation of HACU in both striatal (+34%) and hippocampal (+92%) synaptosomes. The presence of 10μm Morphine in the high-K^+ solution did not affect this increase in either brain region.

This data suggest that high dose morphine treatment decreases cholinergic activity in the hippocampus. This effect is not due to presynaptic inhibition of Ach release in hippocampal terminals, since morphine has no effect on K^+-stimulated release, but is probably due to the sedating effect of high doses of morphine and inhibition of the septal-hippocampal neurons, as is seen with other sedatives such as barbiturates and general anesthetics. Although the striatum is rich in opiate receptors, these results do not suggest a significant interaction of opiate receptors with cholinergic neurons in the striatum.

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256.12 NALOXONE POTENTIATION OF APOMORPHINE STEREOTYPY AND INFLUENCE OF PRETREATMENT WITH μ-, σ- AND κ-OPIATE DRUGS. Raymond M. Quock. Div. Pharmacology, Marquette Univ., Milwaukee, WI 53233.

The dopaminergic agonist apomorphine (APO) evokes stereotypic climbing activity in mice (Protais, Costentin & Schwartz, Psychopharmacol. 50:1, 1976). Previous studies in our laboratory have shown that prior treatment with the narcotic antagonist naloxone (NX) significantly potentiates APO climbing (Quock & Lucas, Life Sciences 28:1421, 1981). We have speculated that the enhanced dopaminergic drug effect might result from NX blockade of presynaptic opiate receptors that are located on dopaminergic nerve terminals. The purpose of this investigation was to ascertain whether the opiate receptor species involved in this interaction might be identified. This was accomplished through treatment of animals with morphine, SKF 10,047 (N-allylnorphenazocine) and ketocyclazocine which compete with NX for binding to μ-, σ- and κ-opiate receptors, respectively. Male ICR mice were used in this investigation. In control experiments, NX (1.0 and 5.0 mg/kg) administered 5 min prior to the APO (2.0 mg/kg) challenge produced significant potentiation of APO climbing activity which was scored 10 and 20 min following the challenge. In one series of tests, mice were pretreated with varying doses of one of the three opiate drugs 10 min prior to APO challenge to ascertain the influence of these drugs upon APO climbing. In another series of tests, mice were pretreated with varying doses of one of the three opiate drugs 5 min prior to NX treatment and 10 min prior to APO challenge to ascertain whether these drugs might competitively reverse the potentiating influence of NX upon APO climbing. Doses of morphine between 1.0 and 5.0 mg/kg exerted no behavioral suppressant effect upon APO climbing; yet these same doses did effectively reverse the potentiating influence of NX upon APO climbing. Doses of SKF 10,047 between 1.0 and 5.0 mg/kg also exerted no behavioral suppressant effect upon APO climbing; yet these same doses did likewise effectively reverse the potentiating influence of NX upon APO climbing. Doses of ketocyclazocine between 1.0 and 10.0 mg/kg did significantly reduce APO climbing; however, these same doses failed to reverse the potentiating influence of NX upon APO climbing. Based upon these experimental findings, we conclude that: (1) potentiation of APO climbing activity in mice by NX may involve NX blockade of μ- and/or σ-opiate receptor types; and (2) κ-opiate receptor types appear not to be involved in the NX/APO drug interaction. (This research was supported in part by a research grant from the American Parkinson Disease Association and also in part by a Faculty Development Award from the Marquette University Graduate School.)

256.13 Neuroleptic-like Actions of Cholecystokinin. Steven L. Cohen,* Carol A. Tamminga, Martha Knight, Thomas N. Chase. NINCDS, NIH, Bethesda, Md., 20205; Maryland Psychiatric Research Center, Baltimore, Maryland 21228.

Cholecystokinin octapeptide (CCK-8) has been localized within the mammalian central nervous system and is thought to function there as a neurotransmitter. It has been identified within cell bodies and terminals of certain dopamine (DA)-containing neurons of the A10 (mesolimbic) DA cell group. Since this sytem has been implicated in mediating the antispychotic effect of neuroleptic drugs, we tested the neuroleptic potential of CCK-8 by performing a group of behavioral, biochemical, and endocrinologic experments. All CCK-8 was tested for purity prior to use and dose dilutions were verified by HPLC after experiments. Classic behavioral effects of neuroleptic drugs in laboratory animals include a reduction in learned conditioned-avoidance behavior, a blockade of apomorphine induced stereotypy, and the production of catalepsy. A conditioned-avoidance paradigm was employed, utililizing a signaled-avoidance task with a tone and light conditioned stimulus, a shuttle box, and a 0.7 ma shock. The effects of CCK-8 (.02-3.8 mg/kg i.p.), haloperidol (100 µg/kg i.p.) and saline were compared in different groups of 3-day trained animals on learned conditioned-avoidance behaviors. Both those groups receiving CCK-8 and Haloperidol evidenced significant reductions in avoidance behavior. Additionally, CCK-8 potentiated the effect of haloperidol on the avoidance task and facilitated extinction of the previously learned avoidance response. Stereotypy induced by apomorphine was diminished by CCK-8. CCK-8 did not induce catalepsy at doses up to 1.28 mg/kg i.p. Biochemical studies focused on elucidating the effect of CCK-8 on DA turnover using the AMPT-induced inhibition of DA synthesis. Male rats pretreated with either AMPT (350 mg/kg) or saline were given CCK-8 in varying doses or placebo by subcutaneous and intraventricular route. Dissections were made of the medial striatum, olfactory tubercle, nucleus accumbens, and median eminence (ME). Tissues for analysis of DA levels were sonicated in 0.4 N perchloric acid, purified over an alumina column, and analyzed with an electrochemical detector on a high pressure liquid chromatography system. Results suggest that CCK-8 decreases the turnover of DA in the ME. Additionally, CCK-8 increases plasma prolactin levels in the unanesthetized male rat. These behavioral and biochemical results suggest that exogenously administered CCK-8 may modify certain functions mediated by central DA systems.

256.14 NEUROENDOCRINE, NEUROANATOMICAL AND NEUROPHARMACOLOGICAL STUDIES OF A PUTATIVE VENTRAL TEGMENTAL AREA/N. TRACTUS DIAGONALIS NEURONAL SYSTEM. E. A. Muth* and D. M. Jacobowitz (SPON: J. A. Moyer). Lab. Clin. Sci., NIMH, Bethesda, MD 20205 and Dept. of Pharmacology, George Washington Univ., Wash., DC 20037.

The central effects of muscarinic and nicotinic cholinergic pharmacological agents on luteinizing hormone (LH) secretion, ovulation, and sexual behavior in the rat have suggested the participation of central cholinergic neurons in the feedback effets of gonadal hormones. In a previous study, both the n. tractus diagonalis (td), a cholinergic cell body area, and the ventral tegmental area (avt), the location of the A10 dopaminergic cell bodies, showed changes in cholinergic activity in male and female rats after gonadal hormone manipulations which altered LH secretion (Muth, Crowley, and Jacobowitz, Neuroendo. 30: 329-336, 1980). These results suggested (a) that these two brain areas may be anatomically related, and (b) that such a system may participate in the feedback control of LH secretion. Subsequent neuroanatomical studies revealed a direct mesolimbic dopaminergic projection from the avt to the td, several limbic cholinergic projections from the td, as well as a direct non-cholinergic projection from the td to the avt. In further neuroendocrine experiments, neither stimulation nor ablation of the avt or the td had any effect on LH secretion. Furthermore, implantation of testosterone directly in the td had no effect on the choline acetyltransferase activity in that nucleus nor in several of its projection areas. In addition, it was shown that hypophysectomy blocks the castration-induced alteration in the choline acetyltransferase activity of the td. Thus, despite its apparent involvement in gonadal hormone feedback effects, the avt/td system was shown to respond to, rather than participate in, the neuroendocrine control of gonadotropin secretion.

The neuropharmacological relationship of the td and the avt was explored in another series of experiments. Acute stimulation of the avt resulted in reduced acetylcholine concentration in the hippocampus, a well-known td cholinergic projection area. In addition, both destruction of afferent catecholamine terminals in the td by 6-hydroxydopamine, and also chronic treatment with the dopamine receptor antagonist, haloperidol, caused alterations in choline acetyltransferase and acetylcholine concentration, respectively, in the avt. These results suggest the presence of a feedback-type functional relationship between the td and the avt which may be pertinent to the cholinergic and dopaminergic neurotransmitter-mediated effects of hormones and psychoactive drugs on behavior.

256.15 PROLONGED TRICYCLIC ANTIDEPRESSANT TREATMENT REDUCES SENSITIVITY OF GABA FACILITATION BY NE IN CEREBELLAR PURKINJE CELLS. H.H. Yeh, H.C. Moises, B.D. Waterhouse and D.J. Woodward, Dept. Cell Biology, Univ. Texas Health Science Ctr., Dallas, TX 75235

Chronic treatment of rats with desipramine (DMI) has been reported to result in a decreased β-adrenoceptor density. The question considered here is whether such biochemical measures are reflected in experimentally demonstrable physiological processes. We have demonstrated previously that the enhancement of GABA-induced Purkinje cell (PC) inhibition by NE can be mimicked by isopreterenol but not phenylephrine and antagonized by sotalol, indicative of a β-receptor mediation. In this iontophoretic study, we examined the possibilty that the sensitivity of GABA facilitation by NE in PC's of normal and DMI rats might reveal a physiological correlate of the proposed alteration in the β-receptor population.

Adult albino rats were pretreated for 11 days with DMI (10 mg /kg. body wt.; i.p.; twice daily). All electrophysiological experiments were performed on the 12th. day. Multibarrel micropipets were used to apply drugs and record extracellular PC unit responses. Inhibitory responses to iontophoretic pulses of GABA were examined before, during and after NE iontophoresis using computer-generated drug response histograms.

PC's of DMI rats fired spntaneously at a mean rate of 23.4 spikes/sec (S.E. +.6, N=80), 1/3 slower than that of normal rats (32.5 spikes/sec, S.E. +1.1, N=91). Interspike interval histograms reveal a marked increase in the frequency of long pauses without significant changes in the number of climbing fiber bursts.

An initial indication that PC's of DMI rats are subsensitive to NE was the finding of a higher mean iontophoretic current needed to induce threshold depressions of spontaneous discharge. Thirty-two PC's from DMI rats were tested for interaction between NE and GABA-induced inhibitions. NE exerted little or no effect in 12 cells and enhanced GABA inhibitions in only 4 cases. A striking finding was that, although PC responsivity to GABA was similar in both groups, GABA inhibitions were effectively antagonized by concurrent NE application in 16 cells.

In summary, the results provide evidence for a major alteration in the response characteristics of β-receptors mediating the facilitating action of NE on GABA. DMI appears to induce a form of subsensitivity involving β-receptor mediated heterosynaptic interactions. These electrophysiological data support the hypothesis of an adaptive down-regulation of β-receptors as one possible mode of action of antidepressant drugs. (Supported by NIDA DA-02338, NSF BNS77-01174 to D.J.W. and the Biological Humanics Foundation.)

256.16 BROMOCRIPTINE INHIBITS PHOSPHORYLATION OF TWO SPECIFIC PROTEINS IN RAT BRAIN. C. A. Stratford* and T. Ueda (SPON: J. H. Woods). Mental Health Research Institute, Depts. of Psychiat. & Pharmacol., Univ. of Michigan, Ann Arbor, MI 48109.

Bromocriptine is an effective agent in the treatment of hyperprolactinemia and parkinsonism, and is believed to achieve its therapeutic effects by a mechanism involving the D-2 dopamine receptor which is not coupled to adenylate cyclase. In an effort to understand some of the biochemical actions of bromocriptine in the central nervous system, crude synaptosome preparations from neostriatum, cerebral cortex and other regions of the rat brain were examined for the effect of the ergot compound on phosphorylation of specific proteins. Bromocriptine was found to inhibit specifically the phosphorylation of 50,000 and 60,000 dalton proteins (BrC-P_{50} and BrC-P_{60}). The bromocriptine-inhibitable phosphorylation was shown to occur on a threonine residue of both proteins. The concentration of the ergot required to cause a 50% inhibition was 8 µM. This inhibitory effect was not observed when dopamine, norepinephrine, isoproterenol, histamine, carbachol, serotonin, GABA, and adenosine were tested at concentrations up to 50 M. However, other ergots, particularly those which have a peptide moiety, such as α-ergocriptine, dihydroergocriptine, and ergotamine, mimicked the inhibitory effect of bromocriptine. Among these ergopeptines, bromocriptine was the most potent and ergotamine was the least potent. Those ergots which lack a peptide moiety, such as ergonovine and methysergide, showed the inhibitory effect only at much higher concentrations. The selective D-2 dopamine receptor antagonists metoclopramide and sulpiride did not block or mimic the inhibitory action of bromocriptine. The dopaminergic ergot lergotrile and the α-adrenergic antagonists phentolamine and yohimbine showed the inhibitory effect at high concentrations; these agents were less potent than ergotamine but more potent than ergonovine. The bromocriptine-sensitive phosphorylation of BrC-P_{50} and BrC-P_{60} was not detected in the anterior pituitary, which contains D-2 receptors but is devoid of D-1 receptors, nor in the goldfish retina, which contains D-1 receptors but is devoid of D-2 receptors. The bromocriptine-sensitive phosphorylation system was found in many regions of the brain but was not detected in the heart, lung, liver or kidney. These observations suggest that the bromocriptine-sensitive phosphorylation system does not represent specific dopaminergic, α-adrenergic, or serotonergic receptors, and raise the possibility that there may be a unique receptor for ergopeptines in the central nervous sytem.

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257.1 Endorphin-related Peptides in the Cerebrospinal Fluid of the Rat and Their Responses to Immobilization Stress. R. Sanford Kiser, Sue Jackson*, Roger Smith*, Lesley H. Rees*, P.J. Lowry*, and G. M. Besser*. Department of Chemical Endocrinology, Pituitary Hormone Laboratory, and Department of Endocrinology. St. Bartholomew's Hospital, London, England EC1A 7BE.

Endorphin-related peptides are found in both plasma and cerebrospinal fluid (CSF). Plasma levels of adrenocorticotropic hormone (ACTH) and β-endorphin rise in response to certain forms of stress, while met-enkephalin levels remain unchanged. Much less is known about the nature or physiology of these peptides in the CSF. The goal of this study was to characterize these peptides in the CSF of the rat and to examine their responses to immobilization stress.

Male albino rats were implanted with chronic cisterna magna cannulas. After the animals had been habituated to handling, daily samples of blood-free CSF were taken and pooled for chromatography on a Sephadex G-75 column. ACTH immunoreactivity was examined utilizing an antiserum with no cross-reactivity with non-ACTH portions of the pro-opiocortin molecule. ACTH immunoreactive peaks were present at the expected positions of the 31K ACTH precursor molecule, the 23K ACTH biosynthetic intermediate, the 14K, glycosylated form of ACTH, and 4.5K ACTH. The major peak was at the position of 14K ACTH. Beta-endorphin immunoreactivity was tested with an antiserum having cross-reactivity with β-lipotrophin but none with ACTH or met-enkephalin. Beta-endorphin-immunoreactive peaks occurred at the expected positions of the 31K precursor molecule, β-lipotrophin, and β-endorphin. The largest peak occurred at the β-endorphin position. Met-enkephalin levels, measured via the specific met-enkephalin assay developed by Clement-Jones et al. (J. Endocr. 86:231-243, 1980), were 48.5 pg/ml.

We then examined the effects of immobilization stress upon the total levels of ACTH, β-endorphin, and met-enkephalin immunoreactivities. CSF samples were taken after one hour of immobilization and compared to baseline samples. No significant changes in ACTH or β-endorphin immunoreactivities were observed (ACTH: baseline = 115 ± 8.8 pg/ml; post-stress = 122 pg/ml; β-endorphin: baseline = 127 ± 15.2 pg/ml, post-stress = 148 pg/ml). Met-enkephalin levels fell slightly to 35 pg/ml.

These results suggest that the CSF of the rat contains β-endorphin, ACTH, and their previously described precursor molecules. Significant levels of met-enkephalin are also present. Immobilization stress produced a slight fall in met-enkephalin levels but had no significant effect upon the immunoreactive levels of β-endorphin or ACTH.

257.2 DISSOCIATION OF BLOOD AND CEREBROSPINAL FLUID β-ENDORPHIN RESPONSES IN THE RAT. M.C. Pian*, M.A. Arnold*, and J.B. Martin. Department of Neurology, Massachusetts General Hospital and Harvard Medical School, Boston, MA 02114.

The effects of various stimuli on β-endorphin (β-E) release into blood and cerebrospinal fluid (CSF) were studied in the rat. Using camel β-E for the RIA standard the assay could detect 2-3 pg/tube; the antibody recognized human β-E and human β-lipotropin on an equimolar basis. The antibody showed negligible cross-reactivity with the enkephalins or with α-endorphin or γ-endorphin. With the use of chronically implanted blood (right atrial) and CSF (cisterna magna) cannulae, blood and CSF samples were withdrawn from unanesthetized, non-stressed male rats for periods of several hours. Gel permeation chromatography of 10 ml rat CSF indicates that 69.4% of the β-endorphin immunoreactivity co-migrates with synthetic β-E. A similar analysis of talc-extracted rat plasma indicates that about 90% of the β-endorphin immunoreactivity co-migrates with synthetic β-E. Immobilization of rats for 30 minutes results in a large increase in mean plasma β-E levels measured over a 1.5 h period (801.1 ± 264.0 in immobilized group vs. 169.4 ± 39.0 pg/ml in control group; mean ± S.E.). CSF β-E levels obtained 10 and 70 minutes after immobilization (147.9 ± 34.7 and 114.2 ± 14.1 pg/ml, respectively) did not differ from pre-immobilization CSF peptide levels (112.9 ± 27.9 pg/ml). Similarily, the iv administration of caffeine (20 mg/kg) more than doubled mean plasma β-E levels measured over a 3 hour period but did not affect CSF β-E 30 and 90 minutes after injection (30 min, 144.4 ± 14.2; 90 min, 178.9 + 23.4 pg/ml) as compared to control levels (-30 min, 147.9 + 17.0 pg/ml). These results indicate that blood and CSF β-E levels are independently regulated in the rat.
(Supported by USPHS Grant AM 26252; M.A.A. is a NIH Postdoctoral Fellow)

257.3 RISE IN PLASMA BETA ENDORPHIN IMMUNOREACTIVITY DURING SURGICAL STRESS, MODIFIED BY EXOGENOUS OPIATES M. Dubois*, D. Pickar*, M. Cohen*, P. Gadde*, T.E. Macnamara* and W.E. Bunney (SPON: R. Coppola). Anesthesia Section, NIH; and Biological Psychiatry Branch, NIMH, Bethesda, MD 20205

Beta endorphin seems to play a definite role in the biological response to stress (Rossier, J., et al, Nature, 270: 618, 1977) and in the endogenous mechanism of pain perception (Hosobuchi, Y., et al, Science, 203, 279, 1979). Exogenously administered opiates during surgery decrease or even supress the activation of the "stress hormones" (i.e. ACTH, Cortisol, etc.) (George, J.M., et al, J. Clin. Endocrinol. Metab., 38:736,1974).

In the present study, a standard general anesthetic technique using no opiates was administered to a first group of nine patients undergoing a staging laparotomy. All patients were medication-free prior to the surgery. In the postoperative period, they were given morphine sulfate for pain relief. In a second comparable group of six patients, selected by the same criteria and undergoing the same type of surgery, fentanyl was administered as main anesthetic drug. In both groups, multiple blood samples were collected prior to, during and after the surgery, following the same time protocol. Plasma beta endorphin immunoreactivity (B-Eir) and plasma cortisol were determined by radio immunoassay. Intra assay variation for plasma cortisol was 6% and for B-Eir 3.5%.

Induction of anesthesia did not raise significantly B-Eir in both groups. Surgery in the first (=no opiate) group was associated with important increases in B-Eir (ANOVA with repeated measures: F=11.2, df=2,14,p=0.001). When morphine was administered in the postoperative period, a significant concomitant decrease in B-Eir was observed (from 74.9±29 pmole/liter after wakening, to 16.6±0.8 pmole/liter, after start of morphine treatment; mean ± SEM). Levels of plasma cortisol followed the same trend in all cases and were significantly related to plasma B-Eir (r=0.61, p<0.01). When fentanyl was given as main anesthetic, plasma B-Eir did not rise during surgery and most levels were undetectable for 90-120 minutes following the injection of the opiate. In three cases, a substantial increase in plasma B-Eir was observed, while the analgesic effect of the drug was disappearing, as monitored by cardiovascular changes during anesthesia or pain on awakening.

In subjects receiving no opiates, surgical stress appears to activate the endorphin system, as it is already known to activate the hypothalamic-pituitary-adrenal axis. On the other hand, the administration of opiates (morphine for postoperative pain relief, and fentanyl during surgery) supresses this activation consistently.

257.4 OPIATE INVOLVEMENT IN ETHANOL INDUCED EFFECTS ON THE CENTRAL NERVOUS SYSTEM IN THE RAT. J.P. Allen, A.K.S. Ho*, T.A. Kepic, L. Biesecker*, A. Mizera*, and B. Hofreiter*. Dept. of Neurosciences and Basic Science, Peoria School of Med., Peoria, IL 61605.

The mechanism through which ethanol effects central nervous system function is incompletely understood. Recent advances in opiate research prompted investigating the role of acute ethanol administration on plasma β-endorphin and ACTH concentrations in the rat. To test whether the opiates are involved in the effects of ethanol on the central nervous system, groups of 200 gm adult male Sprague-Dawley rats were treated with morphine (10 mg/kg, i.p.); ethanol (2g/kg, i.p.); naloxone (0.4 mg/kg, i.p.); morphine (10 mb/kg, i.p.) and naloxone (0.4 mg/kg, i.p.); ethanol (2 g/kg, i.p.) and naloxone (0.4 mg/kg, i.p.); and normal saline (1 cc, i.p.). Naloxone or saline was administered at 0830 and fifteen minutes later the remaining drugs or saline were injected. Sixty minutes after the initial naloxone or saline injection, the rats were decapitated and trunk blood collected for later analysis of β-endorphin and ACTH by radioimmunoassay. There was a significant increase in mean plasma β-endorphin concentration following morphine ($p < 0.001$) or ethanol ($p < 0.01$) compared with controls. This increase induced by either alcohol or morphine was significantly antagonized by naloxone pretreatment ($p < 0.01$). There was no significant difference in the mean plasma β-endorphin concentration between the naloxone and the saline treated controls. In contrast the mean plasma ACTH concentration showed no significant difference between the various treatment groups. We conclude from our data that the central nervous system effects of ethanol may in part be mediated through endogenous opioids and their receptors.

257.5 ENDOTOXIC SHOCK IS ACCOMPANIED BY A NALOXONE-SENSITIVE INCREASE IN NOCICEPTIVE LATENCIES. Gregory Lucas Belenky, Barry A. Ruvio* and John W. Holaday. Dept. Medical Neurosciences, Walter Reed Army Institute of Research, Washington, D.C. 20012.

In earlier work it was shown that endotoxin shock hypotension was rapidly reversed by the opiate antagonist, naloxone (Holaday and Faden, Nature 275, 450, 1978). From this, it was suggested that endotoxemia resulted in the activation of endogenous opiates which contributed to the cardiovascular pathophysiology of shock. The purpose of the present study was to determine if the apparent functional activation of endogenous opiates by endotoxin also resulted in an opiate-like elevation in nociceptive latencies. Male Sprague-Dawley rats (250-300g) were surgically prepared with external jugular-vein cannulae. On the following day, these now-conscious rats were repeatedly exposed to a 52°C hot plate until consistent escape latencies were obtained. Tail-flick latencies and colonic temperatures were also measured. Endotoxemia was produced by the intravenous (iv) injection of 15 mg/kg E. coli lipopolysaccharide endotoxin in rats pretreated 10 min earlier with 10 mg/kg naloxone or saline iv. In saline pretreated rats, a gradual elevation of hot-plate escape latencies was observed over the first 40 min post-endotoxin injection. By contrast, naloxone-pretreated rats showed a decline in escape latencies over this same 40 min interval [ANOVA, $F(3,54)=3.16$, $p=0.032$, n=10 rats/group]. Tail-flick latencies and colonic temperatures were relatively unaffected by endotoxin or drug pretreatment. This dose of endotoxin was 100% lethal in both groups 24 hrs post-injection. The elevation of nociceptive latencies produced by endotoxin, combined with the lack of such effect in naloxone pretreated rats, indicates that endogenous opiate systems may be functionally activated by endotoxins to result in antinociception. Whether this is a direct effect upon endogenous opiate release or an indirect consequence of stress cannot be determined by these experiments. Nonetheless, it appears possible that endotoxemia results in a generalized activation of endogenous opiates to result not only in shock hypotension, but antinociception as well.

257.6 ANALGESIA AND INCREASES IN BRAIN NOREPINEPHRINE METABOLISM PRODUCED BY PERIAQUEDUCTAL GRAY INJECTIONS OF OPIATES. T. G. Reigle. Dept. of Pharmacology, Univ. of Georgia Sch. of Pharmacy, Athens, GA 30602.

Previous work in our laboratory (LoPachin and Reigle J.P.E.T. 207:151, 1978) has demonstrated the ability of systemic opiate injections to produce receptor-specific increases in brain levels of the major norepinephrine (NE) metabolite, 3-methoxy-4-hydroxyphenylethylene glycol sulfate (MOPEG-SO_4) in rats. The present study examined the ability of periaqueductal gray (PAG) injections of morphine (M) and levorphanol (L) to increase limbic and cortical MOPEG-SO_4 and attempted to correlate changes in MOPEG-SO_4 with the analgesic effects of PAG opiates in the same experimental animals. Isotonic solutions of opiates were bilaterally injected into the ventral PAG through permanent cannulae in male Sprague-Dawley rats. Analgesia was determined 30 minutes after opiate injection by measuring changes in tail-flick latency to a fixed heat stimulus and animals were sacrificed immediately after analgesic testing for the determination of brain MOPEG-SO_4. At 30 minutes, bilateral PAG injections of morphine (0.5-5 µg, base) and levorphanol (10-20 µg, base) produced a dose-dependent analgesic effect accompanied by dose-dependent increases in limbic and cortical MOPEG-SO_4. The bilateral injection of an identical volume of saline (1.0 µl) resulted in tail-flick latencies and MOPEG-SO_4 concentrations which were not significantly different from those obtained in untreated controls subjected to analgesic testing. Analgesia and the increases in MOPEG-SO_4 produced by PAG opiates were antagonized by 10 minute pretreatment with systemic naloxone (1 mg/kg, i.p.) and significant correlations were obtained between the degree of analgesia produced by PAG opiates and limbic and cortical concentrations of MOPEG-SO_4 ($r=0.94$ and 0.85, respectively). These findings indicate that analgesia and increases in brain norepinephrine metabolism in two brain regions are mediated by the specific activation of opiate receptors in the PAG and thus provide further evidence for the involvement of brain noradrenergic systems in opiate analgesia. Supported by NIDA Grant no. 02869.

257.7 4-AMINOPYRIDINE REVERSAL OF MORPHINE ANALGESIA. A.S. Tung, M.D* and B.W. Brandom, M.D*. (SPON: J. Roppolo, Ph.D.) Univ. of Pgh. Sch. of Med., Dept. of Anesthesiology, Pgh., PA 15261.

4-Aminopyridine(4AP) reversal of morphine analgesia was assessed because while morphine sulfate(MS) inhibits the release of neurotransmitters involved with pain transmission[1], 4AP enhances calcium-dependent neurotransmitter release[2]. 4AP has been used clinically to reverse non-depolarizing muscle relaxants[3] and ketamine-diazepam anesthesia. The usefulness of 4AP in these circumstances may be due to an ability to block potassium conductance, create a regenerative calcium current and thus enhance neurotransmitter release. These events may also diminish morphine induced analgesia.

Analgesia was measured by tail flick and hot plate tests. The response latencies in these tests were converted to maximal possible effect (%MPE) .MPE = (Postinjection latency - baseline) / (cutoff time - baseline). All drugs were given i.p.

$\bar{X} \pm$ SEM	MS-10	4AP-2	MS & Saline	MS & 4AP-0.5	MS & 4AP-1.0	MS & 4AP-2.0
%MPE TPL	88±10	3±6	65±12	48±32	10±8	22±16
%MPE HPL	36±17	59±14	34±18	48±21	55±21	100±0

Statistically significant ($P<.05$) results are as follows. MS 10 mg/kg elevated both the tail flick (TFL) and hot plate (HPL) latencies. 4AP alone did not affect the TFL but increased the HPL. In addition, all three doses of 4AP caused a significant reduction of the MS induced increase in TFL. On the other hand, 4AP at 2mg/kg resulted in a potentiation of the MS effect on HPL.

As indicated by the results of the TFL (a nociceptive spinal reflex) 4AP did reverse MS analgesia. Analgesia reversal was not demonstrated by the HPL because the end point of the hot plate test was indicated by the animal's ability to lick or tap its hind paw. This behavior would require multisynaptic supraspinal function which was affected by the central excitatory action of 4AP. A clinical implication of these results is that while attempting to antagonize muscle relaxant or anesthetic effects in postoperative patients with 4AP, incidental reversal of much needed narcotic analgesia may occur.

1. Mudge AW, Leeman SE and Fischback GD: Enkephalin inhibits release of substance P from sensory neurons in culture and decreases action potential duration. Proc Natl Acad Sci USA 76:526-530, 1979.
2. Lundh H: Effects of 4-aminopyridine on neuromuscular transmission Brain Res 153:307-318, 1978.
3. Miller RD, Booij LHD, Agoston S, et al: Potentiation of neostigmine and pyridostigmine by 4-aminopyridine in man. Anesthesiology 50:416-420, 1979.

257.8 CONTINUOUS NALOXONE INFUSION INDUCES ABSTINENCE-LIKE SYNDROME. D.H. Malin, M.A. Reagan*, J.G. Leavell*, and C.A. Westmoreland*. Univ. of Houston at Clear Lake City, Houston, Texas 77058.

Continuous blockade of endorphin receptors by subcutaneous naloxone infusion produced behavioral symptoms resembling opiate abstinence syndrome within 28 hours. Rats were implanted subcutaneously (under ether anesthesia) with two Alzet osmotic minipumps delivering a total of 0.625 mg naloxone/hour/kg, or with two control minipumps delivering distilled water only. They were observed for ten minutes under blind conditions at 16 and 28 hours post-implant. After 16 hours, the naloxone-infused rats showed significantly more abdominal writhes than controls (means of 1.17 vs. 0.17, $p<.05$). At 28 hours, the naloxone animals showed significantly more abdominal writhes than controls (means of 1.67 vs. 0.0, $p<.02$), as well as more wet-dog shakes than controls (means of 5.83 vs. 0.83, $p<.05$) and less weight gain since implant than controls (means of 23.3g vs. 30.0g, $p<.005$).

One interpretation of these results is that rats under normal circumstances need at least some daily exposure to endorphergic stimulation in order to maintain a state of comfort or behavioral normality. Another interpretation is that rats suffering from implantation discomfort need endorphinergic stimulation to suppress symptoms of irritation resulting from implantation. In the latter case, severe symptoms should appear in rats implanted with two H_2O containing minipumps, if they are acutely deprived of endorphinergic stimulation at 28 hours post implant by a high dose naloxone injection (3 mg/kg s.c.). However, this treatment produced virtually no wet-dog shakes or abdominal writhes in a group of six rats. A control group of six unimplanted rats also failed to demonstrate these symptoms in response to acute naloxone.

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257.9 MORPHINE DEPENDENCE AND NALOXONE CONTRACTIONS IN ISOLATED GUINEA-PIG ILEUM. R.F. Mucha*, L.E. Robson* and H.W. Kosterlitz. Unit for Research on Addictive Drugs, University of Aberdeen, Marischal College, Aberdeen, Scotland, AB9 1AS.

In morphine-withdrawn ileum isolated from guinea-pigs implanted for 3 days with 2 morphine pellets, the inhibitory effects of the presynaptic α-adrenoceptor agonist clonidine on electrically-induced twitches were much reduced and the dose response (DR) curve was flat. Addition of morphine to the bath restored the inhibitory effect (Gillan et al., Br. J. Pharmac. 1979, 66, 601-608). We used naloxone to produce the morphine-withdrawn state and studied the relation of flattening of the clonidine DR curve to the well-known ability of naloxone to produce contractions in ilea treated with morphine.

Ilea exposed to one of three different treatment conditions were used. Procedures for setting up were described by Gillan et al. (0.5 ms, 0.1 Hz, maximal voltage). The first kind of tissue was removed from guinea-pigs implanted with 2x75 mg morphine pellets for 12, 24, or 48 h. Ilea were set up in Krebs solution containing 1600 nM morphine. The second was tissue removed from naive guinea-pigs and preincubated in Krebs containing 10 μM morphine at room temperature for 24 h. These ilea were then set up in Krebs containing 1600 nM morphine. The third kind of tissue was removed from naive guinea-pigs and set up in normal Krebs. After stabilization, morphine (1600 nM) was added to the Krebs and the tissue was incubated for 3 h.

Segments of whole gut from each of the conditions underwent contractions of few minutes duration on addition of naloxone (400 nM) to the bath fluid. In the tissue treated with pellet implants the mean peak contraction produced by naloxone was not greater than that seen in tissue from the other two treatment conditions. Yet, in the tissue treated with pellet implants, the clonidine DR curve measured in the presence of naloxone was flat, whereas in tissue preincubated for 24 h at room temperature in 10 μM morphine the curve was less flat; in tissue incubated for 3 h in the tissue bath in 1600 nM morphine, the clonidine curve was almost unaltered. Longitudinal muscle strips with the myenteric plexus were also studied. Naloxone contractions were only observed in ilea treated with pellet implants, but at the duration of implantation studied (12 and 24 h), clonidine sensitivity was similar in the presence and absence of naloxone.

The naloxone contraction has generally been considered a manifestation of tolerance produced by morphine treatment. However, it is clear that naloxone contractions are not related in a simple way to tolerance to clonidine seen in the morphine-withdrawn gut. (Supported by the United Kingdom M.R.C. and by NIDA grant DA 00662. R.F.M. is a M.R.C. of Canada fellow)

257.10 DEPRESSION OF THERMOREGULATORY BEHAVIOR AFTER CENTRAL INJECTION OF OPIOIDS IN THE CAT. G.L. Bernardini* and W.G. Clark. Dept. of Pharmacology, Univ. of Texas Hlth Sci. Ctr. at Dallas, TX 75235.

Changes in thermoregulatory activity produced by a drug can be assessed by determining the pattern of changes in core body temperature (Tb) induced over a range of environmental temperatures. The value of this approach is enhanced if the changes in thermoregulatory effector activities which cause or accompany the change in Tb are also noted. Behavioral changes can contribute to the overall determination of drug actions on thermoregulation. The usual pattern is that (1) if a drug alters the level about which Tb is regulated, behavioral activity facilitates the Tb change whereas (2) if the drug depresses thermoregulation or alters thermoregulatory effector activity, behavioral changes tend to oppose the change in Tb. Changes in Tb and behavior produced by morphine, D-Ala^2-Met-enkephalinamide (DAME) and pentazocine (PTZ) were determined in unrestrained cats in which a cannula was implanted for injections into the third cerebral ventricle. Tb was recorded automatically from a thermocouple implanted in the retroperitoneal space. For the behavioral tests each animal was placed in a cold room (4°C) in a cage above which was a battery of infrared lamps. They were trained either (1) to hold down a lever to turn the lamps on and to keep them on as long as the lever was depressed (heat-reinforcement, HR) or (2) to press the lever to turn the lamps off, etc. (heat-escape, HE). The height of the lamps was adjusted for each cat so that it depressed the lever 40-60% of the time after vehicle administration. Data from previous studies were used to select doses of morphine (20 μg) and DAME (25 μg) that increase the level about which Tb is regulated, thereby raising Tb in the cold, and a dose of PTZ (1 mg) which initially depresses thermoregulation, thereby decreasing Tb in the cold. Hence the predicted behavioral responses after all three agents would be enhanced HR activity or decreased HE activity. Contrary to the former expectation, these agents greatly decreased HR activity. On the other hand, in animals tested so far, morphine and DAME either did not change or only slightly decreased HE activity. This indicates that the cats could still sense the heat from the lamps and retained adequate motor capability. Thus these opioids did not evoke the predicted overall pattern of behavioral changes but instead tended to depress thermoregulatory behavior. This decrease in responding, which apparently occurred without associated motor or sensory impairment, may reflect the general ability of opioids to decrease reactions to various sensations such as pain, etc. (Supported by National Institute on Drug Abuse Research Grant # DA02188.)

257.11 BODY TEMPERATURE RESPONSES TO MICROINJECTIONS OF MORPHINE IN BRAIN SITES CONTAINING OPIATE RECEPTORS. J.A.P. Teasdale*, M.A. Bozarth, J. Stewart (SPON: R.B. Malmo) Dept. of Psychology, Concordia University, Montreal, Canada H3G 1M8.

High doses of morphine administered systemically have been shown to produce a biphasic effect on temperature in rats. The first phase of this response is hypothermia followed by hyperthermia. To determine the central sites of action for these morphine effects, rats were given microinjections of morphine in various brain regions.

Male hooded rats were unilaterally implanted with 22 gauge cannulae in one of the following sites: central caudate nucleus (CN), periventricular gray substance (PVG), medial preoptic area of the hypothalamus (MPO), lateral hypothalamic area (LH), or ventral tegmental area of Tsai (VTA). Morphine sulfate was infused through a 28 gauge injection cannula that extended 1 mm beyond the indwelling guide cannula. A 2.5 μg dose of morphine sulfate was dissolved in 0.5 μl of Ringer's solution and delivered over a 28 sec period with an additional 30 sec allowed for diffusion (Bozarth, M.A. & Wise, R.A. J. Neurosci. Meth. 2, 273, 1980). Core temperatures were recorded with a rectal telethermometer 15 min before and 15, 45, 105, and 165 min after the infusion of morphine. Core temperatures following the infusion of morphine were compared with temperatures previously obtained following the infusion of Ringer's solution.

At this dose morphine did not produce hypothermia at any of the brain sites tested. There were no significant temperature increases in response to morphine infusions directed to the CN, PVG, LH or VTA. However a significant temperature rise with fast onset was observed from animals implanted in the MPO. These results suggest, as have other studies, that the MPO is involved in the mediation of morphine's thermic effects. The LH and the CN which contain opiate binding sites have failed to produce any thermal effects to morphine infusions in our study. The PVG which has been implicated in the analgesic effects as well as in physical dependence to morphine also failed to show any mediational role in the thermic effects of morphine. The VTA which has been shown to sustain self-administration of morphine, equally did not show any temperature effects in response to morphine at the dose employed in this study.

From these data it would seem that different responses to morphine such as the ones mentioned above are anatomically separate from the temperature response. (Supported by grants from Medical Research Council of Canada (MA-6678) and from NIDA (DA-02285).)

257.12 MODIFICATION OF OPIOID PEPTIDES LEVEL IN THE HYPOTHALAMUS BY CHRONIC TREATMENT WITH d-FENFLURAMINE. L. G. Harsing, Jr.*, H.-Y. T. Yang*, S. Govoni* and E. Costa. (SPON: I. K. Ho). Lab. Preclin. Pharmacol., NIMH, St. Elizabeths Hosp., Washington, D.C. 20032.

The content of met^5-enkephalin (ME) was measured by radioimmunoassay in various brain structures of rats treated repeatedly with d-fenfluramine (15 mg/kg/day). Groups of animals were killed by exposing the head to microwave irradiation on the 5th, 10th and 15th day of treatment. The hypothalamic ME content was elevated from 6.2±1.0 ng/mg protein to 12.9±0.9, 10.3±1.1 and 9.6±0.7 ng/mg protein (n=8-9, $p<0.02$) on the 5th, 10th and 15th day of d-fenfluramine treatment. The increase of ME content in the hypothalamus was associated with a marked decrease of body weight. The striatal ME content was increased from 12.2±1.1 to 18.2±2.1 ng/mg protein (n=9, $p<0.02$) on the 5th day of d-fenfluramine treatment then returned to the control values. In the frontal cortex, hippocampus, brainstem and pituitary the ME content remained unchanged during the treatment with d-fenfluramine. The content of β-endorphin immunoreactive material (β-E-IR) of hypothalamus was increased from 4.4±0.8 to 9.3±1.8 and 7.0±0.8 ng/mg protein (n=8-10, $p<0.05$) on the 5th and 10th day of d-fenfluramine treatment whereas no change was detected in cortical and hypothalamic content of cholecystokinin like immunoreactive material after 5 days d-fenfluramine treatment. An acute injection of d-fenfluramine (15 mg/kg) or d-amphetamine (3mg/kg) failed to affect the ME content in the frontal cortex, striatum, hypothalamus and brainstem or the β-E-IR in the hypothalamus. Five days treatment with d-amphetamine (3 mg/kg) did not modify the ME content in the frontal cortex, striatum, hypothalamus or brainstem. From these experiments one can infer that an accumulation of hypothalamic ME and β-endorphin may be operative in mediating the anorectic action of d-fenfluramine but not that of d-amphetamine. The present experiments do not allow us to differentiate whether this accumulation is due to an increase of synthesis or a decrease utilization. However since naloxone decreases appetite we are investigating whether the anorectic action is mediated by a decreased utilization of ME and β-endorphin.

257.13 THE RELEASE OF ANTERIOR PITUITARY HORMONES BY WIN 44,441-3; A REPORTED KAPPA OPIATE RECEPTOR ANTAGONIST R. Pechnick*, R. George* and R. Poland* (SPON: D. Janowsky). Dept. of Pharmacology, UCLA and Dept. of Psychiatry, Harbor General Hospital, Los Angeles, CA 90024.

Win 44,441-3 has been reported to be a pure antagonist with antagonist activity at the kappa opiate receptor. This compound is unique in that antagonist activity is usually associated with alkyl substitutions of the N-methyl group. Few compounds that retain the N-methyl group demonstrate antagonist activity. This compound has a saturated ketone side chain attached to position 9 of the metazocine ring. The changes in pattern of release of anterior pituitary hormones following administration of this compound should give an indication of whether the kappa receptor activity can be distinguished from mu receptor activity.

Male, Sprague-Dawley rats were housed in the experimental room under 12/12 hour light dark cycle for eight days prior to the experimental day. Three days prior to the experimental day the subjects were handled and given s.c. saline injections to habituate them to the experimental procedure. On the day of the experiment rats 48 days old with a mean weight of 239.9±2.2 g were randomly assigned to control and treatment groups. Win 44,441-3 was dissolved in 8.5% lactate, the pH brought up to 3.8 by the addition of 0.1 N NaOH and normal saline used to dilute to volume. The control subjects received the vehicle adjusted to a pH of 3.8. The treatment groups received Win 44,441-3 in doses equimolar to 5.0, 10.0 and 20.0 mg/kg of morphine base (equivalent to 13.1, 26.2 and 52.4 μMoles/kg respectively). All injections were given s.c. with a volume of 1.0 ml/kg. 30 minutes following injection trunk blood was obtained for measurement of corticosterone, growth hormone, prolactin, luteinizing hormone and thyroid stimulating hormone via RIA. Statistical analysis was performed on the log transformed data. Analysis of variance was first performed followed by Dunnett's test when initial significant differences were found.

There were no gross behavioral changes seen following the administration of Win 44,441-3. No significant changes were seen in the release of corticosterone, growth hormone or thyroid stimulating hormone. A highly significant increase in the level of luteinizing hormone was seen as well as a significant decrease in the prolactin level was found. These results demonstrate a similar profile to that seen following the administration of naloxone with the exception of no fall in growth hormone levels as reported by several investigators following naloxone administration. (Supported by USPHS DA-01006).

257.14 IN VIVO RELEASE OF ENKEPHALIN FROM THE GLOBUS PALLIDUS IS TONICALLY INHIBITED BY ENDOGENOUS GABA. A. Bayon, R. Drucker-Colin, L. Lugo*. Centro de Investigactiones en Fisiologia Celular. U.N.A.M., Apartado Postal 70-600, Mexico 20, D.F., and W.J. Shoemaker, R. Azad*, and F.E. Bloom. The Salk Institute, P.O. Box 85800, San Diego, CA 92138.

Previous studies demonstrated that endogenous immunoreactive-enkephalin is released in vivo from the globus pallidus; this release is enhanced during local or caudate nucleus stimulation (Bayon et al. Neurosci. Letters. Vol. 23, 1981). Since the globus pallidus is one of the brain regions richest in glutamate decarboxylase, we have investigated the relationship between the GABA-containing system and the enkephalin release in this area. Methodological details of the push-pull perfusion technique used in these experiments have already been reported (Bayon et al., ibid): Cats were chronically implanted under pentobarbital anesthesia with guide-cannulae to allow the stereotaxical placement of concentric perfusion cannulae in the globus pallidus one week later. At that time the fully awake-unrestrained animals were perfused with a modified Krebs-Ringer solution containing BSA (0.1%) and bacitracin (30 μg/ml), to which drugs were added when indicated. The flow rate was held constant at 23 μl/min and perfusates were collected every 15 min. Fractions were assayed by a Leu-enkephalin RIA (3% cross reactivity with Met-enkephalin). As already reported, local perfusion with medium containing Veratrine (60 μg/ml) enhanced the enkephalin release. Exogenous GABA (10^{-4}M) did not elicit a measurable change in resting enkephalin release but significantly inhibited that stimulated by Veratrine. Picrotoxin (5×10^{-4} M), a GABA receptor antagonist, increased the resting enkephalin release (about 3-fold) both in the presence and in the absence of exogenous GABA. This observation suggests that endogenously released GABA may exert a tonic inhibition upon pallidal release of enkephalin. In this context, it is noteworthy that a substantial release of immunoreactive enkephalin (about one half of the tissue stores in 40 min) occurs during resting perfusion of pallidal slices (Bayon et al, PNAS USA, 75, 3503, 1978). Based on our present hypothesis, this massive enkephalin release could be due to the lack in this isolated preparation of the GABA input originating from extrapallidal structures.

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257.15 ENHANCEMENT OF LYMPHOCYTE PROLIFERATIVE RESPONSES BY β-ENDORPHIN. Steven C. Gilman*, Jeffrey M. Schwartz*, Robert J. Milner*, Floyd E. Bloom, and Joseph D. Feldman*. Scripps Clinic and Research Foundation and the A.V. Davis Center at the Salk Institute, La Jolla, CA 92037.

The effects of α and β endorphin on rat lymphocytes were studied using an in vitro culture system. Spleen cells from 3-4 month old Lewis rats were cultured in the presence or absence of purified synthetic α and β-endorphin at final concentrations of 10^{-12} M to 10^{-14}M. Cellular proliferation, measured by the uptake of ^{3}H-thymidine (^{3}H-Tdr), was assessed after 72 hours of culture. α and β endorphin alone had no effect on the proliferation of unstimulated spleen cells. However, the β-endorphin enhanced the proliferative response of spleen cells to the T cell mitogen Concanavalin A (Con A). ^{3}H-Tdr uptake of Con A stimulated cells was increased 2-4 fold in cultures containing 10^{-12}M β-endorphin, compared to uptake in cultures given Con A alone. The enhancing effect of β-endorphin was dose-dependent and was most pronounced when a sub-optimal dose of Con A was used. In contrast, α-endorphin had no effect on Con A responses at any concentration tested. The enhancement of Con A induced ^{3}H-Tdr uptake by β-endorphin was inhibited by naloxone (10^{-5}M). These results suggest that endogenous opiates such as β-endorphin may modulate immune responsiveness and open the possibility of an association between the neuroendocrine and immune systems. Furthermore, given the recent demonstration in rats of immunoreactive β-endorphin secretion in response to stress (Guillemin et al., Science, 1977), the results obtained using this in vitro culture system may reflect cellular mechanisms underlying the psycho-somatic interactions proposed to explain demonstrated modifications of immune function by psychosocial processes (M. Stein et al., Science, 1976). Supported by USPHS Grants AI 7007, MH 07899.

258.1 CORRESPONDENCE OF MELANIN-PIGMENTED NEURONS IN HUMAN BRAIN TO A1-A14 CATECHOLAMINE CELL GROUPS. C.K.Petito and C.B. Saper, Depts. of Neurology and Pathology, New York Hosp.-Cornell Univ. Med. Ctr., New York, NY 10021.

The distribution of catecholaminergic neurons has been plotted in detail for many species, but never for the adult human brain. We report that the adult human brain contains melanin-pigmented and therefore presumably catecholaminergic neurons in a distribution which closely parallels that of the A1-A14 catecholamine cell groups in other species.

Three brainstems and basal forebrains from 56-80 year old people dying of non-neurological causes were obtained at autopsy. After 4-26 weeks formalin fixation the brains were frozen sectioned at 50 u and every tenth section mounted and lightly stained with thionin. Alternate series of sections were stained with PAS; with oil red O; for iron; and with Fontana-Masson stain both with and without hydrogen peroxide bleaching.

The A1 and A2 cell groups, with similar cytological features, were easily distinguished at the level of the dorsal motor vagal nucleus, but no distinct A3 cell group was found. The neurons of the A5 group, near the superior olivary nucleus, the A4 and the A6 groups in the cerebellum and subjacent dorsolateral pons and the A7 cell group in the lateral pontine tegmentum were all larger and tended to be more densely pigmented than the A1 and A2 neurons. The A8, A9 and A10 cell groups, in the substantia nigra pars compacta and associated dorsolateral and medial midbrain tegmentum, also contained primarily larger, densely pigmented and staining neurons. In contrast, the A12, A13 and A14 cell groups in the arcuate and periventricular nuclei of the hypothalamus consisted of much smaller and less densely pigmented neurons. A few similar cells were seen in the posterior hypothalamic area in a cluster which may be homologous to the A11 cell group.

Stains for iron and lipofuscin were not taken up by the pigmentation, but a Fontana-Masson stain for melanin was positive. The pigmentation was bleached, as is appropriate for melanin, with hydrogen peroxide.

These results indicate that in the adult human brain melanin-pigmented neurons are found not only in the locus coeruleus, substantia nigra and dorsal motor vagal nucleus, but more widely in a distribution identical to that of the A1-A14 catecholamine cell groups in other species. As brain melanin is a byproduct of catecholamine synthesis, it appears that melanin may be used as a natural marker in neuropathological assessment of the entire human catecholamine neuron system in post-mortem material.

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258.2 TOPOGRAPHICAL ORDERING OF LOCUS COERULEUS PROJECTION TO THE TELENCEPHALON OF THE RAT. J.M. Cedarbaum and C.B. Saper. (SPON: H.P. Davis). Dept. of Neurology, New York Hospital-Cornell Univ. Med. Ctr., New York, NY 10021.

The noradrenergic neurons of the locus coeruleus (LC) are known to project widely throughout the CNS, including the spinal cord, cerebellum, thalamus, limbic system and neocortex. Single LC neurons may send axon collaterals to multiple terminal fields. Nevertheless, retrograde tracer studies have demonstrated a degree of topographical ordering within the system. Several studies suggest that projections to the spinal cord and the hypothalamus arise from the ventral part of LC, projections to the hippocampus arise from its rostrodorsal portion and that the cortical projection arises diffusely from all portions of the nucleus. No topographical ordering to the LC projection to the cerebral cortex has, however, been described. Using a retrograde transport method employing wheat germ agglutinin conjugated to horseradish peroxidase developed with TMB, we have been able to distinguish a crude topographical ordering within the telencephalic projection of LC.

Labeled neurons were seen in LC both ipsilateral and contralateral to the injection sites (in a ratio of about 10-20:1)in all areas studied. The labeled neurons often appeared to be arranged in clusters or in bands, frequently in relation to small blood vessels. By superimposing results from several experiments with similar injection sites, however, a topographic pattern of labeling emerged. In agreement with previous studies, injections into the septum, the infralimbic cortex and the hippocampus retrogradely labeled neurons in the rostrodorsal LC. A similar pattern was seen after olfactory tubercle, piriform or entorhinal cortex injections. A crude topographical pattern was seen within the LC projection to the neocortex. Medial frontal injections labeled primarily cells in the rostral medial part of LC, whereas lateral hemispheric injections labeled mainly cells in the lateral part of LC and more posterior injections labeled a more caudal population of neurons. More diffuse retrograde labeling within LC was seen following injections into the cingulate cortex presumably due to uptake of label by axons passing through the cingulate bundle. This rough topographic mapping of the LC projection to the cortical surface may provide an anatomical substrate for noradrenergic modulation to be employed differentially in various cortical areas.

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258.3 REGION-SPECIFIC CATECHOLAMINE INNERVATION OF PRIMATE CEREBRAL CORTEX. P.Levitt, P. Rakic and P.S. Goldman-Rakic. Sec. of Neuroanatomy, Yale University Sch. of Med., New Haven, Ct. 06510

The distribution of norepinephrine(NE)-and dopamine(DA)- containing afferents of the cerebral cortex in the adult rhesus monkey was examined using the sensitive SPG fluorescence histochemical method of de la Torre. The density and laminar distribution of catecholamine (CA)-containing axons exhibits remarkable regional specificity that correlates closely with endogenous transmitter levels measured biochemically in the same cortical areas of the rhesus monkey (Brown, Crane & Goldman, Brain Res. 168 '79). In primary sensory areas such as S1 somatosensory, auditory and visual cortices, NE-type axons are distributed primarily in layers II, III and VI. Surprisingly, fluorescent axons are virtually absent in layer I which is usually densely innervated in rodent neocortex. Within secondary sensory cortical areas, CA fibers are similarly distributed, but less dense. In contrast to the sensory areas, motor cortex (Brodmann areas 4,6) contains adrenergic axons in all layers: layers II, V and VI are profusely innervated by NE-type axons, while layers II and III contain both DA- and NE-type axons. Fluorescent fibers are particularly dense medially in the distal hindlimb region as well as more laterally in the distal forelimb and face area. CA-containing axons exhibit a still different pattern of distribution in frontal and parietal association cortex. In these regions, layers II and III are most heavily innervated, and except in Brodmann's area 9 surrounding the principal sulcus, layer I contains few CA fibers. Both NE and DA-type axons are present in anterior cingulate, dorsolateral and orbital cortex of the frontal lobe. Insular cortex has a unique CA fiber distribution, expressed as an extremely dense and homogeneous input to all layers except layer I. Also, unlike other cortical fields, the fluorescent axons in the insula course in only one direction, predominantly dorsoventrally. Additional, more general features of the CA fiber distribution in primate cerebral cortex include a) the presence of a network of long preterminal axons coursing in both anteroposterior and mediolateral directions through the deep strata of layer VI and within the subcortical white matter, b) frequent localization of terminal NE-type axons around interstitial neurons situated within subcortical white matter and c) the more prominent accumulation of CA axons around sulcal invaginations, particularly in more superficial layers.

These general features and the regionally specific patterns and density of NE- and DA-containing afferents in primate cerebral cortex differ substantially from that in rodent. These findings indicate that in the primate, this class of cortical afferents may have specialized functions within each individual cortical field. (Supported by grants NS16666, NS14841 and F32NS06143.)

258.4 SELECTIVE INCREASE OF LOCUS COERULEUS AXONS IN A DEFINED SINGLE GENE MUTATION. Jeffrey L.Noebels and Pat Levitt, Sect. of Neuroanatomy, Yale Univ. Sch. of Med., New Haven, Ct. 06510.

The mutant gene tottering (tg, autosomal recessive, chromosome 8) expresses in the mouse a reproducible alteration in CNS physiology characterized by a delayed developmental appearance of ataxia, spontaneous cortical spike-wave discharges, and focal motor seizures. No disturbances of neuronal organization, regional brain morphology or size have previously been recognized. To further define the cellular expression of the tg allele, the central catecholamine (CA) neuron system of C57Bl/6J tg/tg mice was examined using fluorescence histochemistry and biochemical analysis of CA content. Glyoxylic acid histofluorescence studies revealed 2-3 fold increases in the number of noradrenergic axons within all terminal fields innervated by the nucleus locus coeruleus (LC), including neocortex,hippocampus, cerebellum, and dorsal lateral geniculate nucleus, when compared with age-matched wild type (+/+) mice. This increase was seen uniformly at the time of the earliest behavioral expression of the tg neurological syndrome (4 weeks postnatal) and persisted into adulthood. Concomitant elevations of 100-200% in norepinephrine (NE) levels were detected by high performance liquid chromatography in these terminal regions. In contrast, CA fibers and transmitter content within target areas innervated by a second major NE axon system arising from brainstem lateral tegmental neurons were unaltered. Nuclei receiving a dense dopaminergic innervation were also unchanged in terminal axon number and transmitter content. Despite the striking hypertrophy of the LC axonal plexus, the number and size of LC somata were identical in both tg/tg and +/+ mice, as were the dimensions and cellular organization of all target nuclei. Based on the anatomical segregation of central CA terminal projections, these results show that a single locus mutation can selectively alter the locus coeruleus NE neuronal system while sparing other cell groups sharing the same class of neurotransmitter. The increased number of LC axons in the tg/tg brain differs from the atrophic changes of specific cell types often observed in other neurological gene mutations. This unusual growth may be related to the capacity of central CA neurons for fiber plasticity in developing and adult animals. Unlike sprouting following experimental injury, the inherited lesion displays a generalized proliferation throughout the entire LC axonal arbor, indicating that the expression of a terminal field of defined proportions can be directed by a single gene. The findings described are consistant with a specific gene-linked alteration of developmental events controlling the number of axons produced by a single neuronal population in the mammalian brain. Supported by grant F32NS06143.

258.5 COLLATERALIZATION OF MONOAMINE NEURON PROJECTIONS TO FOREBRAIN
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The monoamine (MA) cell groups projecting to the forebrain consist of dopamine cells in the ventral tegmental area (VTA) and substantia nigra (SN), serotonergic cells in the raphe nuclei, and norepinephrine cells in the locus coeruleus (LC). The efferent targets of these cells have been characterized and the topographical organization of these projections have been the subject of our previous work. These studies suggested that at least some cells in each nucleus could collateralize to innervate more than one structure. The recent development of the fluorescent retrograde tracer techniques has allowed us to address the question of whether individual cells in these nuclei might collateralize to innervate more than one cortical region, or both cortical and subcortical sites. In the present study nuclear yellow, granular blue, true blue, and propidium iodide were injected into MA forebrain fields in the albino rat. The results suggest that each of these MA cell groups differ with respect to their principles of collateralization. VTA cells are rarely double labeled, suggesting that each cell projects to one, or only a few, target areas. SN cells, however, are frequently observed to be double labeled and one cell may project to limbic, striatal, and cortical sites. They are, however, highly organized with respect to this collateralization and only certain combinations of injections result in double labeling. For instance, whereas one cell might project to pregenual and supragenual cortex, one cell does not appear to innervate supragenual and suprarhinal cortex simultaneously. The other MA cell groups, raphe and LC, are highly collateralized. One cell can be observed to innervate cortical and subcortical sites. The innervation of cortex by these groups, however, may be governed by very different organizational principles than those of the DA cell groups. That is, spatial rather than cytoarchitectonic subdivisions may be important. Although one cell may innervate medial pre-frontal and occipital cortex, the same cell is not observed to innervate more lateral regions. The principles governing the collateralization of these projections will be discussed.

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258.6 PROJECTIONS OF THE A1 AND A2 NOREPINEPHRINE CELL GROUPS TO THE SEPTUM OF THE RAT.
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The septum of the rat receives a noradrenergic (NA) innervation from the locus coeruleus and from cells in the medulla. We have made use of the highly selective uptake and retrograde transport of antibodies against dopamine-β-hydroxylase (DBH) by NA axons to determine which of the two medullary NA cell groups (A1 and A2) gives rise to NA fibres in the septum. Rats received a stereotaxic injection of 0.05-0.2 µl guinea pig anti-rat DBH antiserum into the lateral septal nucleus. Antibodies were delivered through a glass micropipette over a period of 40 min. To control for specificity, guinea pig globulins were injected in place of anti-DBH antiserum. After 18 h rats were perfused and 60 µ sections were cut on a Vibratome. Sections were stained with HRP labeled antibodies to visualize retrogradely transported anti-DBH antibodies. Every third section was processed for immunocytochemical staining of DBH.

At the injection site staining for anti-DBH extended from the midportion of the lateral septal nucleus into the medial septal nucleus and the dorsal portion of the interstitial nucleus of the stria terminalis. Positive retrograde labeling of NA cells was indicated by the presence of immunoreactive granules in the cytoplasm and dendrites of NA cells. Strong labeling was observed in a small number of A1 cells bilaterally in the ventrolateral portion of the medulla rostral to the obex; in contrast, very few of the more caudally located A1 cells were labeled. None of the presumably epinephrine containing cells in the rostral medulla contained retrogradely transported anti-DBH antibodies. Numerous labeled A2 cells were found bilaterally at the level of the area postrema but none at the level of the nucleus gracilis. In the pons, a considerable number of labeled cells were present in the rostral and dorsal part of the locus coeruleus. No retrogradely transported anti-DBH was detectable in cells of the A5 and A7 group.

The data indicate that the septal area receives NA fibres from the locus coeruleus and from a restricted portion of the A1 and A2 cell groups. The existence of a NA projection arising from A2 cells, which are embedded in the nucleus of the solitary tract, provides evidence for a direct NA link between this visceral afferent nucleus and the septal area. Support: USPHS NS 16654.

258.7 THE NORADRENERGIC INNERVATION OF PERIAQUEDUCTAL GREY MATTER AS VISUALIZED BY HIGH RESOLUTION RADIOAUTOGRAPHY.
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To examine noradrenergic axon terminals in the periaqueductal grey matter (SGP), light and electron microscope radioautographs were prepared after prolonged (3 h) latero-ventricular instillation of 10^{-4} M tritiated noradrenaline (^{3}H-NA, DL-7-^{3}H-noradrenaline hydrochloride, 200 µCi) in adult rats pretreated with a monoamine oxidase inhibitor. Semi-thin and thin sections taken at various levels across the caudal half of the midbrain displayed labeled axonal varicosities almost exclusively within a distance of 0.5 mm from the ependyma; for example, few were detected inside the nucleus raphe dorsalis proper. A tissue block, centered on the midline and grazing the aqueduct of Sylvius dorsally, was selected for detailed topometric analysis. Radioautographs of serial thin sections were exposed for 6 months, developed with paraphenylenediamine, and all aggregates of silver grains comprising three or more filaments were charted and photographed in a ribbon of 4 sections. Moreover, every axonal process thus recognized as labeled was photographed in each of the sections where it was visible. The region surveyed in this manner measured approximately 0.7 mm^2 and showed 280 different labeled sites. Of these, 57 (20%) could be identified as intervaricose segments belonging to thin unmyelinated axons (mean diameter: 0.3 µm), and 213 (76%) as axonal varicosities, i.e. enlargements containing clustered synaptic vesicles (mean diameter:1 µm). When expressed per unit area, the number of labeled varicosities diminished linearly with increasing distance from the ependyma ($r = -0.96$). A close correlation was also found for the frequency with which the labeling was detected in each section across varicosities seen more than once ($r = -.90$). Only 16 (6%) of the labeled varicosities exhibited a membranous differentiation of synaptic junction, which was invariably axo-dendritic. The junctional profiles were slightly larger (mean diameter: 1.2 µm) than their non-junctional counterparts and appeared randomly distributed with respect to their distance from the ependyma. These data emphasize the restricted penetration of ^{3}H-NA in brain tissue after intraventricular administration. In SGP, the gradient of diffusion actually precluded complete identification of all noradrenergic axon terminals. Nevertheless, topometric analysis of serial thin sections allows the conclusion that the noradrenergic innervation of the SGP is largely non-junctional. (Supported by MRC grant MT-3544).

258.8 AXOPHORESIS OF [^{3}H]DA AND [^{3}H]NA BY CENTRAL CATECHOLAMINERGIC NEURONS IN RAT BRAIN: RADIOAUTOGRAPHIC DEMONSTRATION.
L. Descarries, F. Berthelet* and S. Garcia* (SPON: A. Beaudet). Centre de recherche en sciences neurologiques, Département de physiologie, Université de Montréal, Montréal, Québec, Canada.

To visualize an eventual axonal transport of tritiated dopamine ([^{3}H]DA) or noradrenaline ([^{3}H]NA) in CNS, one or the other tracer (25 µCi/0.5 µl for 10 min) was injected immediately above the substantia nigra (SN) or inside the nucleus caudate-putamen (CP) of adult rats pretreated or not with a monoamine oxidase inhibitor. After 1-24 h survival, the entire brain was radioautographed by exposure of frozen sections on film (LKB) or following perfusion-fixation with glutaraldehyde, paraffin embedding and dipping in liquid emulsion. Labeling at a distance was detected only after pretreatment with monoamine oxidase inhibitor and predominated 3-24 h after the injections, reaching its maximum at 6 and 12 h. The same structures showed labeling with both tracers. 1) After supra-SN injections: rostrally, ipsilateral neostriatum and nucleus accumbens; caudally, ipsilateral fibers in the brainstem tegmentum and nerve cell bodies in the locus coeruleus and in the bulbar groups A-1, A-2 and A-3. A few labeled cell bodies were also seen in the contralateral locus coeruleus. 2) After CP injections: ipsilateral fibers of the nigro-striatal system and nerve cell bodies in the SN, zona compacta, and ventral tegmental area. In addition, a small number of labeled neuronal somata were visible bilaterally, near the midline, in nuclei linearis caudalis and raphe dorsalis; the cells in raphe dorsalis occupied only the rostralmost portion of its medial subgroup (ventral more than dorsal cluster). Concomitant administration of non radioactive serotonin at a concentration 10 times higher than that of [^{3}H]DA had no apparent effect on these patterns of labeling. In rats subjected 15 days earlier to unilateral injection of 6-hydroxydopamine in the zona compacta of SN (8 µg/4 µl), labeling of the nigro-striatal dopaminergic system was almost completely suppressed, which was also the case for the retrograde nerve cell body reactions associated with supra-SN injections. The latter reactions persisted when the rats had been treated with 6-hydroxydopamine after a single dose of desipramine (25 mg/kg i.p., 1 h antea). Two conclusions were drawn: 1) that anterograde and retrograde axophoresis of [^{3}H]-DA or [^{3}H]NA occurs exclusively in catecholaminergic neurons; 2) that the recently described "non-serotoninergic" projection from nucleus raphe dorsalis (and linearis caudalis) to CP in the rat (Steinbusch et al., 1980) is most probably dopaminergic. (Supported by MRC Grant MT-3544).

258.9 UPTAKE OF [^{3}H]ADRENALINE BY CENTRAL MONOAMINERGIC NEURONS. O. Bosler* and L. Descarries (SPON: H.H. Jasper). Département de Neurobiologie Cellulaire, CNRS, BP 71, 13277-Marseille, France, and Centre de recherche en sciences neurologiques, Université de Montréal, Montréal, Qué., Canada.

Neuronal sites of accumulation of adrenaline (A) were investigated with light and electron microscope radioautography after lateroventricular instillation of 10^{-4} M [^{3}H]A (DL [7-^{3}H]adrenaline hydrochloride, specific activity: 11.6-17.3 Ci/mM, 60-350 µCi, for 10 min - 3 h) in adult rats pretreated with a monoamine oxidase inhibitor. Following glutaraldehyde perfusion and osmium postfixation by immersion, there were no aggregates of silver grains suggestive of neuronal uptake and retention of the tracer. In contrast, selective labeling was detected in brains which had been rapidly fixed and postfixed by successive vascular perfusion of the glutaraldehyde and osmium solutions (Nature 284: 620-622, 1980). [^{3}H]A-labeled nerve cell bodies were seen in various regions, all known to contain a catecholaminergic population, wherein they were distributed according to the topographic patterns expected from these elements: preoptic area, periventricular hypothalamus and dorsal thalamus, ventral tegmental area, zona compacta of substantia nigra, locus coeruleus, A-1 (C-1) and A-2 (C-2) areas of the medulla. [^{3}H]A-labeled axonal varicosities were prominent in the caudate-putamen, nucleus accumbens, lateral septum, juxta-ventricular and basal hypothalamus, inner layer of the median eminence, periventricular gray matter, and within the subcommissural organ and above the ependymal lining of several portions of the ventricles. The addition of 10^{-3} M non radioactive serotonin to the [^{3}H]A solution markedly diminished the labeling in the subcommissural organ and the supra-ependymal plexus, but had no apparent effect elsewhere in brain. Prior unilateral injection of 6-hydroxydopamine in the pars compacta of substantia nigra (8 µg/4 µl, 15 days earlier) eradicated the axonal labeling in the ipsilateral neostriatum, but spared that in the nucleus accumbens which receives its dopaminergic innervation mainly from the ventral tegmental area. It was concluded that serotonin-containing axon terminals of the subcommissural organ and the supra-ependymal plexus, as well as dopamine and norepinephrine neurons throughout the neuraxis can take up adrenaline, for which they probably have a lesser affinity than toward their own transmitter. Further work will be needed to distinguish presumptive central adrenergic neurons from their dopaminergic and noradrenergic congeners on the basis of such properties. (Supported by MRC grant MT-3544).

258.10 REGIONAL DISTRIBUTION OF CATECHOLAMINE SYSTEMS IN THE RAT MEDULLA A COMBINED NEUROCHEMICAL AND FLUORESCENCE HISTOCHEMICAL STUDY. M.A. Rea, S.K. Jackson,* D.L. Felten and M.H. Aprison, Inst. of Psychiatric Res., Depts. of Psychiatry, Anatomy and Biochemistry Ind. Univ. Sch. of Med., Indianapolis, IN 46223

Specific catecholamine innervation of medullary nuclei in Wistar rats was carried out with combined neurochemical and histofluorescence methods in an attempt to further characterize adrenergic influences on vagal nuclei. The localization of catecholamine cell bodies and varicosities was achieved with both formaldehyde and glyoxylic acid histofluorescence. For comparative biochemical studies, rats were killed by immersion in liquid N_2 for 30 seconds. The frozen brains were removed in a -30°C cryostat; 300 µm thick coronal sections of brain stem were prepared. Tissue samples containing the descending nucleus of V (DNV), inferior olivary nucleus (ION), medullary Raphe nuclei, hypoglossal nucleus, dorsal motor nucleus of X + nucleus of the solitary tract (DMN+NTS), dorsomedial reticular formation (DMR), ventromedial reticular formation (VMR) and ventrolateral reticular formation including the nucleus ambiguus and vagal preganglionic cell bodies innervating the heart (VLR+NA) were isolated from 6 frozen coronal sections (900 µm caudal to the obex through 900 µm rostral to the obex) with an 18G micropunch. The levels of norepinephrine (NE), epinephrine (Ep) and dopamine (DA) were determined by HPLC with electrochemical detection. Tissue NE levels varied 8-fold among the medullary regions and, in general, correlated well with the density of NE terminals detected by fluorescence histochemistry. The high level of NE in the DMN+NTS (218+10 pmol/mg protein (P)) is consistent with the presence of NE cell bodies of the A_2 catecholamine cell group and the high density of NE varicosities projecting from brain stem cell groups to the DMN of X. Moderately high levels of NE were detected in regions containing DMR (99±5 pmol/mg P), ION (94±4 pmol/mg P) and VLR+NA (80±5pmol/mg P). The DNV, characterized by sparse NE innervation, was found to contain the lowest level of NE (28±1 pmol/mg P). In the VLR+NA punch, cell bodies of the A_1 cell group were found around the lateral reticular nucleus and a moderate density of fluorescent varicosities was found around and dorsomedial to the NA. The current combined neurochemical-histofluorescence observations suggest that the preganglionic parasympathetic cell bodies in the DMN of X are more densely innervated by NE varicosities than the preganglionic cardioinhibitory cell bodies around NA. Although Ep levels did not vary greatly, levels were highest in the DMN+NTS (4.8±0.8 pmol/mg P) providing additional evidence for Ep as a putative transmitter in cell group A_2. DA levels were also highest in DMN+NTS (31±6 pmol/mg P). Supported in part by N.I.H. grant Rol NS 16205.

258.11 BIOCHEMICAL IDENTIFICATION OF A NIGRO-CEREBELLAR PATHWAY. Melvin H. Van Woert, Eunyong Chung Hwang, Mark J. Perlow and Melvin D. Yahr. Dept. of Neurology & Pharmacology, Mount Sinai School of Medicine, New York, N.Y. 10029.

Neuronal connections between the basal ganglia and cerebellum have been suggested, however, the neurotransmitters involved and the precise mechanisms by which they interact to control motor activity is not known. In order to examine for a possible nigro-cerebellar pathway and its neurotransmitter, we lesioned the substantia nigra (SN) and examined high affinity uptake of several neurotransmitters in the cerebellum.

Male Sprague-Dawley rats (150 g) were anesthetized with chloropent and electrothermic lesions of both SN (AP = 2.5 mm, L = ± 2.0 mm and V = 6.8 mm from dura according to Konig and Klippel Atlas) were made with a Grass LM4 lesion maker. 14 days following the lesion, the cerebellum was removed and high affinity (2.5 x 10^{-9}M to 1 x 10^{-7}M) synaptosomal uptake of neurotransmitters was carried out:

Cerebellar Synaptosomal Uptake
(cpm/15 mg/15 min)

Neurotransmitter	Control	SN Lesioned
^{3}H-Norepinephrine (^{3}H-NE)	3810 ± 296	2507 ± 335*
^{3}H-Dopamine (^{3}H-DA)	1158 ± 78	875 ± 182*
^{3}H-Glutamate	2159 ± 186	2106 ± 257
^{3}H-GABA	4646 ± 409	4446 ± 292
^{3}H-Choline	385 ± 88	339 ± 22

* $p < 0.05$ compared to control by t-test.

Cerebellar ^{3}H-NE and ^{3}H-DA uptakes in SN lesioned rats were significantly lower than the controls, while uptake of the other neurotransmitters examined were not significantly different from control.

In order to investigate whether this is a dopaminergic or noradrenergic pathway, we injected 6-hydroxydopamine (6-HODA) into the same region of the SN after pretreatment with desmethylimipramine (DMI) (25 mg/kg ip) which specifically blocks the uptake of 6-HODA into noradrenergic neurons. 6-HODA lesions of the SN produced similar reductions in cerebellar synaptosomal uptakes of ^{3}H-NE and ^{3}H-DA as electrothermic lesions. DMI pretreatment had no effect on the reductions of ^{3}H-NE and ^{3}H-DA uptakes in the cerebellum.

In summary, our results suggest that there is a nigro-cerebellar pathway which appears to be dopaminergic. (Supported by USPHS grant NS 71631).

258.12 BRANCHED COERULEOCEREBRAL-COERULEOCEREBELLAR PROJECTIONS: A DOUBLE RETROGRADE AXONAL TRACING STUDY USING ^{3}H-N-ACETYL WHEAT GERM AGGLUTININ AND HORSERADISH PEROXIDASE. Dennis A. Steindler* (SPON: J. Zacks). Dept. of Anatomy, Michigan State University, East Lansing, Michigan 48824.

The existence of a noradrenergic innervation of the neuraxis and cortical structures arising from neurons of the pontine nucleus locus coeruleus has been well documented in numerous studies. Despite the presupposition that cells in locus coeruleus give rise to collateralized axonal projections, there has been a lack of direct evidence that substantiates the presence of significant numbers of branched neurons within this nucleus. The present double retrograde axonal tracing study was undertaken in order to resolve the existence of collateralized efferent projections of locus coeruleus neurons to both forebrain and cerebellar cortical structures. Injections of horseradish peroxidase (HRP) or ^{3}H-N-acetyl wheat germ agglutinin (^{3}H-acetyl WGA, New England Nuclear, 1.97mCi/mg) were placed within portions of the cerebral cortex, neostriatum, hippocampus and cerebellar cortex of adult Spb;HA(ICR) white mice. Following survival times of 24-36 hr. and aldehyde fixation, sections were processed for combined histochemistry and autoradiography (exposure times varying from 9 days to 4 months) and examined using bright- and darkfield microscopy. The distribution of retrograde labeled neurons within locus coeruleus observed after forebrain and cerebellar cortical injections of the tracers was compared to the distribution of noradrenergic neurons observed in sections treated for aldehyde-induced catecholamine fluorescence. The following observations were made: 1)Bidirectional axonal transport of both tracers was discerned in several structures (e.g. thalamus, substantia nigra, deep cerebellar and precerebellar nuclei). 2) The boundaries of fluorescent noradrenergic locus coeruleus neurons and those revealed by retrograde labeling were strictly coincidental. 3) The distribution of coeruleocerebral and coeruleocerebellar neurons was found to be similar, and the number of retrograde labeled locus coeruleus neurons appeared to be related to the amount of cortical tissue encompassed by the injected material. 4)Injections of both tracers anywhere in the forebrain and cerebellum in the same animal invariably produced double labeling of neurons scattered throughout the ipsilateral locus coeruleus, and of the neurons that were found to be retrogradely labeled from a cerebellar injection, over 50% were also labeled from the forebrain injection of the other tracer. Thus, noradrenergic locus coeruleus neurons achieve a wide spread innervation of the forebrain and cerebellar cortices at least in part through axonal collateralization. (Supported by NIH Grant NS 15931).

259.1 BIOCHEMICAL PROPERTIES OF HUMAN PLATELET IMIPRAMINE RECOGNITION SITES. L.R. Meyerson, B. Beer and L.P. Wennogle*. Dept. of CNS Research, Medical Research Division, American Cyanamid Company, Lederle Laboratories, Pearl River, New York 10965.

The high affinity specific binding of ^{3}H-Imipramine (^{3}H-IMI) has been demonstrated in both neuronal and platelet sources. Suggestions have been advanced relating this recognition site to serotonin reuptake systems, while others have stated that the modulation of this platelet site may represent a biochemical index of depressive illness. Thus, in light of these theoretical constructs we now provide preliminary biochemical evidence and possible metabolic control mechanisms which may influence the expression of this platelet ^{3}H-IMI site. Platelet membrane fractions were prepared by standard procedures either with NaCl-Tris buffers or with similar buffers supplemented with a variety of enzyme inhibitors including antiproteases and EDTA. In the presence of enzyme inhibitors, consistently higher (1.5x) densities of binding sites (Bmax) were observed while Kd values remained unchanged. Experiments were performed in order to assess the contribution of proteins and lipids to this variation. Trypsin and pronase at concentrations of 0.2 and 0.3 mg/ml, respectively, were sufficient to reduce the number of ^{3}H-IMI recognition sites on isolated platelets to 50% of their original value. In contrast, phospholipase A_2 concentrations of only 2 µg/ml were required to reduce the density of IMI sites by 50%. These observations indicate that the IMI site is particularily susceptable to phospholipid perturbations while being fairly resistant to protease degradation. Further studies demonstrated that a calcium activated metabolic process maybe involved in the stability of the ^{3}H-IMI site. Receptor deterioration (75% reduction) is observed in the presence of 3 mM calcium when exposed at 37°C for 20 hours compared to matched controls (-20°C). This loss is further potentiated by the addition of the calcium-ionophore A-23187. Interestingly, this calcium-mediated degradation can be blocked by chelating agents such as EDTA and EGTA. Thermal denaturation of the ^{3}H-IMI site occurs in the same range expected for a typical membraneous entity with a temperature for 50% inactivation ($T_{½}$) of 62°C. The effect of ultrasonic disruption on intact platelet membranes resulted in a negligible loss of total binding sites. However, the specific binding values/mg protein of the resuspended membrane fragments increased probably due to loss of occluded soluble proteins. The results presented herein provide further characterization of the platelet IMI binding site and how possible metabolic processes mediated by divalent cations such as calcium may play an important role in the preservation and maintenance of proteanaceous recognition sites labeled by ^{3}H-IMI.

259.2 GLUCOSE-INDEPENDENT BRAIN RESPIRATORY RESPONSES. J.T. Cummins, M. Juarez* and E. Keller*. Addiction Research Lab., V.A. Med. Ctr., Sepulveda, CA 91343 and Dept. of Psychiatry, UCLA.

The degree of glucose dependency during depolarized respiratory responses was studied in slices from rat brain cortex. The slices, incubated in Krebs-Ringer bicarbonate with and without glucose, were depolarized with electrical pulses (100Hz, 10V, 30 sec.) and the redox responses of the respiratory intermediates, pyridine nucleotides (NADH) and cytochrome a-a_3, were measured by dual wavelength spectrophotometry. Our results demonstrate that certain components of the depolarized respiratory responses do not depend on exogenous glucose. Following electrical depolarization, the reduction of cytochrome a-a_3 is the same with and without glucose, whereas the NADH response increases three-fold from a basal (no glucose present) response in the presence of glucose. Interestingly, depolarized reduction of NAD^+ and cytochrome a-a_3, enhanced by the ß-adrenergic agonist isoproterenol, is not dependent on glucose. These data have suggested that there are several redox responses following depolarization, some possibly using endogenous compounds rather than exogenous glucose to reduce the respiratory intermediates. One of the endogenous redox responses appears to occur in a biochemical compartment high in mitochondrial activity and activated by an adrenergic agent. This differentiation of several brain redox reactions that occur following depolarization may be used to further study the respective brain biochemical compartmentation.

259.3 CHARACTERIZATION OF A HYDROPHOBIC, DIMERIC FORM OF ACETYLCHOLINESTERASE (AchE) FROM TORPEDO ELECTRIC ORGANS. S.L. Lee*, S.J. Camp* and P. Taylor* (Spon: C.E. Spooner). Div. of Pharmacology, University of California at San Diego, La Jolla, CA 92093.

Prior to this study, the native molecular species of Torpedo and Electrophorus AchE which had been purified and characterized were the dimensionally asymmetric, high ionic strength soluble forms of the enzyme. These asymmetric forms of AchE contained a filamentous unit of partial collagen-like composition, which appears responsible for the localization and apparent association of the enzymes with the basal lamina found in the synapse between nerve and muscle cells. This laboratory has reported (Fed. Proc. 39:179, 1980) that following DTT reduction of the asymmetric Torpedo AchE the subunit components consist of the catalytic unit monomer (68K), a partially collagenous subunit (55K), and a relatively protease-sensitive structural subunit (100K). In the absence of DTT, the dimer of catalytic subunits (131K) and an array of 8-12 evenly spaced bands from 405-750K MW are found.

In this presentation, we describe a stable hydrophobic species of AchE which is composed of a dimer of disulfide-linked subunits purified to apparent homogeneity from Torpedo californica electric organs. The enzyme is soluble at low ionic strength in the absence of detergent, but exists as an aggregate. Non-ionic detergent dissociates the complex into a discrete 5.6S species. Although the hydrophobic dimer [5.6S] and the asymmetric forms of AchE [(17+13)S] show immunologic cross-reactivity, a similar carbohydrate content, and neither contains lipids, there are distinct differences between the catalytic subunits. The 5.6S AchE contains a higher content of hydrophobic amino acid residues and a unique peptide map. On SDS gels, ideal migration behavior is observed for the 5.6S dimeric species, while the dimer of the (17+13)S AchE shows atypical migration when mobility is plotted against acrylamide concentration. Since the (17+13)S monomer (68K) and the slightly smaller 5.6S monomer (66K) both show ideal behavior following DTT reduction, the disparate non-reduced migration behavior is likely due to a difference in secondary structure. Hence it is unlikely that the hydrophobic 5.6S and asymmetric (17+13)s AchE species are precursors or products for each other in situ. Rather, they exist as discrete entities with different dispositions within the synapse. Its comparatively hydrophobic amino acid composition and properties of self-association in the absence of detergent suggest that the 5.6S AchE is loosely associated with plasma membrane through hydrophobic interactions. (Supported by GM 18360 and the Muscular Dystrophy Association.)

259.4 ACETYLCHOLINE RECEPTOR SYNTHESIS OF CHICK SKELETAL MUSCLE CELLS IN CULTURE IS ALTERED BY RYANODINE. Leo Pezzementi and Jakob Schmidt. Dept. of Biochemistry, SUNY at Stony Brook, NY 11794

Synthesis of acetylcholine receptor (AcChR) of muscle cells in culture is regulated by the activity of the muscle fibers. We have investigated the effect of ryanodine on AcChR metabolism. Ryanodine is a plant alkaloid which at micromolar concentrations causes contraction of skeletal muscle due to depletion of calcium from the sarcoplasmic reticulum (SR), and at millimolar concentrations stimulates efflux of calcium from the muscle cell. We found that, after a 48-hour exposure of chick myofiber cultures, micromolar concentrations of ryanodine decreased AcChR to approximately 60% of control levels, while millimolar concentrations increased AcChR to over 200% of control. This appears to be a specific effect on the synthesis of the receptor since appearance rates but not degradation rates of AcChR are altered by the drug. Intracellular transport of AcChR is not affected as both surface and total AcChR content are changed in parallel. The differences in receptor levels do not reflect a general alteration of muscle cell metabolism since protein levels, protein synthetic rate, creatine phosphokinase activity, myosin heavy chain levels, and cell surface acetylcholinesterase are not similarly affected. Additionally, after 48 hours total cellular acetylcholinesterase is unchanged from control levels, although there is a transient (ca. 30%) decrease after 24 hours.

The decrease in receptor seen with micromolar concentrations of ryanodine is not affected by either TTX (which blocks sodium channels) or D-600 (a calcium channel blocker), but is inhibited by Dantrolene Sodium (which blocks release of calcium from the SR). Millimolar concentrations of ryanodine overcome the decrease in AcChR elicited by micromolar concentrations of the drug and by caffeine (which releases calcium from the SR), but this effect is blocked by veratridine (a sodium channel activator). These observations lead us to believe that elevated cytoplasmic calcium, released from the SR by micromolar concentrations of ryanodine, acts to shut off receptor synthesis. We also conclude that ryanodine at millimolar concentrations increases AcChR synthesis by causing a decrease in cytoplasmic calcium, through increased calcium efflux from the cell, thus counteracting the effects of micromolar ryanodine and of caffeine, which increase cytoplasmic calcium. Veratridine, by activating sodium channels, allows reentry of calcium into the muscle cell thus inhibiting the increase in receptor synthesis normally seen with high concentrations of ryanodine. (Supported by a grant from the Muscular Dystrophy Association.)

259.5 MODULATION OF ACETYLCHOLINESTERASE ACTIVITY AND ACETYLCHOLINE RECEPTOR NUMBER IN AN ANDROGEN SENSITIVE NEUROMUSCULAR SYSTEM.
W.V. Bleisch and V.N. Luine. Rockefeller University, New York, N.Y. 10021.

The syrinx of the songbird may provide a novel system in which to study the regulation of synaptic machinery in a physiologically relevant context. The syrinx is highly sensitive to androgens, and syringeal weight, protein, acetylcholinesterase (AChE) activity and acetylcholine receptor (AChR) number all decrease following castration of adult males and increase following testosterone (T) treatment of adult females (Luine et al., 1980, Brain Res. 192:89; Harrelson et al., this volume). We now report that T stimulates the syrinx within days and that the effect of castration occurs much more slowly.

Syrinxes were homogenized in buffered 1.5% Triton X-100 and AChE activity was assayed as described in Luine et al. above. For determinations of AChR number, homogenates were incubated at 30° C for 1 hour and spun at high speed. These extracts were then diluted to equal protein concentration and assayed for binding of I-125 α-bungarotoxin (400pM incubated for 3 hours). Bound and free toxin were separated by the method of Kohanski et al. (1977, Anal. Biochem. 80:531). Nonspecific binding was measured by competing with 500 nM gallamine. This assay proved to be sensitive and linear between 0 and 40 fmoles of AChR. Affinity curves for toxin binding to AChR from male and female zebra finch syrinxes were superimposable, indicating no difference in the kinetics of toxin binding.

In order to study the time course of the effects of T, adult female zebra finches were implanted with T (5mm silastic capsules, as in Luine et al. above) at 11, 6, 5, 3, 2 and 1 days before sacrifice. AChR number was significantly elevated 1 day after implantation ($p < .05$) and protein also rose rapidly. In contrast, AChE activity increased little in the first 2 days after implantation, then rose rapidly between 2 and 5 days. Protein, AChR number and AChE activity all appeared to reach a plateau by 5 days.

In contrast to these rapid changes after T stimulation, the effect of castration appeared much more gradually. Adult male zebra finches were castrated at 59, 23 and 11 days before sacrifice. Syringeal weight, protein, AChR number and AChE activity all decreased gradually from 0 to 23 days, after which they appeared to reach a plateau. Half-times for these losses were calculated from a first order model and found to be 6.6 days for total protein, 15.8 days for AChE activity and 12.5 days for AChR number. This last value is consistent with published turnover rates for adult junctional AChR (see Fambrough, 1979, Physiol. Rev. 59:165).

260.1 A NEW CLASS OF NEUROACTIVE COMPOUNDS, THE AVERMECTINS; EFFECTS ON PERMEABILITY PROPERTIES OF THE LOBSTER INHIBITORY NEUROMUSCULAR JUNCTION. T. N. Mellin, R. D. Busch and C. C. Wang. Dept. of Biochemistry, Merck Institute for Therapeutic Research, Rahway, NJ 07065

The avermectins are a new family of anthelmintic macrocylic lactones which paralyze nematodes and insects. One highly potent constituent, avermectin-B_{1a} (AVM), blocks postsynaptic potentials at the lobster nmj by reducing membrane resistance, (Fritz et al., PNAS, 76: 2062, 1979). It also blocks transmission between interneurons and excitatory motorneurons in the ventral cord of the parasitic nematode, Ascaris (Kass et al., PNAS, 77: 6211, 1980).

Our studies with AVM (5 µg/ml), in lobster opener and stretcher muscle, have confirmed earlier observations which showed that AVM caused a rapid disappearance of IPSP's. EPSP amplitude and input membrane resistance were found to decline at a slower rate, with a common time course. A 3-4 mV membrane hyperpolarization was also observed. These effects were not reversed by extensive washing. EPSP amplitude was partially restored by picrotoxin (20 µM). Both GABA (50 µM) and AVM (5 µg/ml) decreased the slope of I/V curves in stretcher muscle, reflecting an increase in membrane conductance. Bicuculline (50 µM) or picrotoxin (20 µM) reduced the conductance changes caused by GABA or AVM. Increases in membrane conductance, induced by various concentrations of GABA, AVM, or avermectin analogs, were determined from changes in resistance. Dose-response curves showed AVM and a 22,23-dihydroavermectin-B_{1b} analog to produce greater conductance increases than GABA. The magnitude of membrane conductance changes induced by avermectin analogs generally paralleled the degree of stimulation of ^{3}H-GABA release from rat brain synaptosomes and paralysis of the free-living nematode, C. elegans (Pong et al., J. Neurochem. 34: 351, 1980). Replacement of 50% of the Cl^- in lobster saline, with equimolar isethionate, resulted in a depolarization of membrane potential by AVM and GABA, rather than the hyperpolarization normally observed. Cl^- replacement did not alter I/V responses to GABA and AVM. Some fibers of the cockroach extensor tibiae muscle lack GABA inervation. Studies on these fibers revealed no effect of AVM on EPSP amplitude.

Our results thus support earlier suggestions that AVM acts to increase Cl^- permeability of the lobster inhibitory nmj.

260.2 INTERACTIONS OF LINCOSAMIDE ANTIBIOTICS WITH IONIC CHANNELS OF NICOTINIC RECEPTORS. S.R. Ikeda*, J.E. Warnick and E.X. Albuquerque. Dept. of Pharmacol. & Exp. Ther., Univ. of Maryland School of Medicine, Baltimore, MD 21201.

The effects of the lincosamide antibiotics clindamycin (CLIN) and lincomycin (LIN) were studied on the frog sciatic-sartorius and cutaneous pectoris muscles using voltage clamp and fluctuation analysis techniques. Both agents interact primarily with the open conformational state of the ionic channel of the nicotinic ACh receptor. CLIN produced a concentration-dependent depression of peak endplate current (EPC) at 25-1000 µM. Nonlinearity of the current/voltage (I/V) relationship was evident at concentrations above 250 µM. EPC decay in the presence of CLIN was single exponential at all concentrations tested with progressive shortening of the time constant of EPC decay (τ_{EPC}) with increasing drug concentration. At concentrations above 250 µM the normal voltage dependence of τ_{EPC} was abolished. The effect of CLIN on miniature EPC (MEPC) peak amplitude and decay (τ_{MEPC}) was similar to that of EPCs. Noise analysis of EPC fluctuations in response to iontophoretically applied ACh revealed a concentration-dependent decrease in mean channel lifetime and single channel conductance. Power spectra at all concentrations of CLIN could be adequately fitted to a single Lorentzian component. In contrast, LIN produced a progressive lengthening of τ_{EPC} above 250 µM. In addition, a second fast component was evident in τ_{EPC} with higher concentrations (400-1200 µM) of LIN and hyperpolarized membrane potentials (-90 to -160 mV). EPC peak amplitudes in the presence of LIN were depressed in a dose-dependent manner with significant nonlinearity of the I/V relationship evident at concentrations above 100 µM. Studies on τ_{MEPC} revealed two components at concentrations of LIN greater that 50 µM and at all membrane potentials tested (-60 to -120 mV). The fast component of τ was relatively insensitive to drug concentration and membrane potential while the slow component of τ increased with hyperpolarization and drug concentration. The amplitudes of the fast and slow components were insensitive to both drug concentration and membrane potential. Noise analysis in the presence of 50 µM LIN or greater produced spectra consistent with two kinetic components. These results suggest that LIN and CLIN react primarily at the ionic channel in the open conformation and may explain the inability of Ca^{++} and anticholinesterase agents to reverse lincosamide-induced neuromuscular blockade. Furthermore, studies with LIN may provide information about factors controlling τ_{EPC} and τ_{MEPC}. (Supported in part by a grant from The Upjohn Co. and USPHS grant NS-12063.)

260.3 BIOCHEMICAL CHARACTERIZATION OF KETAMINE INTERACTIONS WITH THE NICOTINIC RECEPTOR-ION CHANNEL COMPLEX. Latha Narayanan* and Robert S. Aronstam (SPON: J.J.Buccafusco). Department of Pharmacology, Medical College of Georgia, Augusta, GA 30912.

The interactions of ketamine with the nicotinic receptor-ion channel complex in Torpedo electric organ membranes were investigated using radiolabelled probes for sites associated with the receptor and the ion channel. Receptor binding was measured with 0.1 µM [^{3}H]acetylcholine ([^{3}H]ACh) and [^{3}H]d-tubocurarine ([^{3}H]d-TC) using equilibrium dialysis techniques. "Ion channel" binding was measured with [^{3}H]perhydrohistrionicotoxin ([^{3}H]$_{12}$-HTX) and [^{3}H]phencyclidine ([^{3}H]PCP) using a filtration assay. Ketamine depressed the binding of 2 nM [^{3}H]PCP and 1 nM [^{3}H]H_{12}-HTX to the channel in a dose-dependent fashion; IC50 values ranged from 3 to 8 µM. The ability of ketamine to inhibit the binding of either ligand was enhanced 2 to 3 fold in the presence of 1 µM acetylcholine. Ketamine was equally effective in depressing [^{3}H]PCP and [^{3}H[H_{12}-HTX binding. The inhibition was reversed upon removing the ketamine by centrifugation.

In contrast, ketamine at concentrations up to 1 mM did not depress the binding of 0.1 µM [^{3}H]acetylcholine or [^{3}H]d-tubocurarine to the acetylcholine receptor. This binding was measured using equilibrium dialysis techniques. This suggests that the interference of ketamine with neuromuscular transmission detected in biophysical studies (Alkadhi et al., Fedn. Proc. 40:263, 1981) reflects interactions of the drug with sites removed from the receptor itself.

Probe	Concentration, nM	Ketamine IC50, µM
[^{3}H]ACh	100	>1000
[^{3}H]d-TC	100	>1000
[^{3}H]H_{12}-HTX	1	6.2
[^{3}H]H_{12}HTX/ACh	1	3.1
[^{3}H]PCP	2	7.9
[^{3}H]PCP/ACh	2	3.2

*In these cases binidng was measured in the presence of 1 µM ACh.

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260.4 THE SYNERGISTIC INTERACTION OF 4-AMINOPYRIDINE AND NEOSTIGMINE AT THE NEUROMUSCULAR JUNCTION. Peter C. Tierney*, Yong I. Kim* and T.R. Johns. Department of Neurology, University of Virginia School of Medicine and the University of Virginia Jerry Lewis Neuromuscular Center, Charlottesville, Virginia 22908.

4-Aminopyridine (4-AP) facilitates nicotinic neuromuscular transmission by an increase in the release of acetylcholine (ACh) from motor nerve terminals. Clinical trials of this compound indicate that it is a potent antagonist of the actions of non-depolarizing neuromuscular blocking drugs and that it produces an improvement in muscle strength and neuromuscular transmission in patients with Eaton-Lambert syndrome or myasthenia gravis. Using intracellular microelectrode recording techniques, we investigated the interaction between 4-AP and neostigmine at the rat neuromuscular junction. Our results demonstrate a synergistic increase in neuromuscular facilitatory action when the two drugs were used simultaneously in vitro.

End-plate potentials (EPPs) were measured from forearm flexor digitorum longus muscles exposed to a physiological buffer containing 1.2 µg/ml of d-tubocurarine chloride (TC). Control measurements of EPPs from 15 different end-plates were followed by a one hour perfusion with a solution containing either 4-AP, neostigmine or both. EPPs were recorded again from 15 different end-plates following perfusion. The drug concentrations used were 0.5, 1 and 2 µM 4-AP and 2 and 8 nM neostigmine.

The increase in EPP amplitude when 0.5 µM 4-AP was combined with either 2 or 8 nM neostigmine was equal to or slightly greater than the summation of their individual effects when applied separately. When 1 µM 4-AP was combined with 2 or 8 nM neostigmine, a 20% greater than additive increase in EPP amplitude was seen. An effect 50% greater than additive was produced when 2 µM 4-AP was used with either neostigmine concentration.

This study suggests that a significant portion of the synergism between 4-AP and neostigmine on facilitating neuromuscular transmission occurs at the junction and that this synergism is concentration dependent. Future investigations will attempt to further determine the site of interaction between the two drugs.

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260.5 THE EFFECTS OF PROCAINAMIDE ON NEUROMUSCULAR TRANSMISSION. David C. Lee*, Yong I. Kim* and T.R. Johns (SPON: G.R. Hanna). Department of Neurology, University of Virginia School of Medicine and the University of Virginia Jerry Lewis Neuromuscular Center, Charlottesville, Virginia 22908.

Procainamide has been shown to produce clinical exacerbations in patients with myasthenia gravis when utilized for the control of cardiac arrhythmia. We wished to characterize the neuromuscular effects of procainamide at therapeutic doses on the rat flexor digitorum longus muscle.

Using intracellular microelectrode recording techniques, control measurements were made for spontaneously occurring miniature end-plate potentials (MEPPs) from 15 end-plates and then from 15 end-plates after perfusion with procainamide. This was performed at 1, 2, 4, 6 and 8 times the maximum therapeutic level (MTL-16 mg/l). Similar measurements were made for neurally evoked end-plate potentials (EPPs) on muscles treated with 0.6 µg/ml of d-turbocurarine chloride at 1, 4 and 8 MTL.

MEPP amplitude decreased in a dose dependent manner with no detectable MEPPs at 8 MTL. MEPP frequency was reduced significantly only at 6 MTL. The resting membrane potential (RMP) was stable. EPP amplitude was decreased to a comparable degree with that of MEPP with no significant effect on RMP. The half-decay time demonstrated a marked prolongation, while the rise time was slightly reduced. MEPP and EPP measurements made at 4 MTL on single end-plates revealed a reduction in amplitude similar to that of the multiple puncture studies. The effects on both MEPPs and EPPs were reversible with washing with control solution.

Direct quantal analysis was performed using a physiologic buffer containing a high magnesium concentration (10 mM Mg^{++}). Single junction studies were made for 1 and 4 MTL and a decrease in both EPP and MEPP amplitudes was demonstrated. The EPP reduction was greater than that of the MEPP and thus there was a reduction in the number of ACh quanta released from the motor nerve terminals. Both pre- and postjunctional blockades produced by the drug were reversed with a control buffer wash.

This study demonstrates that procainamide decreases postjunctional sensitivity to ACh as well as reduces the quantal content of ACh at clinically significant doses. This direct inhibitory action of procainamide at the junction may account for the clinical exacerbations produced by this drug.
(Supported in part by a center grant from the Muscular Dystrophy Association).

260.6 EVIDENCE FOR GENERATION OF THE SLOW IPSP BY THE DIRECT MUSCARINIC HYPERPOLARIZING ACTION OF ACETYLCHOLINE IN BULLFROG SYMPATHETIC GANGLIA. Peter A. Smith and Forrest F. Weight, Department of Pharmacology, University of Alberta, Edmonton, Alberta, TGG 2H7 Canada and Laboratory of Preclinical Studies, National Institute on Alcohol Abuse and Alcoholism, Rockville, MD 20852

The synaptic pathway and the neurotransmitter mediating the slow IPSP in bullfrog sympathetic ganglia has been controversial for several years. To study the slow IPSP, the nicotinic fast EPSP must be reduced or blocked with antagonists such as nicotine or d-tubocurarine (dTC). Weight & Padjen (Brain Res. 55, 225, 1973) found in nicotine blocked bullfrog ganglia, that the muscarinic hyperpolarizing action of ACh was present in a low Ca/high Mg Ringer; they proposed that the slow IPSP is generated by a direct muscarinic hyperpolarizing action of ACh released from the cholinergic preganglionic fibers that synapse on C neurons. Libet & Kobayashi (J. Neurophysiol. 37, 805, 1974) confirmed that observation, but reported that in curarized ganglia low Ca/high Mg abolished the hyperpolarizing response to ACh or methacholine (MCh); they concluded that the slow IPSP in curarized ganglia involves an adrenergic interneuron. We have re-examined the generation of the slow IPSP and the effect inhibiting transmitter release on the MCh-hyperpolarization (MCh_H) in curarized ganglia. The experiments were performed on the IXth or Xth paravertebral sympathetic ganglion of the bullfrog in the presence of 70 uM dTC. With intracellular recording, stimulation of the VIIIth spinal nerve generated a slow IPSP that could be recorded intracellularly from C neurons; the iontophoretic administration of ACh to C neurons elicited a slow hyperpolarizing response in those neurons. In sucrose gap experiments, Ringer's solutions containing the following were used to inhibit transmitter release: (i) tetrodotoxin (10^{-5} M); (ii) 4 mM cobalt; (iii) Ca-free (no Ca^{2+} added); (iv) Ca-free with 10^{-4} M EGTA; or (v) Ca-free with 10 mM Mg^{2+}. Although each of these treatments effectively inhibited synaptic transmission, none blocked the MCh_H. In fact, the MCh_H was increased in amplitude by Ca-free Ringer with 10^{-4} M EGTA. If an interneuron is involved in the generation of the slow IPSP, the MCh_H should be abolished by conditions that prevent neurotransmitter release. On the basis of the observations reported here, the MCh_H appears to be a direct muscarinic postsynaptic response in curarized, as well as nicotinized ganglia. This suggests that the slow IPSP results from a direct muscarinic hyperpolarizing action of ACh released from cholinergic preganglionic fibers that synapse on C neurons, regardless of the nicotinic antagonist.

260.7 EFFECT OF PHENTOLAMINE ON SYNAPTIC TRANSMISSION IN BULLFROG SYMPATHETIC GANGLIA. Parviz Yavari* and Forrest F. Weight, Laboratory of Preclinical Studies, National Institute on Alcohol Abuse and Alcoholism, Rockville, MD 20852

The neurotransmitter and pathway mediating the slow IPSP in the sympathetic ganglia of bullfrog has been controversial for several years. One hypothesis proposes that the slow IPSP is mediated by a direct muscarinic action of acetylcholine released from cholinergic preganglionic fibers (Weight & Padjen, Brain Res., 55:225, 1973). A second hypothesis proposes that the slow IPSP is mediated by a catecholamine released from an adrenergic interneuron (Libet & Kobayashi, J. Neurophysiol., 37:805, 1974). The latter hypothesis is based, in part, upon the observation that the alpha-adrenergic antagonist phentolamine in a concentration of 40 and 200 µM partially depresses the slow IPSP. We have used the sucrose-gap recording method to study the effect of phentolamine on synaptic transmission in the IXth or Xth paravertebral sympathetic ganglia of bullfrogs. Preganglionic B fibers were stimulated in the sympathetic chain between the VIth and VIIth ganglion, and preganglionic C fibers were stimulated in the VIIIth spinal nerve. In a concentration of 1 or 10 µM, phentolamine did not have any appreciable effect on the amplitude of the transmitted action potential of either the B or the C fiber pathway. However, when the concentration of phentolamine was increased to 40 µM, the amplitude of both the B and the C volley was consistently reduced. Phentolamine in a concentration of 40 µM for 30 min. reduced the B action potential 13 ± 2% (mean ± SEM; n = 8) and the C spike 12 ± 3% (n = 4). In other experiments, when 40 µM phentolamine was perfused for 60 min, the reductions were 23 ± 5% for B (n = 4) and 20 ± 7% for C spikes (n = 3). At a concentration of 200 µM, phentolamine produced a 38 ± 6% (n = 3) reduction in the height of the B volley when it was perfused for 30 min. Since synaptic transmission in bullfrog ganglia is mediated by a nicotinic cholinergic mechanism, the results indicate that in concentrations greater than 10 µM, the alpha-adrenergic antagonist phentolamine can have non-specific depressant effects on synaptic transmission.

260.8 EFFECTS OF ACIDIC AMINO ACID DERIVATIVES ON PERFORANT PATH EVOKED RESPONSES. Eric W. Harris, James F. Koerner* and Carl W. Cotman. Department of Psychobiology, Univ. Calif. Irvine, Irvine, CA 92717 and Department of Biochemistry, Medical School, Univ. Minn. Minneapolis, MN 55455.

Acidic amino acids, in particular L-glutamate, have been proposed as candidates for neurotransmitters in the central nervous system. There is especially strong supportive evidence that glutamate is the transmitter for the entorhinal cortical projection to the dentate gyrus (the perforant path). Using the isolated hippocampal slice preparation, we investigated the effects of superfusion with several putative glutamate antagonists and found a very potent anatgonism by the L-stereoisomer of 2-amino-4-phosphonobutyric acid (L-APB). Micromolar L-APB antagonized the evoked response in the outer-most portions of the dentate dendritic field (Koerner and Cotman, Brain Res., in press). The input to this region arises from the more lateral portions of the entorhinal cortex. The projection from more medial entorhinal cortex, terminating in the middle part of the dentate dendritic field, is is less sensitive and exhibits a compound inhibition by L-APB. The L-APB-sensitive lateral component was less sensitive to the D-stereoisomer of APB and to the two homologues D,L-2-amino-3-phosphonopropionic acid and D,L-2-amino-5-phosphonovaleric acid.

To further characterize the receptors activated by the perforant path, we have examined the effects of D-α-aminoadipic acid (DαAA) and glutamic acid diethyl ester (GDEE), which block the classes of receptors activated by N-methyl-D-aspartic acid and quisqualic acid, respectively (see Evans and Watkins, Life Sci., 28, 1303-1308, 1981). Evoked responses along the lateral component of the perforant path were much less sensitive to antagonism by DαAA than by APB. The perforant path appears to be uniformly sensitive to antagonism by DαAA, and is relatively insensitive to the L-stereoisomer of αAA, or to GDEE which produced significant reduction in the evoked response size only at very high concentrations (>4mM). At concentrations of either APB or DαAA which yielded assymptotic levels of antagonism, addition of the other compound produced a further response decrement, suggesting that the compounds act on non-identical populations of receptors.

Our results indicate that transmission at the perforant path to granule cell synapse involves a novel type of receptor, that, for the lateral component, is sensitive to L-APB, and that does not fit the prevailing acidic amino acid receptor classification scheme.

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260.9 PHARMACOLOGICAL CHARACTERIZATION OF THE ELECTROPHYSIOLOGICAL AND BEHAVIORAL EFFECTS OF ADENOSINE AGONISTS. Thomas V. Dunwiddie and Thomas Worth*. Dept. of Pharmacology, University of Colorado Health Sciences Center, Denver, CO 80262

Biochemical experiments have suggested that adenosine may interact with at least two different types of receptors in brain tissue (Bruns et al.,PNAS 77:5547,1980). We have characterized physiological responses to adenosine analogs in order to compare their pharmacological actions to their relative affinities for adenosine receptors.

Adenosine, related purines, and analogs of adenosine were examined in terms of their effects on synaptic transmission and anticonvulsant activity in vitro, and for sedative, anticonvulsant, and hypothermic actions in vivo. Several non-metabolizable analogs of adenosine were found to be extremely potent in antagonizing synaptic transmission, and in inhibiting the rate of interictal discharge in the hippocampus in vitro, with EC50's in the low nanomolar range.

In vivo, these same analogs exhibited sedative, hypothermic, and anticonvulsant activity in both mice and rats. Several results suggest that these adenosine analogs do not act via a homogeneous population of receptors to produce these effects in the intact animal. First, the potency of a given agonist in producing sedative effects could be as much as an order of magnitude greater than its potency as an anticonvulsant. Secondly, some agonists were relatively better anticonvulsants, while others were more potent as sedatives. Finally, when the anticonvulsant actions of adenosine agonists were examined with respect to a variety of convulsants, some dissimilarities between different agonists were readily apparent. Cyclohexyladenosine and 2-chloroadenosine appeared quite similar, being very potent in antagonizing picrotoxin-induced seizures, and relatively weak as anticonvulsants of pentylenetetrazol-induced convulsions. Conversely, L-phenyl-isopropyladenosine was effective against a range of different convulsants including pentylenetetrazol. If all adenosine analogs acted on a single population of receptors to produce their effects one would expect that, although some might be weaker agonists than others, all would show the same general profile of relative potency when tested in various ways. This clearly was not the case.

The results from both in vitro and in vivo experiments suggest that the receptors mediating these responses are similar to those tentatively characterized as A1 receptors. However, the heterogeneity of the responses seen in vivo suggests that other types of receptors may be implicated as well.

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260.10 BUSPIRONE'S EFFECTS ON FELINE SPINAL REFLEXES. G. Keith Matheson. Indiana University, School of Medicine, Evansville, IN 47732.

This study was undertaken in order to determine the acute effects of buspirone on the monosynaptic reflex (MR) and on modulatory mechanisms regulating the MR. Buspirone HCl is a nonbenzodiazepine anti-anxiety agent with a markedly different psychopharmacologic profile from other known antianxiety agents. Unlike the benzodiazepines, buspirone has no anticonvulsant activity, interacts minimally with CNS depressants, does not cause muscle relaxation and lacks abuse potential. In this study random source cats of either sex were anesthetized with alpha-chloralose (60mg/kg, i.p.), intubated, and catheterized. A paraffin boat was formed around the lumbar region of the exposed spinal cord. Some of the dorsal roots between L-6 and S-1 were attached to Pt bipolar stimulating electrodes. Platinum recording electrodes were fixed to ventral rootlets at L-7 and/or S-1 and to some dorsal rootlets at L-7. The animals were artificially ventilated and paralyzed with gallamine triethiodide. Stimulus rates were not allowed to exceed 0.5 pulses per second. Control MR potentials were collected at thirty minute intervals for 1 to 2 hours. Buspirone increased the amplitude of the MR and the dorsal root reflex by 100 percent or more. The effect peaked after the third hour and returned to pre-drug levels by the sixth hour. A dual pulse condition--test stimulation technique was used to examine the effects of buspirone on spinal excitatory and inhibitory mechanisms. During the excitatory phase the amplitude of the MR was neither enhanced nor diminished, indicating the test pulse may already be eliciting a maximal excitatory response from the neuron pool. During the post-synaptic inhibitory phase the inhibitory effect was increased nearly one-fold, attenuating the MR to approximately fifty percent of control levels. During the pre-synaptic inhibitory phase the MR was not significantly attenuated suggesting that this inhibitory mechanism was almost totally supressed by buspirone. These findings suggest that buspirone may affect spinal reflex activity by supressing pre-synaptic inhibitory mechanisms, thereby allowing maximal activity in post-synaptic excitatory and inhibitory pathways impinging on the spinal alpha-motor neuron.

260.11 PROTECTIVE EFFECTS OF L-LYSINE ON PENTYLENETETRAZOL-INDUCED SEIZURES AND DEATH. N.R.Myslinski, B.Soohoo‡ K.Thuang‡ and Y.Chang‡ Depts. of Physiology and Biochemistry, University of Maryland Dental School, Baltimore, Md. 21201.

Recent evidence indicates that certain lysine metabolites significantly influence synaptic transmission of gamma-aminobutyric acid (GABA) in the brain. Since GABA is involved in the mechanism of action of many anticonvulsant agents we studied the effects of L-lysine and its metabolites on pentylenetetrazol(PTZ) induced seizures. Male Swiss-Webster mice weighing between 15 and 29 g were used. PTZ was administered IP at 80 mg/kg, after which the animals were observed for 15 min and then sacrificed. Significance of most data was determined by the Mann-Whitney U test.

In double blind experiments using saline controls L-lysine (1M,0.01 ml/g of mouse) significantly prolonged the latency of seizures when administered either as a single dose 15 min before PTZ ($P<0.002$), or in 5 doses during the preceeding 3 days ($P<0.006$). When administered intraventricularly (ICV)(0.5 ul/g of mouse) at 1,2,5, or 10 min before PTZ, L-lysine prolonged seizure latency at both 0.2M ($P<0.05$) and 1.0M ($P<0.05$) with the greatest effect occurring when lysine was given 5 min before. The most profound effect of ICV lysine was its prevention of death. Seventy-three percent of the saline pretreated mice died, but only 21% of the L-lysine pretreated mice died as a result of the PTZ-induced seizures. Piperidine (ICV at 0.2 M), a metabolite of L-lysine, also prolonged the latency of seizures ($P<0.05$) when given 5 min before PTZ. It also reduced the death rate to 44%. Two other metabolites of L-lysine were administered ICV 5 min before but had opposite effects. L-pipecolate (0.2M) shortened the seizure latency ($P<0.02$) and increased the death rate to 90%. L -α- aminoadipic acid (0.2M) shortened the seizure latency ($P<0.05$) and increased the death rate to 100%.

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260.12 BRAIN PERFUSATE DRUG KINETICS. H. D. Christensen, Berhane Gherezghiher* and Tseggai Gherezghiher*. Dept. of Pharmacology, Univ. of Oklahoma Health Sciences Center, Coll. of Medicine, McGee Institute and V.A. Medical Center, Oklahoma City, OK 73190.

One of our laboratory goals is to correlate pharmacological or behavioral responses of CNS active drugs with their pharmacokinetic parameters and modification of endogenous neurochemicals. The use of push-pull perfusion of selected brain regions, liquid chromatographic separation and multiplexing of detectors offer some advantages over more traditional approaches. Adequate serial samples can be collected for appropriate disposition kinetics, and repeated evaluations can be made in the same animal under various conditions.

If moderate concentrations of caffeine (10 mg/kg) are administered to an alert non-restrained cat, then the cortex perfusate concentrations of caffeine are eliminated slightly slower than in the plasma confirming the apparent difference observed after sacrifice of the animals. This also indicated that the brain perfusate concentrations will probably give no better correlates than the plasma caffeine concentrations to either cue discrimination or neuronal activity. By separating the compounds in the perfusate by means of a liquid chromatography column with both UV and electrochemical detectors, it was determined that the amines fluctuated slightly but adenosine showed a consistent transient increase. A very preliminary study would indicate that cyclic AMP levels, as measured by RIA, decreases in the cortex perfusate after caffeine administration.

A modification of the conceptual model for synaptic transmission in the oculomotor nucleus occurred when the mechanism of αmethyldopa (MD) induced mydriasis was investigated by analysis of oculomotor perfusate concentrations of MD and its metabolites. When MD is administered i.v. to the pentobarbital anesthetized cat, it rapidly penetrates into the brain and is eliminated from a single compartment with an elimination rate constant of 0.015 min^{-1} and volume of distribution of 1.84 L/kg. MNE is formed in the neuron with rate of conversion-synaptic vesicle transport of 0.018 min^{-1} and released into synaptic cleft at a rate of 0.007 min^{-1}. The elimination from the perfusate space is 0.003 min^{-1}. The MD induced mydriasis could be correlated ($r=0.94$) with the MNE perfusate concentrations which were dependent upon the kinetic constants and initial dose of MD. The reuptake mechanism is non-functional for αmethylated compounds in these neurons. Thus MNE cannot return to the neuron; its compartment space is about 30% of that of MD, the same as for 3-O-methyl-α-MD. This property plus the direct contact of neurons with capillaries offer an explanation of why some drugs with no intrinsic receptor activity can be ineffective given directly to brain area but are very effective when administered intravenously.

260.13 FOOD DYES ALTER SPONTANEOUS RELEASE OF NEUROTRANSMITTER FROM FROG NEUROMUSCULAR JUNCTION: A STRUCTURE-ACTIVITY STUDY. K. C. Wadhwani* and H. Levitan. Department of Zoology, University of Maryland, College Park, Maryland 20742.

The widely used food dye erythrosin B (FD&C Red No. 3) has previously been shown to alter the release of transmitter from the frog neuromuscular junction (Science 207:1489(1980)). In an attempt to better understand the basis for the ability of this xanthene dye to enhance the frequency of release, and to determine whether other food dyes commonly used in the U.S. have similar effects on transmitter release, we have examined the effects of several azo, triphenylmethane and other fluorescein dyes on the spontaneous release of transmitter from nerve terminals innervating cutaneous pectoris muscle. In experiments carried out in the dark (i.e. at light intensities at least six orders of magnitude less than ambient room light) analogs of fluorescein increased mepp frequency exponentially with time at a rate which was dependent upon the particular dye and the concentration applied. The dye concentrations producing a 10-fold increase in mepp frequency within 120 min were(in micromoles/l):Rose Bengal=1;Phloxine B=3; Erythrosin B=20;Eosin B=25; Eosin Y=80; Fluorescein=1300. The more lipid soluble the dye the more potent it was. The effects of the dyes appeared to be reversible in the dark in contrast to the effects of erythrosin B examined previously in normal ambient light. The mepp frequency also increased exponentially with time on exposure to the triphenylmethane dye FD&C Green No. 1. The azo dyes Alura Red AC(FD&C Red No.40) and Tartrazine (FD&C Yellow No.5) increased the mepp frequency only when applied in millimolar concentrations. In these azo dyes the frequency increased very rapidly to a steady-state level which was dependent upon the dye concentration applied, and remained at that level for at least as long as an hour. Upon washing out the dye the mepp frequency rapidly returned to control levels. We conclude that the xanthene and triphenylmethane dyes interact with hydrophobic regions of the nerve terminal to enhance release while the azo dyes augment release by an osmotic mechanism.

261.1 ABNORMAL KINASE ACTIVITY AND DEFICIENT ENDOGENOUS PHOSPHORYLATION IN BRAINS OF EPILEPTIC FOWL. J.M. Tuchek*, D.D. Johnson, S. Polvi* and R.D. Crawford*. Dept. of Pharmacology, Univ. of Saskatchewan, Saskatoon, Sask. S7N 0W0 Canada.

We have previously reported an abnormality of endogenous phosphorylation in epileptic fowl. The high seizure susceptibility in this model of epilepsy is due to an autosomal recessive mutation. Homozygotes (epileptics) undergo spontaneous seizures and convulse in response to photic stimulation whereas heterozygotes (non-epileptics) do not develop seizures. Three proteins with approximate molecular weights of 72, 60 and $16x10^3$ in brain homogenates from epileptics incorporated less ^{32}P from radiolabelled ATP than did the same proteins isolated from their non-epileptic hatchmates. Subfractionation of the homogenates indicated that the low molecular weight protein was associated with the myelin fraction whereas the two higher molecular weight bands were present in the 100,000xg supernatant fraction indicating a cytosolic origin. Tissue culture studies of neuronal and glial cells revealed that the proteins were of neuronal origin.

Protein kinase activity was assayed in the 100,000xg brain supernatants according to the method of Witt and Roskoski, Analytical Biochem. 66: 253 (1975). Kinase activities in the brain fractions obtained from epileptics were lower than the enzyme activities in non-epileptic brain homogenates. When assayed in the presence of 100 µM EGTA, the protein kinase could be stimulated by adding Ca^{++}, with optimal activities being achieved in the presence of 150-200 µM added Ca^{++}. This Ca^{++} stimulatory effect was diminished by the addition of $5x10^{-5}$ M cAMP. Autoradiographic data on the 72,000 and 60,000 molecular weight cytosolic proteins which were phosphorylated in vitro at 30°C for 30 seconds with endogenous kinases, confirmed the Ca^{++} stimulatory effect and the antagonism of this effect by cAMP that was seen in the protein kinase assays where histone was used as the receptor protein.
(Supported by the MRC of Canada, Grant #MT-5893.)

261.2 A MECHANISM OF BARBITURATE ENHANCEMENT OF SPIKE FREQUENCY ADAPTATION. G. Evans*, J.R. Huguenard*, W.A. Wilson*, D.V. Lewis* (SPON: T. Slotkin). VA Epilepsy Center and Depts. of Pharmacology and Medicine, Duke Univ., Durham, NC 27710

Barbiturates are widely used as anticonvulsants. We have shown that the anticonvulsant barbiturates phenobarbital and pentobarbital enhance the process of spike frequency adaptation (SFA), the decremental change in neuronal firing rate in response to a prolonged constant depolarization. Zbicz and Wilson (JPET, 217:222-227, 1981) demonstrated that in Aplysia neurons R2 and LP1, SFA has a fast (<10sec) and slow phase. The fast phase is a pronounced decrement in firing rate during the first 10 sec of depolarization; the slow phase is a gradual decrement in firing that continues for tens of seconds. Barbiturates do not alter the fast phase of SFA but dramatically potentiate slow SFA.

The currents underlying SFA may be elucidated by voltage clamping. Aplysia neurons (LP1 and R2) were clamped to -50mV (near resting potential) and then step depolarized to -30mV (near spike threshold) for 60 seconds. During the depolarization, a K^+ dependent slow outward current (SOC) develops which can be increased by 50-200µM pentobarbital. Upon repolarization to -50mV, tail currents that represent the exponential decay of the SOC are observed.

The kinetic parameters of the tails were examined with a curve peeling technique (compartmental analysis). Analyses consistently derived two first order decay processes for tail currents. In normal sea water half-lives for these two processes are approximately 10 and 50 seconds; the fast component having an amplitude 4 to 5 times larger than that of the slow component. However, in 100µM pentobarbital the amplitude of the slow component is markedly increased with little effect on the amplitude of the fast component or the kinetics of either component. The net result is a major contribution by the slow component to the total amplitude of the tail current. This is not unexpected since the barbiturates potentiate the slow phase of SFA in these neurons.

Compartmental analysis of tail currents thus appears to be an appropriate means of analyzing and quantitating the effect of barbiturates on SFA mechanisms.

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261.3 REGIONAL ELECTRICAL EFFECTS OF TOPICALLY ADMINISTERED GAMMA-HYDROXYBUTYRIC ACID. W. O. Haggard*, L. J. Bearden, E. M. Wilson*, and O. C. Snead. Departments of Biomedical Engineering, Neurology, Pediatrics, and the Neurosciences Program, University of Alabama in Birmingham, Birmingham, AL 35294.

There is considerable evidence that the endogenous compound gamma-hydroxybutyric acid (GHB) provides an appropriate model for the study of petit mal epilepsy. Experiments have shown that the behavioral and electrical effects produced by GHB are very similar to the abnormalities observed during petit mal seizures. It has also been found that these effects are suppressed or aborted by anticonvulsants which are clinically successful against seizures of petit mal epilepsy. Other studies have found that the peptides leucine enkephalin, methionine enkephalin, and beta-endorphin also produce behavioral and electrical effects like those observed during petit mal seizures, and that these effects can be blocked by naloxone (Urca et al., Science 197:83, 1977; Frenk et al., Brain Research 147:327, 1978; Henriksen et al., Proc. Natl. Acad. Sci. U.S.A. 75:5221, 1978). Work from our laboratory has further shown that naloxone blocks the behavioral and electrical effects of GHB (Snead and Bearden, Neurology 30:832, 1980), and that the petit mal anticonvulsants (ethosuximide, trimethadione, valproic acid) effectively block the convulsive effects of leucine enkephalin. Therefore, there are significant similarities between the seizure-like effects of gamma-hydroxybutyric acid and those of the opiate peptides. We have pursued these studies further by examining the electrical effects of GHB and leucine enkephalin following injections of these compounds into discrete areas of the brain.

Adult male rats (250-350 g) were stereotaxically implanted with epoxylite insulated tungsten macroelectrodes to permit recording of the EEG from the cortex, hippocampus, thalamus, and caudate-putamen of the brain. Following recovery from surgery, animals were anesthetized with chloral hydrate, repositioned in a stereotaxic apparatus, and control EEG was recorded from the indicated sites. Subsequently, stereotaxically placed injections of GHB or leucine enkephalin were made into the brain. These injections were made via a glass micropipet, and were accomplished by means of a pressure microinjection system. Such experiments have shown that the electrical effects of both GHB and leucine enkephalin are variable between selected regions of the brain.

261.4 FOLATES MIMIC DISTINCTIVE FEATURES OF KAINATE (BUT NOT GLUTAMATE) NEUROTOXICITY. J.W. Olney, T.A. Fuller, T. deGubareff*, and J. Labruyere*, Washington Univ. Sch. Med., St. Louis, MO.

Prompted by the report of Ruck et al. (Nature 287, 852, 1980), that methyltetrahydrofolate (MTHF) competes strongly for kainic acid (KA) binding sites on neural membranes and exerts KA-like depolarizing action on frog spinal neurons, we compared a series of folic acid (pteroyl-L-glutamic acid, PGA) derivatives, including MTHF, with several glutamic acid (Glu) analogs, including KA, for their neurotoxic properties when administered by various routes, including direct injection into several brain regions. Consistent with prior observations, Glu and its linear analogs, such as DL-homocysteate (HCA) and N-methyl-aspartate (NMA), when injected directly into brain (e.g., striatum or amygdala), reproduced the local brain damaging action of KA (NMA >HCA >Glu) without inducing the sustained seizures and distant disseminated lesions that characteristically accompany direct injection of KA into brain. Conversely, folates effectively reproduced the convulsant and distant brain damaging actions of KA without damaging neurons at local brain injection sites, e.g., high intrastriatal doses did not injure striatal neurons but destroyed many neurons in extrastriatal regions typically damaged by intrastriatal KA. Diazepam (sc) protected against the entire pattern of folate-induced damage (Fuller et al.,Neurosci.Abst. 1981). PGA and folinic acid (N-5-formyltetrahydrofolate) were the most powerful folates tested, whereas MTHF and dihydrofolate were the weakest (minimal effective intra-amygdala dose for inducing a seizure-brain damage syndrome = 5-10 nmoles for the former two agents and 100-300 nmols for the latter two). When injected into the lateral ventricle, PGA tended to give an all or none response (either rapidly lethal seizures or no effect); although intra-amygdala injection of PGA (>50 nmoles) sometimes induced a typical KA pattern of hippocampal damage, intra-hippocampal PGA (50 nmoles) caused rapidly lethal seizures without appreciable hippocampal damage. When administered systemically, PGA did not reproduce the Glu type of neurotoxic action in circumventricular organs, even at doses that induced lethal convulsions. We propose that KA neurotoxicity may have a compound mechanism, one component (seizure-mediated distant damage) involving folate-sensitive receptors and another (local neuron-necrotizing action) involving folate insensitive receptors. Since PGA is readily available, inexpensive and powerfully mimics seizure-related features of KA neurotoxicity but not seizure-unrelated features, it may be at least as useful as KA for studying mechanisms of epilepsy and epileptic brain damage. Moreover, since folates are present in foods and stored in the body, including the CNS, it warrants consideration that they might play a pathogenic role in human epilepsy or epileptic brain damage. Supported by USPHS grants NS 09156-DA 00259, RSA MH38894(JWO)&MH 00330 (TAF).

261.5 DIAZEPAM MARKEDLY ATTENUATES THE NEUROTOXICITY OF FOLIC ACID. T.A. Fuller, J.W. Olney and V.T. Conboy*, Washington Univ., Sch. Med., Dept. Psychiatry, St. Louis, MO.

Kainic acid (KA), a cyclic glutamate (Glu) analog, is a powerful convulsant and neurotoxin which, when injected directly into the striatum or amygdala, destroys neurons both locally and in several remote regions of brain. Ben-Ari et al. (Br Res 165,632, 1979) have attributed the remote disseminated lesions to a seizure mechanism since the anti-convulsant, diazepam (DZ) protects against these lesions but not the local neuron-necrotizing action of KA. Folic acid (pteroyl-L-glutamic acid, PGA), also a cyclic Glu analog, was recently shown (Olney et al., Neurosci Abst,1981) to induce KA-like seizures and a KA-like disseminated pattern of brain damage when injected into various brain regions, but did not appear to exert local neurotoxic action. If PGA reproduces only the seizure-related disseminated component of KA neurotoxicity, one might expect DZ to protect against the entire PGA pattern of damage; the experiments described here were undertaken to test this hypothesis. Sixteen adult male rats were injected unilaterally with 50 nmole PGA into either the striatum or amygdala under halothane anesthesia. Half of each group received PGA only and the other half received DZ (15-20 mg/kg sc) 20 min prior to and 1 hr after PGA. The animals were perfused transcardially 24 hrs after PGA for histopathological evaluation of their brains. All rats (n=8) that received only PGA manifested repetitive seizures and moderately to extremely severe brain damage. Regions most consistently and severely damaged by either striatal or amygdala PGA were the ipsilateral amygdaloid complex, claustrum, piriform cortex and fronto-parietal cortex. The most severe local damage, after intra-amygdala injection, was not concentric about the injection site (as is the usual pattern with KA injections) but rather was often more severe at a variable distance from the injection. Following intra-striatal injection, there was no damage at or near the injection site but subacute degenerative changes were noted at the lateral margin of the striatum in those brains displaying severe lesions in the superjacent cerebral cortex. In DZ/PGA-treated rats, 1 of 8 had seizures and a very mild focus of tissue pathology (swollen dendritic processes) in the neocortex, and another, in the absence of seizures, had a questionable focus of dark cell degeneration in the piriform cortex. Our findings support the conclusion that PGA reproduces one component (seizure-related disseminated damage) of KA neurotoxicity. Further studies comparing the properties of folates with those of KA seem warranted for the light they may shed on excitatory neurotoxic phenomena and on mechanisms of epilepsy and epileptic brain damage.
Supported by USPHS grants NS-09156, DA-00259, RSA MH-38894 (JWO) and RSDA MH-00330 (TAF).

261.6 SODIUM CHANNEL BLOCKING ACTION OF PHENYTOIN IN MAMMALIAN NEURONS IN TISSUE CULTURES. N. Matsuki*, F.N. Quandt*, J.Z. Yeh* and R. Ten Eick* (SPON: C.H. Wu). Dept. of Pharmacology, Northwestern Univ. Med. Sch., Chicago, IL 60611.

Three mechanisms have been proposed to explain the anticonvulsant action of phenytoin (DPH): direct actions on membrane ionic channels, on synaptic membranes, and on metabolic processes. Using cultured neuroblastoma cells (NIE105) we investigated the effects of DPH on the Na channel. The single suction pipette method developed by Lee et al. (1978) for snail neurons was adapted for voltage clamping neuroblastoma cells. This technique also provides for an exchange of the internal milieu. Na currents were isolated from the other ionic currents by replacing internal and external K ions with Cs ions.

DPH (25-100 μM) applied externally produced a dose-dependent resting (background) block of Na current which depended on the level of holding potential. When held at -60 mV, in the control state, 50% of the Na channels were available for opening in response to depolarizing pulses; whereas at the same holding potential when exposed to 75 μM DPH only 10% were available. This was a reflection of a hyperpolarizing shift in the slow inactivation curve which resulted in inactivation of most of the Na channels at normal levels of resting potential. However, when holding potential was -100 mV, no resting block was observed. During repetitive pulsing the block produced by DPH was enhanced. The additional block is termed "conditioned block". The extent of conditioned block was augmented by increasing pulse rate, duration, amplitude and number and by decreasing interpulse interval and potential. For example, during 30 conditioning pulses from -80 to +10 mV for 40 msec with an interpulse interval of 500 msec DPH produced an additional 25% decrease from control in Na current. In contrast, when the holding potential was increased to -100 mV the same pulse protocol produced less than a 5% decrease.

Recovery from the conditioned block was faster at -100 mV than at -80 mV suggesting that at -100 mV the decreased effectiveness of DPH to produce conditioned block is due to the more rapid disappearance of block. It was also found that a single long pulse was more effective than several shorter pulses. Since the duration of the open state of Na channels is much shorter than the duration of the pulses used to produce conditioned block, we conclude DPH binds primarily to inactivated channels.

In summary, DPH's Na channel blocking action can be enhanced by (1) lowering the membrane potential and (2) stimulating the neuron at high frequency. Both actions are thought to be related to anticonvulsant efficacy because seizures are associated with high frequency firing and depolarization of the neuronal membranes. (Supported by Epilepsy Foundation of America).

261.7 REDUCTION OF SEIZURE THRESHOLD BY INDOMETHACIN. T. W. Lysz, P. Needleman* and J. A. Ferrendelli. Div. of Clinical Neuropharmacology, Depts. of Pharmacology and Neurology. Washington Univ. School of Medicine, St. Louis, MO 63110.

Previous studies have suggested that prostaglandins in CNS may be involved in pathophysiological mechanisms of seizures. In an attempt to define the relationship(s) between prostaglandins and seizures the effects of indomethacin a cyclooxygenase inhibitor, on pentylenetetrazol-induced seizures in adult, Swiss-Webster mice was studied. Pretreatment of animals with 10 mg/kg of indomethacin one hour prior to the administration of pentylenetetrazol consistently reduced the CD_{50} for tonic convulsion from 75 to 64 mg/kg but had no effect on clonic seizure threshold. Dose response studies revealed that indomethacin concentrations of 1 mg/kg or greater were capable of reducing tonic seizure threshold.

Measurement of cyclooxygenase activity in brain microsomes revealed that tissue from animals systemically treated with 10 mg/kg of indomethacin had a decrease in prostaglandin synthesis. This is consistent with the report of Steinhauer et al. (Naunyn-Schmiedeberg's Arch. Pharmacol. 310, 53-58 (1979)) demonstrating that PGE_2, $PGF_{2\alpha}$ and TxB_2 synthesis in mouse brain, in vivo, is markedly decreased by treatment of the animals with 1 or 10 mg/kg indomethacin.

The present data in conjuction with reported results demonstrate that indomethacin reduces seizure threshold at the same concentration that it inhibits prostaglandin synthesis in brain. This suggests that prostaglandin or some other product of arachidonic acid metabolism has an anticonvulsant action in mammalian brain. Support in part by NIH grants NS-14834, HL-14397 and HL-20787.

261.7 INTERACTIONS BETWEEN VASOPRESSIN- AND PENTYLENETETRAZOL-INDUCED CONVULSIONS IN RATS. D.M. Burnard, Q.J. Pittman and W.L. Veale. Dept. of Medical Physiology and Pharmacology, University of Calgary, Calgary, Alberta, Canada T2N 1N4.

Arginine vasopressin (AVP) has been shown recently to cause convulsions when administered into the lateral cerebral ventricles (ICV) of rats. This occurs via a sensitization process, whereby AVP initially causes short periods of immobility and staring, but the same dose causes myoclonic-myotonic convulsions upon the second injection two days later. Pentylenetetrazol (PTZ)-induced clonic convulsions can also be produced over several days by repeated administration of initially subconvulsive doses. The present work was designed to determine if AVP would alter the typical response to a threshold dose of the convulsant PTZ, and to establish whether or not a generalized PTZ-induced convulsion would result in sensitization of the brain to the seizure-inducing effect of AVP.

Male Long Evans rats were implanted stereotaxically with a guide cannula directed towards a lateral cerebral ventricle. Following recovery, rats were divided into two groups, the first of which was given 1.0 μg AVP ICV, followed by PTZ intraperitoneally two days later. The first administration of AVP resulted in staring and immobility, as expected, followed by burrowing behavior. Subsequent to a threshold dose of PTZ on Day 3, behaviors such as rearing, myoclonic jerks of the head and forelimbs, twitching of facial musculature and/or long periods of locomotor immobility were observed. Only the last two behaviors were observed in the controls.

A second group of rats received a dose of PTZ (40.0 mg/kg) on the first day which induced myoclonic jerks of the head and body, followed by immobility, staring and a loss of postural equilibrium in some rats. Two days later, after administration of 1.0μg AVP ICV, some rats exhibited stereotyped behaviors such as burrowing, periods of immobility and staring, abnormal, exaggerated scratching, and/or locomotor difficulties. We had not previously observed the last two behaviors following a single AVP injection.

These observations suggest that AVP may be acting within the CNS to alter or modify the typical response to a threshold dose of the convulsant PTZ, and that a previous PTZ-induced convulsion modifies the typical response to a first ICV injection of AVP. Whether or not PTZ and AVP are acting to lower the convulsive threshold and potentiate the response to other such agents is yet unclear, and awaits further investigation.

This work was supported by the Medical Research Council of Canada. D.M.B. is supported by AHFMR.

261.9 EFFECTS OF BICUCULLINE ON THE SENSORY RESPONSES OF NEURONS IN BRAINSTEM RETICULAR FORMATION AND PERICRUCIATE CORTEX. C.L. Faingold and W.E. Hoffmann*. Department of Pharmacology, Southern Illinois Univ. Sch. Med., Springfield, IL 62702.

Our previous studies indicate that the sensory responses of brainstem reticular formation (RF) neurons are greatly enhanced following subconvulsant doses of pentylenetetrazol, strychnine, bemegride, or physostigmine. The responses of most lateral geniculate and hippocampal neurons are not greatly affected. RF neuronal response enhancement correlates with convulsant-mediated increases in the amplitude of RF sensory-evoked field potentials (SEPs), but SEPs in non-primary sensory cortex are also extensively enhanced (see Faingold, C.L., Prog. NeuroPsychopharm., 2, 401, 1978).

This study examined the effects of bicuculline (BIC) on the neuronal responses to sensory stimuli in RF and pericruciate cortex. BIC was infused (.025-.06 mg/kg/min i.v.) or applied iontophoretically (onto RF neurons) in locally anesthetized, paralyzed and respired cats or rats (Sprague-Dawley); and ketamine anesthetized rats. Neuronal responsiveness to visual, auditory, and somatosensory stimuli was analyzed using peristimulus time histograms (PrSTHs). Most mesencephalic (93%) and medullary (84%) RF neuronal responses were extensively enhanced in at least one sensory modality following iontophoretic or systemic BIC. RF neurons which were unresponsive before drug became quite responsive after subconvulsant doses of BIC. Pericruciate neurons (71%) showed sensory response enhancement in many cases simultaneous with RF enhancement. The degree of cortical enhancement was somewhat less extensive than that seen in MRF, and the latencies of response peaks in cortical PrSTHs were considerably longer. Drug-induced inhibition without response enhancement was observed in 10% of cortical neurons but was rarely seen in RF neurons.

Our iontophoretic findings in the RF and the previous finding that the receptive fields of neurons in visual cortex are enhanced after BIC iontophoresis (Tsumoto, T. et al., Exp. Brain Res., 34, 352, 1979) suggest that effects on RF and cortex can be mediated directly. However, RF neurons almost invariably respond to the same stimulus with a shorter latency than cortical neurons, conduction from the RF is implicated in the generation of secondary components of cortical SEPs, and a direct influence of the RF on the pericruciate neurons has been observed (Spehlmann, R. and Daniels, J.C., Brain Res., 48, 320, 1972). These findings suggest the possibility that enhanced RF neuronal responsiveness may play a role in the changes observed in cortical neurons following systemic administration of convulsant drugs, but resolution of this question requires further experimentation. These data further support the hypothesis that enhanced neuronal responsiveness to sensory stimuli in certain non-primary sensory brain regions is a general action of convulsant drugs. Since sensory stimuli can often trigger seizure generalization after subconvulsant doses of convulsant drugs, this neuronal effect may reveal information about the mechanisms of seizure initiation under these conditions. (Supported in part by CRC funds, SIU Foundation and NIH Grant NS 13849).

261.10 EVIDENCE FOR THE NEOCORTICAL MICROCIRCUITRY INVOLVED IN EPILEPTOGENESIS. A.B. Chatt and J.S. Ebersole. Dept. of Neurology, Yale Univ. School of Medicine, New Haven, CT 06510 and VA Epilepsy Center, West Haven, CT 06516.

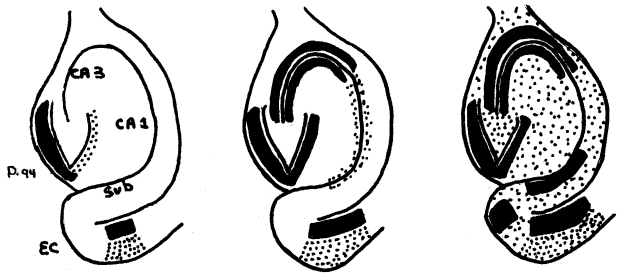
A developmental laminar analysis was conducted of interictal epileptiform potentials, induced by microinjection of nanoliter volumes of 47 mM penicillin (NaPen) at various depths in cat visual cortex and evoked by field-specific photic stimulation. Recordings were obtained from triple-barrel microelectrodes with longitudinal tip separations which permitted recording from two cortical layers while injecting in one. In several animals, ^{14}C NaPen was used and tissue excised following the development of epileptic abnormalities. The tissue was processed for diffusable-substance autoradiography and the results of NaPen spread compared to focal epileptic development in different cortical layers.

Inter-laminar induction of NaPen related abnormalities was most effective in cortical layer 4. These abnormalities included an initial enhancement of the primary latency evoked response(EPR) followed by the development of longer latency, negative epileptiform potentials. Similar negative potentials could also be induced in layers 2-3 and 5-6, but more NaPen was required and development was slower. Epileptiform potentials were not evoked from these superficial and deep cortical foci without diffusion of NaPen into layer 4 as manifest by an enhancement of the primary potential recorded there and corresponding autoradiograms. Added responsivity within layer 4 to the geniculo-cortical afferents and a corresponding enhanced output to layers 2-3 and 5-6 appears to be required to elicit epileptiform potentials from these layers even in the presence of NaPen. Evolution of epileptiform abnormalities in layer 4, however, proceeds rapidly enough to exclude a significant diffusion of NaPen into adjacent layers as requisite for epileptogenesis there(also confirmed by corresponding autoradiograms). The cellular interconnections, then, within layer 4 are apparently sufficient to elaborate an EPR into an epileptiform abnormality.

Inter-laminar projection of epileptic potentials appears to follow anatomically established pathways upwards(Gilbert and Wiesel, Nature, 1979). Abnormalities induced in layer 4 are simultaneously recorded in magnified form in superficial layers 2-3 probably reflecting the collateral afferent output from spiny stellate cells to layer 2-3 pyramidal cells. The positive "mirror" potential recorded deep to laminar epileptic foci in 4 probably reflects a current source for the epileptiform potential sink in 4. This current may be carried by layer 5-6 pyramidal apical dendrites which extend up to layer 1. An abnormal utilization of normal cortical serial processing, then, appears to underlie this enhancement of focal epileptic abnormalities initiated in layer 4.

261.11 FUNCTIONAL ANATOMY OF FOCAL LIMBIC SEIZURES IN RAT Robert G. Tearse*, Robert C. Collins, and Eric W. Lothman Neurology, Wash. Univ. Med. Sch., St. Louis, MO 63110

Relationships between site and intensity of seizure discharges, anatomical spread, and changes in behavior are not well known for limbic systems. We have studied focal seizures in entorhinal cortex (EC) in three groups of albino rats. One group (n=8) was given Na penicillin (250 units/µl) through a 33 ga recording cannula into EC at 0.1 µl/hr. A second group (n=8) was infused with picrotoxin (0.3 µg/µl), and a third group (n= 5) received bipolar tetanic stimuli. The dose of convulsant and stimulation intensity were varied to cause seizures of increasing severity judged by focal and dorsal hippocampal (HC) electrodes. Rats were studied by quantitative deoxyglucose (DG) autoradiography during local, mild, and strong seizure states.



Local (ipsilateral) | Mild (ipsi > contra) | Severe (ipsi = contral)

Hippocampal Pattern of DG Use During Focal Seizures of Entorhinal Cortex

Local seizures (<10 spikes/min) confined to EC caused no behavioral change and only a 30 to 50% increase in DG in EC and ipsilateral dentate gyrus (infra > supra). Mild seizures (>20 spikes/min) were associated with normal behavior or mild staring spells. DG metabolism was increased in EC, HC, and lateral septum. Strong seizures (prolonged afterdischarges) were associated with staring, sniffing, facial twitching, wet dog shakes, and occasional rearing. DG was markedly increased bilaterally (100-300%) in EC, HC, septum, amygdala, and substantia nigra.

These studies indicate that prolonged focal afterdischarges are necessary for seizure spread to contralateral limbic and subcortical extralimbic sites and the expression of behavioral convulsions.

261.12 FACILITATION OF KINDLING BY CONVULSIONS PRODUCED BY COCAINE OR LIDOCAINE BUT NOT PENTYLENETETRAZOL. Jeffrey S. Stripling and Curtis Hendricks*. Department of Psychology, University of Arkansas, Fayetteville, AR 72701.

Convulsions induced by cocaine have been reported to facilitate subsequent kindling in rats (Kilbey et al., Exp. Neurol., 64: 306-314, 1979). The purpose of the experiments reported here was to determine whether this effect is a general property of convulsive agents or is related to a specific pharmacological action of cocaine. Male Long-Evans rats chronically implanted with an intravenous catheter and an electrode in the olfactory bulb were convulsed by an intravenous infusion of cocaine, lidocaine, or pentylenetetrazol (PTZ), or received a control infusion of saline. Beginning eight days later each animal received daily electrical stimulation of the olfactory bulb until fully kindled seizures occurred. Animals which had been convulsed by cocaine or lidocaine kindled significantly faster than saline controls. PTZ-convulsed animals did not differ significantly from controls. A second experiment was conducted to determine if an effect of PTZ on kindling could be obtained with repeated convulsions. Animals received three convulsions produced by cocaine or PTZ, spaced three days apart, or control infusions of saline. Kindling by daily stimulation of the olfactory bulb was begun on the eighth day following the last drug treatment. Cocaine-convulsed animals again kindled significantly faster than saline controls, while PTZ-convulsed animals did not. This effect was a substantial one, with two of the cocaine-treated animals exhibiting clonus on the first day of electrical stimulation. Furthermore, the facilitation of kindling was not transitory: cocaine-treated animals still exhibited a fully kindled response when stimulated 21 days after the kindling criterion was reached. These results indicate that the facilitating effect of cocaine-induced convulsions on kindling is not a general property of all convulsive agents but is a more specific effect which is apparently shared by other local anesthetics. This effect persists for at least eight days after a convulsion is produced by cocaine, and may represent not merely a modulation of the kindling process, but a form of kindling by the drug convulsion itself, since some of the cocaine-convulsed animals exhibited a well-kindled response on the first day of electrical stimulation. In this regard it is interesting to note that previous studies have provided indirect evidence that local anesthetic convulsions may be generated within the olfactory forebrain.

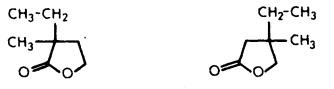
261.13 CONVULSANT AND ANTICONVULSANT PROPERTIES OF ALKYL-SUBSTITUTED γ-BUTYROLACTONES. W.E. Klunk*, D.F. Covey* and J.A. Ferrendelli. Div. of Clinical Neuropharmacology, Depts. of Pharmacology and Neurology, Washington University School of Medicine, St. Louis, MO 63110.

Gamma-butyrolactone (GBL) is a naturally occurring substance in mammalian brain and when administered systemically to experimental animals produces seizures which resemble petit mal absences in humans. In an effort to define the mechanism of action of GBL we have synthesized several alkyl substituted gamma-butyrolactones and have tested their convulsant and anticonvulsant properties in mice and guinea pigs.

Addition of an ethyl and a methyl group on the β-carbon of GBL (β-EMGBL) produces a convulsant agent that causes clonic and tonic convulsions which resemble pentylenetetrazol-induced seizures and are behaviorally different from those produced by GBL. β-dimethyl GBL has effects similar to those of β-EMGBL but is less potent. Seizures induced by these compounds are prevented by ethosuximide but not by phenytoin. In contrast, ethyl-methyl or dimethyl substitution at the α-carbon produces a highly effective anticonvulsant that prevents pentylenetetrazol-, β-EMGBL-, and picrotoxin-induced seizures but has no effect against maximal electroshock. Gamma-ethyl-γ-methyl GBL is also anticonvulsant but is much less potent than α-EMGBL. Alkyl substitution at both the α and β positions produces compounds with convulsant activity. These are more potent than β-substituted compounds if the α substituent is hydrophobic and less potent if it is hydrophilic. Structure activity analyses lead to the suggestion that alkyl-substituted gamma-butyrolactones act at the same site as picrotoxin which contains a β-alkyl-γ-butyrolactone that is essential for activity.

The present data demonstrate a new class of convulsant and anticonvulsant compounds which may be useful in the investigation of mechanisms of seizures and actions of antiepileptic drugs.

(Supported, in part, by USPHS Grant NS-14834)



α-Ethyl-α-Methyl GBL (α-EMGBL)

β-Ethyl-β-Methyl GBL (β-EMGBL)

261.14 EFFECTS OF PROSTAGLANDIN SYNTHESIS INHIBITORS ON ANIMAL MODELS OF EPILEPSY. M.C. Wallenstein and E.A. Mauss* Dept. Physiology, New York Univ., New York, NY 10010.

Even though agents of widely divergent physical and chemical properties can induce epileptiform activity in animals, the resulting bioelectric alterations underlying the epileptogenic process tend to be very similar. It is possible that a final common pathway such as an autocoid, is involved. Prostaglandins (PGs), are autocoids and have a role in modulating neuronal activity. An investigation of the effects of pretreatment with PG synthesis inhibitors on convulsions and electrocortical seizures induced by epileptogenic agents, was undertaken. In the first study, convulsant and postural responses were scored and timed. Both paracetamol (150-300 mg/kg) and indomethacin (10-20 mg/kg) tended to reduce the convulsive effects of strychnine or picrotoxin. However, the data were not always replicable. In the second study, the electrocorticogram (ECoG) was recorded from chronically implanted supradural electrodes. Behavior was observed via video monitor. Pretreatment with indomethacin (10 mg/kg) significantly attenuated electrocortical seizure activity induced by picrotoxin but not that induced by pentylenetetrazol. Pretreatment with paracetamol (400 mg/kg) attenuated seizure activity induced by picrotoxin and by penicillin but not that induced by pentylenetetrazol. These preliminary experiments suggest that PGs may have a role in the epileptogenic process. Furthermore, the specificity of interaction of class of PG synthesis inhibitor with the particular epileptic model may help to localize the site of PG action.

Supported by BRSG RR05332 and NYSHRC Grant 9-021

261.15 *ZINC BINDING BY VALPROIC ACID.* R.W. Hurd*, B.J. Wilder, J.J. Street* and G.L. Sciscent* Dept. Neuroscience, Univ. of Fla., Neurology Section, VA Medical Research Center and Dept. of Soil Science, IFAS, Univ. of Fla., Gainesville, FL 32610.

Valproic acid (VPA) is an anticonvulsant with a broad spectrum of activity against generalized seizures including absence, generalized tonic-clonic and myoclonus. Recent incidences however, of drug related hepatic damage and deaths have concerned physicians. As of Jan. 1981 a total of 43 deaths have occurred with VPA usage.

In reviewing the side effects of VPA, we noted a striking similarity of these with symptoms of zinc deficiency. Side effects produced by VPA which are also found with zinc deficiency or the zinc responsive disease of acrodermatis enteropathica include gastrointestinal disturbance, drowsiness, hair loss, anorexia, tremor, skin rash, bad taste, decreased platelet count, hyperammonemia, pancreatitis and hepatic dysfunction. Two other symptoms of zinc deficiency, decreased thymus weight and testicular atrophy have been produced in animals on high dosage VPA. Since other 5 carbon fatty acids shown to bind zinc (Martel & Smith, Critical Stability Constants, Vol. 3) we investigated the zinc binding of VPA using differential pulse polarography. Initial results indicate significant binding of zinc by VPA.

Zinc has a concentration of 1 μg/ml in plasma while therapeutic levels of VPA range between 50-100 μg/ml indicating that even with a small binding efficacy, VPA might compete favorably with other zinc binders which are present at much lower concentration.

Phenytoin has previously been shown to bind zinc (J. Invest. Dermal. 71:396, 1978) although no overt signs of zinc deficiency are evident with this anticonvulsant. Zinc levels are currently being determined in patients and animals receiving VPA. At present, we do not know if actual zinc deficiency occurs with VPA usage contributing to the above symptomatology.

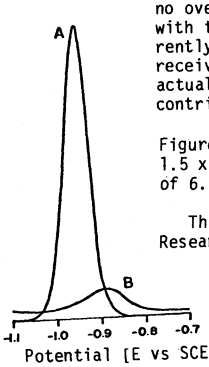


Figure: Differential Pulse Polarography of 1.5×10^{-5} Zn^{++} alone (A) and in the presence of 6.9×10^{-6}M VPA (B).

This research supported by the Epilepsy Research Foundation of Florida.

261.16 THYROTROPIN RELEASING HORMONE (TRH) INCREASES SEIZURE SUSCEPTIBILITY AND AROUSAL BEHAVIORS IN THE MONGOLIAN GERBIL. J.G. Bajorek*, R. Lee* and P. Lomax. Department of Pharmacology, School of Medicine and the Brain Research Institute, UCLA, Los Angeles, CA 90024.

Seizure sensitive Mongolian gerbils from our breeding colony at UCLA exhibit characteristic motor and EEG seizures upon exposure to novel environments or upon handling stress. As part of a series of studies examining the role of neuroendocrine agents released by stress as endogenous modulators of seizure susceptibility in the gerbil, we injected TRH (1-50 μg) intraventricularly (i.c.v.) and observed the effects on seizures and behavior. Compared to control injections of artificial cerebrospinal fluid (ACSF) (2 μl), injections of TRH (10 μg) produced a 50% increase in the incidence of seizures, a 78% increase in the severity of the seizures, and a 10.7 fold increase in seizure duration. Behavioral observations noted that a specific arousal response, 2/sec foot stomping by the rear legs, was present in 90% of the animals following the TRH injection. In some animals this behavior continued over 60 min (after TRH injection the mean duration of foot stomping behavior is 47 fold greater than in control injected animals). It was associated with EEG desynchrony and appears similar to a previous report of foot stomping in the gerbil after self stimulation or foot shock (Routtenberg, 1967). In our colony foot stomping is sometimes noted after handling, and seldom lasts over 10 sec. Higher doses of TRH (50 μg) produce toxic reactions including tremor, paw and chewing automatisms, and abnormal EEG spiking. At these higher doses foot stomping is markedly reduced to below control levels. The significance of this behavior and of the effects of TRH in the pathogenesis of the seizures are yet to be elucidated. However, our previous reports on the anticonvulsant properties of β-endorphin in these animals and the existence of foot stomping behavior under normal conditions suggests that 1) foot stomping may be an endogenous TRH mediated response, while 2) the proconvulsant effects of TRH may have a function in the partial modulatory control of seizure susceptibility in concert with β-endorphin. Routtenberg, A. and Kramis, R.C. Nature 214:173-174 (1967). (This work supported by grant ONR N00014-75-C0506).

261.17 CHANGES IN CONVULSIVE SUSCEPTIBILITY AND SEVERITY AND ANTICONVULSANT DRUG EFFECTS IN ADULT RATS ADMINISTERED 6-HYDROXYDOPAMINE OR 5,7-DIHYDROXYTRYPTAMINE DURING POSTNATAL DEVELOPMENT. Gary G. Buterbaugh and Steven B. Waller*. Dept. of Pharmacology and Toxicology, Univ. of Maryland Sch. of Pharmacy, Baltimore, MD 21201.

Marked monoaminergic involvement in electroshock convulsive thresholds and patterns has been demonstrated in neonate rats during the first three postnatal weeks (London, E.D. and Buterbaugh, G.G., J. Pharmacol. Exp. Ther. 206:81, 1978) when convulsive thresholds and patterns and central monoaminergic systems are undergoing maturation. To determine if disruption of monoaminergic systems in neonate rats would cause long-lasting changes in convulsive behavior and anticonvulsant drug action in adult rats, rats were injected intracisternally with the selective neurotoxins 6-hydroxydopamine (6-OHDA) or 5,7-dihydroxytryptamine (5,7-DHT) during two age periods in the maturation of electroshock thresholds and patterns: the first postnatal week (early, slow maturation) and the third postnatal week (rapid maturation). The rats were tested when 26 weeks old and compared to rats treated with the neurotoxins when 25 weeks old. The doses of the neurotoxins were selected to produce equivalent monoamine reduction in all three age groups at 26 weeks: 6-OHDA caused an average 49% and 33% reduction of brain norepinephrine and dopamine, respectively, with no change in serotonin; 5,7-DHT caused an average 45% reduction in brain serotonin with no change in catecholamines. The tonic electroshock convulsive threshold was significantly decreased by the same amount in rats injected with 5,7-DHT at all three ages: 6-OHDA had no effect on the threshold. The severity of the tonic convulsive pattern in all three age groups receiving 5,7-DHT was only slightly increased. In contrast, although 6-OHDA significantly increased convulsive severity in all groups, the effect was greatest (45% increase) in rats injected during the first postnatal week. The ability of phenytoin or phenobarbital to protect against electroshock tonic convulsions was competitively antagonized by both 6-OHDA and 5,7-DHT. This effect was greatest in rats injected with 6-OHDA during the first postnatal week or with 5,7-DHT when 25 weeks old. Kindled convulsions were produced by daily injections of lidocaine, 65 mg/kg, to 26 week old rats injected with the neurotoxins during the first postnatal week. The rate of kindling was accelerated by 6-OHDA but not by 5,7-DHT which caused a delayed reduction in the severity of the kindled convulsions. In summary, disruption of central monoaminergic systems in neonate rats was associated with changes in convulsive thresholds and severity in adult rats. These changes were sometimes related to the age at disruption, suggesting possible critical periods in the maturation of the interaction between monoamines and convulsive behavior.

261.18 INCREASED CONVULSIVE THRESHOLD IN INFANT RATS ASSOCIATED WITH CHRONIC ADMINISTRATION OF PENTYLENETETRAZOL. James A. McCaughran Jr.* and Nisson Schechter. (SPON: I. Fand). Department of Psychiatry and the Long Island Research Institute, SUNY at Stony Brook, New York 11794.

Starting at 5 days of age, groups of rat pups were administered 20, 50, or 90 mg/kg of pentylenetetrazol (PTZ). Drug injections were administered subcutaneously according to a number of regimens: once daily, on alternate days, or every fifth day. An additional group of infants were administered an ascending series of doses starting from 50 mg/kg and ending with 90 mg/kg in increments of 10 mg/kg/day.

Infants that received 20 mg/kg PTZ daily failed to develop any signs of convulsive behavior over the course of the study. In the group that received 50 mg/kg daily, 40% displayed convulsions at 5 days of age but 0% by 9 days of age. Administration of 90 mg/kg daily elicited convulsions from 100% of the group at 5 days of age but from only 25% of the group by 9 days of age. Littermates of these two groups were administered a single dose of 50 or 90 mg/kg at 9, 14, or 19 days of age. At 50 mg/kg, the incidence of convulsions was 40, 33, and 38% respectively (in contrast to 0, 8, and 0% in the daily PTZ group). At 90 mg/kg, the incidence of convulsions was 100% at 9 and 14 days of age (in contrast to 25 and 19% in the daily PTZ group). In the group that was administered 90 mg.kg on alternate days, the incidence of convulsions remained at 100%. In addition, there was a high mortality rate associated with this regimen. The administration of an ascending series of doses was associated with an increasing incidence of convulsions. The incidence rose from 40% (50 mg/kg at 5 days of age) to 90% (90 mg/kg at 9 days of age). When the dose of 90 mg/kg was maintained, the incidence of convulsions on subsequent days declined to 20%.

The results of this study indicate that in the infant rat the chronic administration of convulsive doses of PTZ is associated with a transient increase in the threshold to subsequent convulsions. This elevation is evident at 24 hrs but not at 48 hrs. Age-related changes in the dose response curve (Vernadakis and Woodbury; Epilepsia, 10, 1969) could be ruled out since convulsions were effectively elicited when PTZ was administered on alternate days or every fifth day. The present results are in marked contrast to the progressive increase in seizure susceptibility that chronic administration of PTZ evokes in the adult rat.

261.19 CONTRASTING EFFECTS OF CARBAMAZEPINE, PHENYTOIN, AND CLONAZEPAM ON FOCAL AD THRESHOLDS IN AMYGDALA AND CORTEX. Penny Albright*, K.E. Livingston, and W.M. Burnham. Dept. of Pharmacology, University of Toronto, Can.

Traditionally carbamazepine and phenytoin are believed to act by blocking seizure spread whereas the benzodiazepines and drugs used in absence epilepsy are thought to elevate seizure threshold (1). The present study examined the effects of anticonvulsants from both these categories on focal AD threshold. Due to reported differences in drug action on cortical and amygdala foci (2), drug effects on thresholds from both areas were measured.

Seventy-two rats were implanted with electrodes in either the amygdala (36 rats) or the anterior neocortex (36 rats). Multiple doses of carbamazepine, phenytoin, or clonazepam were given to each cortical and amygdala group (12 rats each) and thresholds were measured under each dose. Drug thresholds were compared to baseline measures taken 3 days before and after each dose. In the cortex, all drugs produced significant elevations in seizure threshold. Carbamazepine was the most potent in this respect and phenytoin and clonazepam were slightly less effective. All drugs were less active in raising AD thresholds in the amygdala than in the cortex. Carbamazepine was the most effective here and phenytoin was somewhat less useful. Clonazepam did not elevate amygdala thresholds significantly at any dose.

These results indicate that in contrast to the classical view, carbamazepine and phenytoin are very effective in raising seizure thresholds in this study. Clonazepam, which theoretically should be the most effective, is the least potent of the 3 drugs. These data suggest a re-appraisal of the accepted mechanism of action of these drugs. Moreover these results support the proposed use of the amygdala focal seizure as a model of complex partial epilepsy (2). Like the clinical condition, the amygdala threshold is resistant to anticonvulsants but is more sensitive to the standard therapeutic agents (phenytoin and carbamazepine).

(1) Kamei et al. Epilepsia, 1978, 19, 625-636.
(2) Albright & Burnham. Epilepsia, 1980, 21, 681-689.

261.20 LONGTERM DEFICITS IN LEARNING FOLLOWING EXPERIMENTAL FEBRILE CONVULSIONS. Corinne Manetto*, James A. McCaughran, Jr.*, and Nisson Schechter. (SPON: T. I. Lidsky) Departments of Psychology, Psychiatry, and Long Island Research Institute, SUNY at Stony Brook, New York 11794.

In a previous study we reported that hyperthermia convulsions (HC) in the infant rat can produce longterm increases in seizure susceptibility. Although the human infant can be similarly affected by febrile convulsions, prolonged or recurrent seizures in the human infant are also associated with later deficits in learning. In the present study, the longterm effects on learning following experimental febrile convulsions during infancy in the rat were assessed in order to clarify this issue.

Infant rats were subjected to either a single hyperthermia convulsion at 5, 10, 15, or 20 days of age or to a series of HCs, at the average rate of 1/2days, starting from 5 days of age and ending at 20 days of age. Litter-mate control rats comprised two groups: one group was subjected to hyperthermia alone and not allowed to have a convulsion; the other group was subjected to handling. At 50 days of age, the ability of each rat to acquire a step-down passive avoidance response was assessed.

Rats subjected to a single convulsion at 10, 15, or 20 days of age required significantly more trials to acquire the passive avoidance response (4.1, 4.4, and 4.7 trials to criterion, respectively) than their respective hyperthermia controls (3.2, 3.0, and 2.8 trials to criterion) - or handled controls (2.8, 2.5, and 2.6 trials to criterion). Rats subjected to a single HC at 5 days of age did not differ from their respective controls (2.8 vs 2.6 and 2.6 trials to criterion). However, rats subjected to multiple HCs were not only slower than rats subjected to single HCs at 10, 15, or 20 days of age (5.1 vs 4.1, 4.4, and 4.7 trials to criterion, respectively) but also show the greatest deficits compared to their respective hyperthermia and handled control groups (2.7 and 2.4 trials to criterion).

The results of this study indicate that experimental infantile febrile convulsions can have longterm effects on the ability of rats to acquire a simple learning task. In addition, the observation that rats subjected to a single HC at 5 days of age fail to display this deficit suggests that the substrate(s) necessary for the development of passive avoidance responding have either not developed at 5 days of age (and therefore have been spared) or have been affected, but show a recovery of function. The increased deficits in responding that were observed following multiple HCs are consistent with the clinical data which indicate that infants who experience recurrent febrile convulsions have a higher incidence of neurological sequelae.

261.21 BRAIN VASCULATURE AND INDUCED ISCHEMIA IN SEIZURE PRONE AND NON-SEIZURE PRONE GERBILS. M. Donadio*, P. Kozlowski*, H. Kaplan, H.M. Wisniewski*, and J. Majkowski*. N.Y.S. Institute for Basic Research in Developmental Disabilities, Staten Island, N.Y. 10314

The relationship of seizure propensity to the morphology of the anterior cerebral arteries and to stroke induced by unilateral ligation of the common carotid artery was investigated using seizure prone (SP) and nonseizure prone (NSP) gerbils. Subjects were selected from the well-differentiated IBR colony, which regularly undergoes standard seizure tests and is now in the 10th to 12th generation of sib-sib matings. Twenty SP and 20 NSP gerbils were perfused with a latex dye and the vascular patterns of the anterior cerebral artery were analyzed. In addition, 30 SP and 30 NSP gerbils underwent unilateral ligation of the common carotid artery and the number developing stroke (determined both by clinical observation and by microscopic examination of the brains) was recorded. In all 40 gerbils, the 2 anterior cerebral arteries joined to form one central vessel that vascularized the olfactory bulbs.They also gave rise to two large lateral vessels (rostral arteries) that vascularized a previously undescribed nasal plexus. There was, however, much variability in the anatomical arrangement of these vessels, which were grouped into 5 patterns. Four of these were 'stroke-prone' patterns, i.e. there was either no or insufficient communication between right and left anterior cerebral arteries. We found no significant relationship between the anatomical pattern of the anterior cerebral arteries and seizure propensity in the gerbil. While 12 SP and only 7 NSP gerbils had stroke-prone patterns, this difference was not significant. There was also no significant difference in the number of SP and NSP gerbils developing stroke after common carotid artery ligation (9 NSP and 13 SP). Thus, in conflict with the report of Schonfeld and Glick (Brain Res. 1979, 173: 147), we found no statistically significant relationship between seizure propensity, the vascular anatomy of the anterior part of the brain and the occurrence of stroke induced by unilateral ligation of the common carotid artery. The evidence indicates, rather, that the mechanism responsible for naturally occurring seizures in the gerbil, and thet responsible for stroke susceptibility are not identical.

(This research was supported in part by NIH Grant 5 R01 NS 15724-02.)

261.22 VARIETIES OF ANTICONVULSANT BLOCKING ACTIONS ON NA CHANNELS IN SKELETAL MUSCLE. K. R. Courtney and E. F. Etter.* Palo Alto Medical Foundation, Palo Alto, CA 94301.

Several anticonvulsants were studied for their effects on skeletal muscle Na currents of bullfrog, these Na currents being easier to measure than similar Na currents in myelinated nerve. The single cell, Vaseline gap, voltage-clamp procedure was utilized. Five anticonvulsants have been looked at (table) and they all depress sodium currents. Half-effective blocking concentrations (D50, infrequent depolarizations) are listed below for normal holding potentials where 35% of channels are inactivated. Proper care was taken to avoid possible channel unblocking effects of hyperpolarizing prepulses (Courtney, BBA 642:433, 1981) which are normally used to condition Na inactivation.

Three of these drugs show clear frequency-dependent changes in channel block as indicated by step-by-step changes in peak current levels after insertion or removal of hyperpolarizing prepulses before the channel opening depolarizations.

Time constants governing recovery from frequency-dependent changes in channel block differ considerably among these anticonvulsants (T in table). Such time constants help determine what action potential frequencies an excitable cell can follow, a capability of potential therapeutic significance. (Time constants could not be measured for ethotoin and diazepam which showed no frequency-dependent blocking at pulse rates of up to 10 Hz.)

Finally there appeared to be an "inactivation shift" produced by all drugs except phenytoin. The inactivation parameter h is set near 0.65 before drug treatment and falls by the amount indicated in the table (Δh) during treatment with drug concentrations near the half-blocking dose. Such observations could indicate relatively fast channel unblocking during the hyperpolarizing prepulse (see reference above).

In summary, these five anticonvulsant drugs all block Na channels but they do so with considerably differing potencies and kinetic detail. Future investigations along these lines will hopefully reveal some new structure/activity relationships useful for design of more effective anticonvulsant drug structures.

Supported by NIH grant NS15914.

drug	D50	f-dep?	T	Δh
diazepam	51 μM	no		.22
phenytoin	36	some	4	none
carbamazepine	71	yes	1.6 sec	.16
phenobarbital	800	yes	0.4	.12
ethotoin	17	no		.14

262.1

WITHDRAWN

262.2 ANTIDOTE EFFECT OF SODIUM FLUORIDE AGAINST SOMAN POISONING IN MICE. J. G. Clement*, M. G. Filbert* and Daniel L. Rickett. Biomedical Section, Defence Research Establishment Suffield, Ralston, Alberta, Canada, TOJ 2NO; and Physiology Branch, US Army Biomedical Laboratory, Aberdeen Proving Ground, MD 21010.

Sodium fluoride (NaF) has long been known to: (a) inhibit both acetylcholinesterase and butyrylcholinesterase reversibly (1); (b) reactivate cholinesterase inhibited by the nerve agents sarin and tabun but not by soman (2,3); facilitate the sensitivity of the motor end plate to the stimulating action of acetylcholine (4) and activate the enzyme adenylate cyclase (5).

The facilitating action of NaF on acetylcholine's stimulation of the motor end plate was recently shown by Akasu and Karczmar (6) to be due to slowing of the progress of desensitization as well as to an acceleration of recovery from desensitization. Anticholinesterase compounds result in persistence of acetylcholine and therefore accelerate the process of desensitization. The object of this study was to determine if NaF had any antidotal properties against the lethality of soman, an organophosphorus, anticholinesterase agent.

Male mice received subcutaneous injections of soman 5 minutes after an intraperitoneal injection of either atropine (17 mg/kg) alone or a mixture of atropine + NaF. Low doses of NaF (5 mg/kg and 15 mg/kg) significantly increased the LD50 of soman from a control value of 162 µg/kg to 202 µg/kg and 208 µg/kg, respectively. When 8 mg/kg NaF was combined with atropine and pyridostigmine, a carbamate cholinesterase inhibitor, the mixture given prophylactically, the LD50 of soman was increased to 540 µg/kg.

Measurement of brain cholinesterase determined that the protective action of NaF against soman lethality is not due to reactivation of centrally inhibited enzyme. The antidesensitizing effect of NaF is a reasonable hypothesis to account for the observed antidotal effect against soman. Furthermore, desensitization of cholinergic receptors may contribute significantly to the lethality of refractory nerve agents such as soman.

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262.3 COMPARISON OF END-PLATE ACTIVATION AND DESENSITIZATION AT SNAKE SLOW AND TWITCH MUSCLE FIBERS. R.M.Schnitzler*, E.A. Connor*, J.F. Fiekers and R.L. Parsons. Dept. Anatomy and Neurobiology, University of Vermont College of Medicine, Burlington, VT 05405.

Desensitization of the end-plate receptor-channel complex produced by sustained exposure to agonists is well documented, but the mechanism(s) responsible for the loss of chemosensitivity remains unknown. Recently, the activation kinetics of receptor-channel gating were found to differ at snake twitch and slow muscle fiber end-plates (Dionne and Parsons, J. Physiol. 310:145, 1980; Dionne, J. Physiol. 310:159, 1981). In the present study we are investigating the influence of this difference in activation kinetics on the development of desensitization in these two fiber types. The kinetics of carbachol-induced desensitization are being compared in voltage-clamped twitch and slow muscle fibers from costocutaneous muscles of the garter snake maintained in a HEPES-buffered isotonic potassium proprionate solution (mM:NaCl 159, KCl 2.15, $CaCl_2$ 1.0, $MgCl_2$ 4.0, CsCl 5.0; pH=7.2; 19-21°C).

In initial experiments we determined that slow fiber MEPC decay was significantly longer and exhibited considerably less voltage dependence than twitch fiber MEPC decay as reported previously for muscles maintained in sodium solution. With both fiber types, microperfusion of 216 µM carbachol induced a transient EPC_{carb} superimposed on the holding current which developed with a time to peak of a few seconds and then decayed slowly towards the baseline in the continued presence of agonist. The timecourse of EPC_{carb} decay is used to estimate the timecourse of desensitization onset. At twitch fiber end-plates, EPC_{carb} decay timecourse can be described by a single exponential function at all voltages; however, at slow fiber end-plates the EPC_{carb} decay at voltages more negative than -40mV often exhibits two distinct exponential components. Desensitization appears to develop more rapidly at twitch fiber end-plates than at slow fiber end-plates, but the difference is not significant at all voltages. The timecourse of desensitization onset is voltage dependent at both end-plate types; the magnitude of the voltage dependence for twitch fibers (0.0096 mV^{-1}) being comparable to that reported previously for amphibian twitch end-plates (Fiekers et al., J. Gen. Physiol. 75:51, 1980). The equilibrium level of desensitization, obtained over a wide voltage range (-80 to +50 mV) after a 3 minute exposure to 216 µM carbachol is voltage dependent in both fiber types. The voltage dependence of the equilibrium level appears to be less in slow fibers than in twitch fibers. Additional experiments are in progress to determine the concentration dependence of desensitization kinetics in both fiber types. (Supported by the MDA).

262.4 DECREMENT IN RESPONSE OF HIPPOCAMPAL PYRAMIDAL NEURONS TO LONG IONTOPHORETIC PULSES OF GABA. R. H. Thalmann and Norman Hershkowitz*Depts. of Cell Biology, Neurology and Program in Neuroscience, Baylor College of Medicine, Houston,TX 77030

We have further studied the response decrement which has been reported when GABA is continuously ejected near the somata of hippocampal neurons(Ben-Ari et al, Can. J. Physiol. Pharmacol., 1979,57,1462-1466). In addition, we have similarly studied the depolarizing response which follows GABA application near the apical dendrites of these neurons. Responses of CA1 neurons of the rat hippocampal slice were measured in terms of change in membrane conductance. When GABA was ejected near the soma for 10-60sec,conductance increased to a maximum within about 3sec,then declined to approach an asymptote which was always greater than the resting conductance. The half-decay time of this decline was typically about 5 sec. This response decrement could be demonstrated during purely hyperpolarizing responses, or during the biphasic response which occurs during more intense iontophoretic currents. In the range of ejection currents tested, greater conductance decrements were produced by higher ejection currents. The response decrement also occurred when the bathing medium contained 10^{-6}M TTX,0Ca and 2mM Mn. In contrast, the response to GABA ejected near the apical dendrites appeared to be quite different. In this case, a given iontophoretic current tended to produce a smaller peak response, and a smaller response decrement than when applied to the somatic region. In fact, in some cases, a gradual increment in conductance occurred during the initial 10-30sec. When the TTX, 0Ca, 2mM Mn medium perfused the slices, however, only decrements in the dendritic response have been observed. The decrements in GABA responses which have been reported here were not due to decreased ejection of GABA during the long GABA pulses: Control experiments with two GABA barrels showed that the initial peak of the GABA response which was elicited by one GABA barrel was reduced when it was preceeded by a 30sec GABA pulse via the second barrel. The response decrements were also probably not secondary to changes in voltage since they occurred whether the response was hyperpolarizing or depolarizing, or when the membrane potential was held at or near the reversal potential of a given response to GABA. Finally, the occurrence of such decrements did not appear to require Ca conductances, or TTX-sensitive Na conductances, since the decrements could be readily observed even following 2 hours of perfusion with the TTX,0Ca, 2mM Mn medium. Supported by NIH grant NS 11535.

262.5 ALTERATION OF ALPHA AND BETA ADRENERGIC RESPONSES OF RAT AORTA CAUSED BY WEEK-LONG EXPOSURE, VIA MINIPUMP, TO APPROPRIATE AGONISTS. C.J. Sun* and J.P. Hanig* (SPON: J. Kenimer) Division of Drug Biology, Food and Drug Administration, Washington, D.C. 20204

Decrease in adrenergic responses or receptor density can be achieved rapidly in vitro by incubation of rat aorta or brain slices with appropriate agonists. In vivo, this desensitization or downregulation phenomenon can also be elicited following a long period of administration of antidepressants or monoamine oxidase inhibitors. This is due to an indirect increase in the effective concentration of neurotransmitter at the synapse. The present study was performed to determine whether a constant 1 week exposure to either alpha or beta agonists in vivo would allow alteration or manipulation of the responses of rat aortic alpha and beta adrenergic receptors. ALZET osmotic minipumps delivering either phenylephrine (PE) or isoproterenol (ISO) for 7 days at a constant rate of 1.0 µl/hr and a dose of 4.2 and 3.2 mg/kg/day, respectively, were implanted in male Holtzman rats under halothane anesthesia. The minipumps were designed to maintain steady-state blood levels of the drugs. Seven days later, rats were killed and aortic ring preparations were set up to measure their alpha and beta adrenergic responses. In PE pretreated rats, alpha adrenergic responses, as measured by contractions induced by PE, were markedly reduced ($P < 0.05$) across a dose range from 10^{-9} to 10^{-6}M. In contrast, in these same PE pretreated preparations, the beta adrenergic responses involving ISO-induced relaxation were significantly increased across a dose range of 10^{-7} to 10^{-5}M. In a separate series of experiments, ISO pretreatment with minipumps for 7 days resulted in a significant reduction of beta adrenergic aortic relaxation, whereas the alpha adrenergic responses to PE remained unchanged compared to controls. These findings indicate that the alpha agonist-induced desensitization or downregulation of the alpha adrenergic response after in vivo pretreatment also induces reciprocal changes in the functionally related beta adrenergic apparatus. This suggests linkage between these two receptors. In contrast, the beta receptor appears to desensitize or downregulate in response to beta agonist exposure in a manner that appears to be independent of or to operate in the absence of an alteration of the alpha receptor.

262.6 DENERVATION PRODUCES α_1-ADRENOCEPTOR SUPERSENSITIVITY IN BRAIN: PHYSIOLOGICAL AND RECEPTOR BINDING STUDIES. D.B. Menkes, D.W. Gallager, J.F. Reinhard, and G.K. Aghajanian, Depts. Pharmacol. and Psychiat., Yale Univ. Sch. Med., New Haven, CT 06508.

Noradrenergic denervation is known to enhance postsynaptic responses to α-adrenoceptor stimulation in peripheral systems (Trendelenburg et al., J. Pharmacol. Exp. Ther., 172: 91, 1970). In the brain, such denervation has been reported to increase ^{3}H-WB-4101 binding sites (U'Prichard et al., Molec. Pharmacol., 16: 47, 1979) suggesting that α-adrenoceptor supersensitivity to norepinephrine (NE) may develop under these conditions. The present study examined the development of α-adrenoceptor denervation supersensitivity in brain using both single-unit recording and receptor binding techniques.

Male albino rats (200-250 g) were given a single intracerebroventricular injection of the catecholamine neurotoxin 6-hydroxydopamine (6-OHDA, 200 µg free base). Pretreatment with fluoxetine (10 mg/kg, i.p., 1 hr before 6-OHDA) was used to protect serotonergic neurons. Control animals were treated in the same way, except vehicle without 6-OHDA was injected. Five to eight weeks later, animals were transected at the level of the caudal mesencephalon and mounted in a stereotaxic apparatus. Neurons in the dorsal lateral geniculate (LGNd) were identified and their sensitivity to iontophoretic phenylephrine, NE, and carbachol was determined as previously described (Menkes and Aghajanian, Eur. J. Pharmacol., in press).

Previous studies have indicated that the activation of LGNd neurons in response to NE or phenylephrine is mediated by a classical α_1-adrenoceptor (Rogawski and Aghajanian, Brain Res., 182: 345, 1980). Carbachol, by contrast, produces a similar activation through a muscarinic receptor. Sensitivity of LGNd cells to NE and phenylephrine was found to be enhanced more than twofold in the 6-OHDA group ($p < .01$) while responses to carbachol were unchanged.

Following recording, animals were killed by decapitation and cerebral cortices were dissected and used for biochemical determination of NE levels and for ^{3}H-prazosin binding. Liquid chromatography showed that the 6-OHDA treatment caused a profound (> 90%) depletion of NE levels. Density of ^{3}H-prazosin-labelled α_1-adrenoceptors was found to be increased 40% ($p < .01$) by denervation with no change in Kd. These results indicate that destruction of central NE systems results in physiological supersensitivity to α_1-adrenoceptor stimulation which is correlated with an increase in α_1-adrenoceptor density.

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262.7 ALPHA AND BETA RECEPTOR BINDING IN RAT SPINAL CORD FOLLOWING COMPLETE CORD TRANSECTION, L.A. Hershey, D.R. Kopaniky,* R.F. Macko,* and J.S. Brodkey. Laboratory of Neurology and Neurosurgery, Case Western Reserve Sch. of Med., Cleveland, OH 44106.

Evidence is accumulating to suggest that norepinephrine (NE) is a neurotransmitter in the spinal cord. NE appears to act through alpha and beta receptors in the ventral horn to modulate stretch reflexes. It appears to inhibit pain transmission via its action on alpha receptors in the dorsal horn. Radioligand binding methods for measuring postsynaptic noradrenergic receptors in rat spinal cord have been described recently. We compared binding of ^{3}H-dihydroalprenolol (± cold propranolol) in membranes prepared from control cords to that from cords of rats surviving complete cord transection (T2-3) for 7 days. These animals could eat and drink, but they were paraplegic. We found the number of beta-adrenergic binding sites in transected cords to be nearly double that measured in controls. When ^{3}H-prazosin binding (± cold prazosin) in control cords was compared to that measured in transected cords, there was no difference to suggest a change in alpha$_1$ receptor density or affinity. There are two alternative explanations for the difference in behavior of the alpha$_1$ and beta receptors following cord transection: 1) Both types of receptors are denervated, but they respond differently to denervation. 2) The alpha$_1$ receptors are not denervated - either because the receptors are mostly located on non-neural tissue (blood vessels, etc.) or because the receptors are driven by neurons not contained in the descending bulbospinal tracts.

262.8 ROLE OF NEURONAL SIGNAL INPUT IN THE DOWN-REGULATION OF CENTRAL NORADRENERGIC RECEPTOR FUNCTION BY ANTIDEPRESSANT DRUGS. Aaron Janowsky[1], Larry R. Steranka[2], David D. Gillespie[1] and Fridolin Sulser[1], Vanderbilt University School of Medicine[1], Nashville, TN 37232, and Indiana University[2], Gary, IN 46408.

It is now well established that antidepressant drugs, if administered on a clinically relevant time basis cause sub-sensitivity of the norepinephrine (NE) receptor coupled adenylate cyclase system. To ascertain the role of NE signal input in this noradrenergic down-regulation, desipramine (DMI) and iprindole (IP) were administered for a period of two weeks (15 mg/kg/day i.p.) to animals whose right locus coeruleus had been lesioned electrolytically two weeks prior to treatment. Successful lesions caused a selective depletion of NE in the ipsilateral cortex to less than 25% of its contralateral control value. The responsiveness of the cAMP generating system to NE (100 µM) was slightly enhanced on the lesioned side. While chronic administration of the NE uptake inhibitor DMI reduced the maximal responsiveness of the cAMP generating system to NE from 44.4 ± 5.8 (controls) to 19.9 ± 3.2 (non-lesioned side) pmoles cyclic AMP/mg/protein ± SEM, the drug did not significantly alter the responsiveness to NE on the lesioned side (36.8 ± 5.4 pmoles cyclic AMP/mg/protein ± SEM). IP which does not change the availability of NE also down-regulated the NE receptor coupled adenylate cyclase system on the non-lesioned side while not significantly reducing the sensitivity to NE on the side with the locus coeruleus lesion (49.4 ± 3.9 (controls) and 43.3 ± 6.7 (IP) pmoles cyclic AMP/mg protein ± SEM). These results support the view that NE signal input and thus the formation of the NE-receptor complex is one of the pre-requisites for down-regulation by antidepressant drugs. However, since iprindole shared the effect with DMI, but unlike DMI, does not inhibit the uptake of NE, other regulatory factors (co-transmitters?) are implicated in the process of drug induced desensitization of the NE receptor coupled adenylate cyclase system in brain.

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262.9 CORTICAL DESENSITISATION TO NORADRENALINE BY OXAPROTILINE: EFFECT OF THE TWO ENANTIOMERS. H. Olpe* (SPON: G. Lynch) Ciba-Geigy Ltd., Biology Research Laboratory, Pharmaceuticals Division, 4002 Basle, Switzerland.

Oxaprotiline (CGP 49802) is a potential antidepressant which is chemically related to maprotiline (Ludiomil). It is a potent and highly selective inhibitor of noradrenaline (NA) uptake with an ED 50 of 4 to 7 mg/kg p.o. depending on the test system used. It lacks anticholinergic and 5-HT potentiating properties. Oxaprotiline is a mixture of two enantiomers, one being a potent inhibitor of noradrenaline uptake (CGP 12104), the other being devoid of this property (CGP 12103).

Chronic administration of a number of tricyclic antidepressants or of monoamine oxidase inhibitors has been reported to reduce the number of cortical β-receptors, to attenuate the adenylate cyclase response to NA and to reduce the sensitivity of cortical neurons to microiontophoretically administered noradrenaline. It has been speculated that this desensitisation is not coupled with the NA-uptake-inhibiting property since chronically administered iprindol also induced the same effect without being a blocker of NA-uptake. With the help of the two enantiomers of oxaprotiline, it was possible to test whether the phenomenon of desensitisation is coupled to the NA-uptake inhibiting property or some other properties of the tricyclic structure. Following a ten-day treatment of rats with either CGP 12104 or CGP 12103, the sensitivity of cingulate cortical neurons to microiontophoretically administered NA was compared in chronically- and acutely-treated animals. The acutely-treated rats received a single injection of either of the two enantiomers 24 hours before the start of the experiment. Rats treated chronically with CGP 12104 but not those treated with CGP 12103 showed a marked reduction in sensitivity to NA as compared to the acutely treated rats. The cell-inhibiting property of NA was reduced with respect to both the duration of firing-inhibition and the amplitude of firing-reduction. These findings indicate that the phenomenon of NA-desensitisation is coupled with the NA-uptake-inhibiting property and not with the chemical structure of tricyclics per se.

262.10 CHRONIC ANTIDEPRESSANT TREATMENT PRODUCES SUPERSENSITIVITY TO THE ELECTROPHYSIOLOGICAL RESPONSE OF HISTAMINE IN GUINEA PIG HIPPOCAMPUS WITHOUT A CHANGE IN THE SENSITIVITY OF THE HISTAMINE SENSITIVE ADENYLATE CYCLASE. M. C. Olianas*, A. P. Oliver* and N. H. Neff. Lab. Preclin. Pharmacol., NIMH, St. Elizabeths Hosp., Washington, D.C. 20032.

Typical and atypical antidepressant drugs have been shown to block histamine sensitive adenylate cyclase of guinea pig brain. We have previously shown that histamine, increases the excitability of guinea pig hippocampal pyramidal cells via an H_2 receptor. In this study, we compared the interactions of histamine and antidepressants on adenylate cyclase activity in a homogenate in guinea pig hippocampus and the interictal spike frequency in the region CA-3 of a perfused hippocampal slice. Imipramine, amitriptyline, iprindole and mianserin block histamine activated adenylate cyclase with an IC_{50} in the micromolar range. In the hippocampal slice, imipramine, desmethylimipramine or chlorimiincrease neuronal excitability but do not block the histamine effect while mianserin which does not have a direct effect significantly blocked the histamine response at about 1μM. After chronically treating animals with imipramine (10mg/kg i.p.) or mianserin (10mg/kg i.p.) twice a day for 14 days there is supersensitivity of the electrophysiological response to histamine with a shift of the dose-response curve to the left and an increase of the maximal response. When we tested the responsiveness of the adenylate cyclase in the hippocampal homogenate of the same animals, however, there was no change of the response to histamine. Many factors could be responsible for the different effects of the chronic antidepressant treatment on the two histamine dependent responses. Among the possibilities are that the homogenate preparation may lack an essential factor, the two systems may not be strictly related and the electrophysiological response may be indirect.

262.11 ESTROGEN MODULATION OF SEROTONIN RECEPTORS IN THE RAT BRAIN B.S. McEwen and A. Biegon* (Sponsor D.J. Micco, Jr.) The Rockefeller University, New York, NY 10021

Serotonin (5HT) receptor density fluctuates in the basal forebrain during the estrous cycle of the rat (Biegon et al., Brain Res. 187:221, 1980). The underlying hormonal mechanisms were studied in ovariectomized (OVX) rats. In each of 2-5 experiments, estradiol (E) in ethanol/saline or estradiol benzoate (EB) in oil, in doses ranging from 2 to 50μg, were injected subcutaneously into two animals; two OVX controls received the appropriate vehicle. The animals were decapitated 1-2h later and the hypothalamus, cortex or the whole forebrain were assayed for ^{3}H-5HT binding using previously described methods. A Scatchard analysis of the binding data revealed a marked crease in the density (Bmax) of 5HT receptor following these treatments: 2μg E reduced the binding by 30%; the higher doses inhibited up to 70-80%. EB at 2μg was only marginally effective, but 5μg EB produced a 30% decrease. Incubation of brain membranes with 5HT and E simultaneously resulted in a decrease in Bmax only at high (10-5M) concentration of E. However, when brain membranes were preincubated with E in polyvinyl pyrrolidone for 2h prior to assay, a 25% reduction in 5HT binding was evident with E concentrations as low as 1nM.

Delayed effects of EB injection were also studied. Ovariectomized rats were injected with 10μg/rat/day for 2 d and killed 48h after the second injection. Under these conditions, there is a selective increase in Bmax of ^{3}H-5HT binding to membranes in estrogen concentrating brain regions - 40% in the preoptic area, 28% in amygdala, and 39% in hypothalamus. No change occurs in the cortex and caudate. Progesterone, 2.5 mg, given to estrogen-primed rats 3h before sacrifice had no additional effect on 5HT binding; neither did hypophysectomy.

The time course and anatomical localization of the increase in 5HT receptors following EB treatment suggest an estrogen-receptor-mediated mechanism, while the widespread decrease 1-2h after an acute injection of E or EB, which can be mimicked under certain conditions in vitro in a preparation free of cytoplasmic E receptors, is suggestive of a direct membrane effect of the steroid. These two mechanisms may both be operating in the estrous cycle in the rat and thus help account for 40-50% fluctuation in 5HT receptor density in the basal forebrain during the cycle.

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263.1 PARTICIPATION OF CHOLINERGIC MECHANISMS IN THE CONTROL OF ARTERIAL PRESSURE BY THE NUCLEUS TRACTUS SOLITARII OF THE RAT. L. Criscione*, W. T. Talman, and D. J. Reis. Laboratory of Neurobiology, Cornell University Medical College, N. Y., N. Y. 10021.

The nucleus tractus solitarii (NTS) is the site of termination of baro- and other cardiopulmonary receptor afferents. The presence within the NTS of acetylcholine (ACh), cholineacetyltransferase, and cholinergic (muscarinic) receptors suggests a cholinergic innervation in the region. We have thus investigated whether cholinergic mechanisms in NTS participate in regulation of arterial pressure. Rats were anesthetized with halothane or chloralose. Microinjection (0.1 µl) of carbachol (C) or ACh unilaterally in NTS produced a dose dependent fall in mean arterial pressure (MAP) and heart rate (HR). For C, the threshold dose was 4.4 pmoles and the maximal response (fall of MAP of 41 ± 6 mm Hg from a baseline of 94 ± 6 mm Hg and of HR of 19 ± 5 bpm from a baseline of 322 ± 12 bpm; n=7; $p<.005$ and $p<.05$ respectively; mean ± S.E.M.) occurred at 44 pmoles. For ACh the dose-response curve was shifted to the right and a maximal res ponse (fall of MAP of 35 ± 2.7 from a baseline of 80 ± 3.3 mm Hg and fall of HR of 10 ± 2.2 from a baseline of 341 ± 17.4 bpm; n=5; $p<.05$) occurred at 0.28 nmoles. Within the brain stem the effective sites for C were restricted to the intermediate NTS; however, high doses injected over the dorsal surface of the brain stem at the level of the calamus scriptorius transiently elevated MAP and HR. Atropine (0.12 nmoles) injected unilaterally into NTS did not affect MAP or HR but produced complete blockade of cardiovascular responses to locally injected C. The same dose did not alter the effect of the local injection of l-glutamate (0.3 nmoles). Hexamethonium (0.21 nmoles) had no effect on C. Physostigmine (1.6 nmoles) injected unilaterally did not alter MAP or HR. Atropine (0.12 nmoles) injected bilaterally into NTS increased MAP by 17 ± 2 mm Hg in rats anesthetized with chloralose and produced a 45% inhibition ($p<.05$) of the reflex fall of HR induced by raising MAP (45-50 mm Hg) with phenylephrine (2 µg/kg, i.v.). Physostigmine (1.6 nmoles) did not affect baroreceptor reflexes. We conclude that; (a) cholinergic agents microinjected into the NTS simulate baroreflexes, (b) the receptors involved are muscarinic, and (c) muscarinic blockade produces signs of arterial baroreceptor inhibition. The study suggests that muscarinic receptors on neurons in NTS play a role in the mediation of baroreflexes and control of arterial pressure. (Supported by NIH Grant HL 18974; WTT is an Established Investigator for the American Heart Association; LLC is a CIBA-GEIGY visiting research associate).

263.2 CENTRAL NERVOUS SYSTEM CHOLINERGIC RECEPTOR SITE ACTIVITY IN THE DAHL RAT MODEL OF HYPERTENSION. R. Friedman*, E. Edwards, W. McNally*, J. McCaughran*, J. Iwai* and N. Schechter. (SPON. B. Twarog) Department of Psychiatry and the Long Island Research Institute, SUNY at Stony Brook, N.Y. 11794.

The Dahl model of experimental hypertension consists of two lines of rats with opposite, genetically determined predispositions to hypertension. One line, the Dahl hypertension-sensitive (DS) line, rapidly and predictably develops severe hypertension when fed a high salt diet, but remains normotensive on a low salt diet. The other line, the Dahl hypertension-resistant (DR) line, exhibits no change in blood pressure when maintained on either diet. Altered central cholinergic receptor binding activity has been reported in other models of experimental hypertension and therefore, we have determined the number of cholinergic receptor sites (muscarinic and nicotinic) in cortex, medulla, and hypothalamus in DS and DR rats on both high and low salt diets.

After three weeks on their respective diets and following blood pressure determination, brain regions from DR and DS rats were homogenized and aliquots were incubated in the presence of ^{125}I-alphabungarotoxin (aButX) for nicotinic receptors and ^{3}H-quinuclidinyl benzilate (QNB) for muscarinic receptors. Non-specific binding was determined by parallel incubations with several 100-fold excess of curare or atropine respectively. All determinations were performed at saturation. A detailed method has been previously described (Brain Res., 183 (1980) 224-228).

As anticipated, the high salt diet resulted in significant elevations in blood pressure in DS rats but was without effect in DR rats. In the low salt condition, where both lines are normotensive, there was a tendency for the DS rats to have a higher number of QNB binding sites than DR rats in all brain areas studied with the most pronounced difference occurring in the cortex. The DS rats fed high salt exhibited more QNB binding sites than low salt DS rats in cortex, medulla and hypothalamus. This contrasts with the observation that high salt DR rats had slightly fewer QNB binding sites than DR rats fed low salt. There were no differences in aButX binding sites in any area examined in the four line x diet groups.

The results suggest that DS rats in the low salt condition exhibit central cholinergic receptor activity which distinguishes them from DR rats. Furthermore, when exposed to the hypertensinogenic stimulus, DR rats tend to show modest reductions in QNB binding sites while DS rats exhibited significant increases concomitantly with elevations in blood pressure.

263.3 RELEASE OF 3H-L-GLUTAMATE AND 3H-D-ASPARTATE FROM THE AREA OF THE NUCLEUS TRACTUS SOLITARIUS BY ELECTRICAL STIMULATION OF THE VAGUS NERVES. A.R. Granata*, A.F. Sved and D.J. Reis. Lab. of Neurobiology, Cornell Univ. Med. College. New York, NY 10021

We have proposed that L-Glutamate (L-GLU) is a neurotransmitter released in the nucleus tractus solitarius (NTS) by excitation of baroreceptor and other primary afferent fibers of the vagus nerve (Talman et al. Science 209:815-819. 1980). Such release appears to mediate the reflex hypotension and bradycardia of the baroreflex. In the present study we sought to determine if electrical stimulation of afferent vagal fibers with stimuli sufficient to elicit hypotension and bradycardia would elicit release of labelled L-GLU or its stable analogue D-Aspartate (D-ASP) from the area of the NTS. Rats were anesthetized with urethane, paralyzed and ventilated. The cervical vagi were transected bilaterally and the central ends placed on stimulating electrodes. One ul of artificial CSF containing 3H or 14C-GLU or 3H-D-ASP was microinjected into the NTS in order to preload endogenous stores. 14C-urea, 14C-sucrose or 14C-glycine were injected along with the 3H-amino acids as markers of non-specific release. A push-pull cannula consisting of two fused pipettes 150 u each was placed in NTS. The area of NTS was then perfused with artificial CSF at 40 ul/min and samples were collected at three minute intervals thereafter. The vagi were stimulated bilaterally to elicit a maximal fall of arterial pressure (-56 ± 3mm Hg) (6 min stim train, 2 ms pulse, 5 Hz at 15 x threshold). 3H-L-GLU, 14C-L-GLU, or 3H-D-ASP but not 14C-urea, 14C-sucrose or 14C-glycine were released during vagal stimulation. The release of 3H-L-GLU, calculated as a percent of increase in radioactivity above the pre-stimulation level, was a 140 ± 24.7% (N=11) during bilateral vagal stimulation and 47.7 ± 16.4% (N=7) during unilateral stimulation and ($P<.001$). Release, was detected only when the cannula was within the NTS area, was proportional to the magnitude of the stimulus intensity (which in turn was generally related to the magnitude of reflex hypotension), and when systemic arterial pressure was acutely elevated over 50mmHg by aortic compression. Analysis of the perfusate by thin layer chromatography in experiments utilizing D-ASP indicated that greater that 90% of the radioactivity could be recovered as D-ASP. We conclude that electrical stimulation of fibers of small diameter in the vagus nerve eliciting baroreflex-like responses will release L-GLU from NTS. The results are consistent with the hypothesis that L-GLU is a neurotransmitter of neurons in the NTS mediating the baroreflex.
(A.R.G. is a fellow from Consejo Nacional De Investigaciones Cientificas Y Tecnicas De La Republica Argentina)

263.4 BLOCKADE OF FOREBRAIN GABA RECEPTORS INHIBITS BAROREFLEX-INDUCED BRADYCARDIA IN THE ANESTHETIZED CAT. J.A. DiMicco*. (SPON: M. Aprison) Department of Pharmacology, Indiana University School of Medicine, Indianapolis, Indiana 46223

The neurotransmitter γ-aminobutyric acid (GABA) appears to play an important inhibitory role in central nervous control of autonomic outflow to the cardiovascular system (Gillis et al., Brain Res. Bull. 5 [suppl. 2]: 303-315, 1980). Thus, bicuculline methiodide (BMI), a GABA antagonist, elicits contrasting but characteristic effects on basal arterial pressure and heart rate at different CNS sites in the anesthetized cat. In the medulla, BMI elicits vagal bradycardia by blockade of GABAergic inhibition at the nucleus ambiguus (DiMicco et al., Science 204: 1106-1109, 1979). In contrast, BMI elicits hypertension and tachycardia mediated by the sympathetic nervous system when this agent is restricted to the forebrain (lateral and third) ventricles (Williford et al., Neuropharmacol. 19: 245-250, 1980). Electrical stimulation of forebrain areas eliciting similar cardiovascular changes also suppresses baroreflex-induced bradycardia (Gebber and Klevans, Fed. Proc. 31: 1245-1252, 1972). The purpose of this study was to determine whether blockade of forebrain GABA receptors with BMI might similarly alter medullary cardiovascular reflex function.

Adult mongrel cats were anesthetized with chloralose/urethane (50/500 mg/kg i.p.), artificially ventilated and paralyzed with decamethonium 0.25 mg/kg i.v., supplemented as needed. Arterial pressure and heart rate were continuously monitored. Reproducible reflex bradycardia (ranging from -42 to -165 beats/min. in different preparations) was elicited by baroreceptor stimulation (phenylephrine HCl 20-100 µg i.v.). BMI (1-32 µg) injected into, and restricted to, the forebrain (lateral and third) ventricles, suppressed baroreflex bradycardia in a dose-related fashion. Maximal effect was observed within 10 minutes of injection and consisted of 30±10% reduction at 1-4 µg (n=6), 66±8% reduction at 5-10 µg (n=4), and 88±4% suppression at 16-32 µg (n=3). The highest doses also elicited transient increases in arterial pressure and heart rate. However, significant suppression of reflex bradycardia (>50%) persisted after recovery of original baseline parameters, and after administration of lower doses of BMI which failed to alter arterial pressure and heart rate by more than 3 mmHg or 5 beats/min., respectively. Forebrain administration of the GABA agonist muscimol (10 µg), which had no effect on baseline arterial pressure and heart rate or on baroreflex-evoked bradycardia in untreated cats, completely reversed the effects of BMI on these parameters.

These data suggest that blockade of forebrain GABAergic inhibition suppresses baroreflex bradycardia.

263.5 PERIPHERAL OR CENTRAL ADMINISTRATION OF THE GABA RECEPTOR AGONIST, THIP, RESULTS IN A CENTRALLY MEDIATED HYPOTENSION.
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Intracerebroventricular infusion of 40 µg/kg of the GABA agonist 4,5,6,7-tetrahydroisoxazolo[5,4-c]pyridin-3-ol(THIP) over a 10 min period lowers blood pressure 40 ± 9% ($p < 0.05$) and heart rate 20 ± 3% ($p < 0.05$) in chloralose anesthetized cats. In contrast, the intravenous (IV) administration of THIP (40 µg/kg) failed to alter blood pressure (8 ± 4% N.S.) and heart rate (5 ± 2% N.S.). Increasing the dose of THIP to 6-8 mg/kg IV resulted in a significant fall in blood pressure (40 ± 9%, $p < 0.05$) and heart rate (20 ± 4%, $p < 0.05$). The onset of the fall in blood pressure occurred within 1 min and was maximal within 5 min after the bolus IV injection of THIP. The fall in heart rate was evident within 1 min following the injection of THIP and reached its nadir 20-30 min later. The magnitude of the fall in heart rate appeared to be related to the resting heart rate prior to THIP. In 4 cats in which THIP (8 mg/kg IV) was administered, the increase in blood pressure produced by a bolus injection of norepinephrine (0.03 - 1.0 µg/kg IV) or 1,1 dimethyl-4-phenyl piperazinium iodide (DMPP, 1-10 µg/kg IV) were not reduced by THIP. Similarly, the vasoconstrictor response evoked during a 30 sec bilateral occlusion of the carotid arteries was not altered by THIP. In 3 cats, the GABA antagonist bicuculline methiodide (B) was infused into the cerebral aqueduct (0.5 µg/kg/min) without altering blood pressure or heart rate. During the infusion of B, THIP (6 mg/kg IV) was administered as a bolus and the resultant THIP-induced fall in blood pressure was attenuated by the B infusion. The data suggest that THIP like other GABA receptor agonists acts centrally to lower blood pressure and heart rate. There is no evidence of peripheral α-receptor or ganglionic blockade by THIP. The antagonism by B suggests that the cardiovascular actions of THIP are mediated through activation of central GABA receptors, results consistent with the previously demonstrated ability of THIP to bind to GABA receptors.

263.6 BEHAVIORAL, NEURAL AND CARDIOVASCULAR INFLUENCES OF TAURINE.
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Taurine may be a neurotransmitter or modulator and has both an inhibitory effect and membrane stabilizing properties. In the adult rat the acute effects of taurine include a gradual fall in blood pressure (BP) with intraventricular application and a more rapid fall with intracisternal (IC) application. Locomotor activity (LCMA) can be decreased by intraperitoneal injections.

We examined the effects of acute taurine administration in chloralose anesthetized spontaneously hypertensive rats (SHR). Intravenous injection of 12mg/kg taurine in saline produced no change in BP over one hour. IC application of 750 micrograms taurine in 20 microliters of vehicle solution delivered over 90 seconds produced a fall in BP to 52±8% of control within two minutes. Hypotension remained for 30-60 minutes. Heart rate (HR) fell to 78±6% of control and sympathetic nerve activity (SNA), recorded from the splanchnic, was reduced to 57±15% of control at the peak of the effect. Raising pressure with phenylephrine further reduced SNA. IC injections of isoosmotic saline had no effect.

The levels of taurine in brain can be elevated chronically by providing rats with drinking water containing 3% taurine (Nara et al, Biochem. Pharm. 27:2689:78). We examined the effects of oral taurine in adult (25 week) SHR's. BP was measured repeatedly for one week prior to onset of treatment and then for 10 days during treatment. BP fell significantly by day 3 and remained reduced until day 6. By day 10, BP had returned to pretreatment levels.

Nara et al (78) report that chronic oral taurine given to SHR beginning at age 4 weeks alters the course of blood pressure development. We have measured both BP and LCMA from age 4 to 16 weeks in treated and untreated SHR. Both groups display an increase in pressure throughout the period but the rise in BP is retarded in the treated animals (Two way ANOVA $p < .001$).

LCMA was measured at 12 and 16 weeks of age using an automated activity cage. Cumulative activity during 15 minutes was significantly lower in treated animals at both ages (Two way ANOVA $p < .001$).

These data show: 1) the fall in BP with acute IC taurine injection involves a reduction in both HR and splanchnic SNA. 2) BP is reduced in adult SHR within 3 days of the onset of chronic oral taurine treatment. 3) Both BP and LCMA are reduced by chronic administration in the developing animal. (Support from HL24110 and Vermont Heart Association).

263.7 COMPARABLE DEPRESSION OF THREE CENTRAL SYMPATHETIC PATHWAYS BY CLONIDINE AND ALPHA-METHYLDOPA THROUGH ACTIVATION OF ALPHA-2 RECEPTORS.
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Previous studies have demonstrated a dose-dependent (2.5-50 ug/kg) depression of transmission through central sympathetic pathways by clonidine (Pharmacologist 22:301, 1980). Sympathetic discharges, recorded from upper thoracic preganglionic rami, were evoked by spinal reflex (SR) or intraspinal (IS) pathways in unanesthetized spinal cats or by long spinal-bulbospinal (SBS) reflex pathways in debuffered, chloralosed cats. The SBS pathways were more sensitive to depression by clonidine than the IS pathways, but both could be depressed completely. The SR pathway was least sensitive and could not be depressed below 40% of control values even by larger doses. The depression by clonidine could be prevented or reversed by the alpha-2 receptor antagonists, yohimbine or tolazoline.

In the present study, alpha-methyldopa (MD) was tested on the same three pathways. Although 150 mg/kg of MD alone usually produced only a slight enhancement, subsequent injection of 5 mg/kg of reserpine after at least 2 hr produced a prompt depression of transmission through each pathway. Reserpine alone generally produces only a modest increase in transmission. The SBS and IS pathways were completely depressed whereas the SR pathways were depressed to only 40% of control. Depression was rapidly reversed by yohimbine (0.5 mg/kg) or tolazoline (2-5 mg/kg). Pretreatment with these antagonists also blocked the depression produced by reserpine. A minimum period of about 45 min after MD injection was necessary before reserpine could produce depression, suggesting that the action of MD requires the formation of an active metabolite, probably alpha-methylnorepinephrine which, like clonidine, has a high affinity for alpha-2 receptors. Reserpine apparently releases the intraneuronal stores of this active metabolite which is synthesized as a false transmitter.

The results indicate that both clonidine and MD act at sites in both the brainstem and spinal cord through activation of alpha-2 receptors. In the spinal cord, these receptors appear to be postsynaptic on sympathetic preganglionic neurons.

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263.8 ENHANCED TRANSMISSION TO SYMPATHETIC PREGANGLIONIC NEURONS DURING SELECTIVE INHIBITION OF EPINEPHRINE SYNTHESIS.
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Sympathetic preganglionic neurons (SPGNs) receive a dense innervation of norepinephrine (NE) terminals and a lesser but almost exclusive innervation of epinephrine (EPI) terminals from neurons associated with autonomic centers in the brainstem. As yet, no concensus regarding their respective functional roles has been reached.

Our own previous studies, conducted on unanesthetized spinal cats and designed to analyze sympathetic discharges recorded from upper thoracic preganglionic rami and evoked by microelectrode stimulation of descending excitatory pathways in the cervical dorsolateral funiculus, suggest that the NE pathways activate adenylate cyclase to generate cyclic AMP which appears to increase the excitability of SPGNs. Clonidine depresses SPGNs by activation of alpha-2 receptors which appear to inactivate adenylate cyclase (Neurosci. Abstr. 6:165, 1980).

Since the affinity of EPI for central alpha-2 receptors is about twice that of NE, the possibility that EPI may function as an endogenous ligand for the alpha-2 receptors on SPGNs was tested with a selective inhibitor of PNMT, LY134046 (8,9-dichloro-2,3,4,5-tetrahydro-1H-2-benzazepine HCl) which markedly depletes central EPI within a few hours (Fuller et al., Biochem. Pharmacol., 1981, in press). Within about 0.5 hr after LY134046 (20 mg/kg, i.v.), intraspinal transmission consistently increased linearly with time to an average of 220% of control values (N=5; range, 170-300%) at 4.5 hr. This dose of the inhibitor appeared to exert little blocking action on alpha-2 receptors since neither the typical depressant effect of clonidine nor its antagonism were noticably impaired.

These results support the proposal that EPI pathways to SPGNs depress their excitability by activating postsynaptic alpha-2 receptors which are negatively coupled to adenylate cyclase. Although the synaptic coupling of EPI and NE terminals may differ, this proposal also suggests a mechanism whereby excessive activity in the excitatory NE pathways could become self-limiting, by activating the alpha-2 receptors to depress adenylate cyclase and suppress cyclic AMP synthesis.

(Supported by NIH grants HL-24085 and GM-07579.)

263.9 THE EFFECT OF A NEW POTENT ANTAGONIST OF VASOPRESSIN ON THE PRESSOR RESPONSE OF INTRACEREBROVENTRICULARLY ADMINISTERED HISTAMINE IN UNANESTHETIZED FREELY MOVING RATS. P.J. Gatti* and S.B. Gertner* (SPON: G.A. Condouris). Dept. Pharmacol., N.J. Med. Sch.-CMDNJ-Newark, N.J. 07103.

Intracerebroventricular (i.c.v.) injections of histamine in conscious freely moving or anesthetized rats have been shown to produce a rise in mean arterial blood pressure (MAP). This increase in MAP has been reported to be due to an increase in sympathetic outflow from the central nervous system to the peripheral vasculature. Studies in our laboratory have shown that in the conscious rat, these reports cannot explain our experiments which have shown that hexamethonium, a ganglionic blocker, does not block the pressor effect of i.c.v. histamine. Likewise, 6-hydroxydopamine pretreatment does not attenuate the pressor response of i.c.v. histamine. Since others have shown an antidiuresis after centrally administered histamine in the rat, cat and goat, we investigated the possibility that arginine-vasopressin, released from the posterior pituitary, may play a role in the pressor response to i.c.v. histamine. Control MAP in 20 male Sprague-Dawley rats was 100±4.0 mmHg. I.c.v. injections of histamine dihydrochloride (1 µg) into the lateral ventricles produced a rise in MAP of 29±1.4 mmHg compared to controls. When rats were pretreated with 5 µg of [1-(β-mercapto-β,β-cyclopentamethyleneproprionic acid),2-(O-methyl)tyrosine] arginine-vasopressin intravenously, and then given 1 µg of histamine 5 minutes later, the rise in MAP was reduced 72% to 8±1.1 mmHg (n=8). This was significantly lower than control ($p<.05$). However, in 6 other animals also given the same dose of blocker, the response was not attenuated at all; the rise in MAP in these animals after histamine was 31±3.3 mmHg which was not different than control ($p>.05$). It appears that in this latter group, something other than arginine-vasopressin is contributing to the pressor response of i.c.v. histamine. Work is currently being done in our laboratory to investigate the factors responsible for the hypertensive response to histamine in those animals which are not blocked by the vasopressin antagonist.

263.10 REDUCTION OF THE HARMALINE CARDIOVASCULAR RESPONSES BY DIAZEPAM ADMINISTERED IN THE CEREBELLUM. K. J. Dormer. Department of Physiology and Biophysics, University of Oklahoma College of Medicine, Oklahoma City, OK 73190.

Harmaline produces cardiovascular effects in dogs in addition to the relatively minor tremorogenic effects. These effects include elevated arterial pressure and heart rate for doses 0.5-1.5 mg/kg (1.v.). Since specific interactions are reported to occur in the inferior olivary complex which gives climbing fibers to the cerebellum, including the fastigial nucleus (FN), it has been suggested by Dormer and Brown (Soc. Neurosci. Abst. 6:163, 1980) that the cardiovascular effects resulted from FN activation. Lesions in rostral FN or cerebellectomy essentially abolished the cardiovascular responses. Since, harmaline has been associated with benzodiazepine receptors, in this study diazepam was administered subdurally to the cerebellum to observe its effects on the harmaline cardiovascular responses. Mongrel dogs (12-15 kg) were chronically instrumented with a solid-state pressure transducer (Konigsberg P-4) in the descending aorta and a Doppler ultrasonic blood flow transducer on the left renal artery. Two weeks following recovery multipolar semimicro stainless steel electrodes were implanted into the rostral FN and a PE-50 cannula was placed subdurally above folium 4 on the cerebellar cortex. After 5-10 days recovery harmaline was injected (1.5 mg/kg i.v.) and the cardiovascular responses recorded. Mean arterial pressure increased 40%, heart rate increased 61% and renal arterial blood flow velocity increased 31% (all mean responses). These changes were observed within 50 seconds of infusion and were proportional to the 6-10 cycle/sec. tremors evoked. 24-48 hours later the experiment was repeated but 10 minutes following 1.5 mg diazepam administered subdurally to the cerebellum. Arterial pressure and heart rate fell initially and subsequent harmaline administration did not elevate pressure (6% decrease from control). HR was decreased 15% and renal flow velocity increased 31%. No tremors were observed. These observations suggest the harmaline may also have direct effect on the cerebellum since diazepam abolishes the cardiovascular changes which have previously been associated with the FN. Supported by NIH Young Investigators Award HL22747.

263.11 BENZODIAZEPINE AND OPIATE RECEPTOR LEVELS IN DIFFERENT BRAIN REGIONS AND THE KIDNEY OF SPONTANEOUSLY HYPERTENSIVE RATS. Leonard G. Davis and William F. Herblin. Central Research and Development Dept., E. I. du Pont de Nemours & Co., Wilmington, Delaware 19898.

Several reports exist that describe altered neurotransmitter systems in rats with spontaneous or induced hypertension. The major change appears to involve the adrenergic system yet other receptor populations are affected. Benzodiazepines have been used by physicians in the management of essential hypertension yet these drugs have only been shown to be effective in hypertensive patients reporting psychological stress. On the other hand, opiate agonists have long been known to reduce blood pressure. These clinical observations have been partially supported by in vitro animal studies: 1) Regan et al. (Life Sci 28: 991, 1981) reported that CNS benzodiazepine receptors were unaltered in deoxycorticosterone acetate (DOCA)-salt induced hypertension but they did demonstrate that the number of kidney receptors in the DOCA-treated animals was elevated. 2) Martucci and Hahn (Endocrine Res. Comm. 6: 291,1979) reported that the number of CNS opiate receptors in spontaneous hypertensive rats (SHR) was elevated without any change in their affinity for ^{3}H-naltrexone, an antagonist. We have investigated benzodiazepine and opiate receptors in various brain regions and in the kidneys of SHR and control rats.

Utilizing a pneumatic pressure sensor and a tail-cuff, we determined the systolic blood pressure of unanesthetized rats. As expected, the blood pressures of SHR and control animals were statistically different (160 ± 9 vs. 107 ± 2, respectively). The following day, these animals were sacrificed and various brain regions were dissected free while the kidney was desheathed and removed. A crude membrane fraction was prepared from each brain region and kidney by standard centrifugation procedures after hypoosmotic shock for immediate use in standard saturation binding assays with either ^{3}H-diazepam for benzodiazepine receptors or ^{3}H-etorphin, an agonist, for opiate receptors. The data were transformed for Scatchard analysis. Although the number of benzodiazepine receptors in each brain region differed, minimal differences were detected between SHR and control rat brain regions. However, the number of kidney receptors was markedly elevated in SHR when compared to controls. Opiate receptors showed less dramatic changes in all areas studied.

The significance of the profound change in peripheral benzodiazepine receptors in hypertensive rats will be further explored as well as the possible influence of altered agonist/antagonist configuations of opiate receptor populations.

263.12 CENTRAL CARDIOVASCULAR EFFECTS OF PROSTAGLANDIN E_2 and I_2. G. Feuerstein, D.M. Jacobowitz and I.J. Kopin. Lab. of Clinical Science, NIMH, Bethesda, MD 20205.

Prostaglandins E_2 and I_2 are important modulators of the cardiovascular system through multiple sites of actions -- i.e., blood vessels, cardiac functions, platelets homeostasis, renin catecholamines and vasopressin release. Prostaglandins are also synthesized by brain tissue, but their role in central regulation of the cardiovascular system is largely unknown. The following experiments were designed to investigate further the role of vasodilator prostaglandins - PGE_2 and PGI_2 in central cardiovascular and sympathetic regulation in halothane (0.8% in oxygen) anesthetized rats.

Injections of PGE_2 (0.5-5 nmol/kg) into the lateral cerebral ventricle (i.c.v., 5µl) elicited a dose dependent increase of heart rate up to + 117 ± 14 (n=8) beats/min.; blood pressure was also elevated, up to +27 ±6 mm Hg. These effects subsided after 30 min. At time of maximal heart rate and blood pressure responses, plasma norepinephrine (NE, radioenzymatic assay) increased up to 802 ± 103 pg/ml (from 294 ± 29 pg/ml at the control period) and plasma epinephrine (EPI) increased up to 116 ± 21 pg/ml (from control level of 41 ± 6 pg/ml). The increases in plasma catecholamine levels were also dose dependent, and tapered back to control levels after 30 min. Injection of PGE_2-0.5 nmol/kg (0.5 µl) into discrete hypothalamic regions -- the dorsomedial (DMN) and posterior hypothalamic (PHN) nuclei, elicited a moderate increase in blood pressure, up to +22 ± 2 mm Hg (n=8) and a marked increase in heart rate, up to +175 ± 23 beats/min. Plasma NE, but not EPI was increased up to 533 ± 60 pg/ml (versus 186 ± 25 pg/ml at the control period) at the time of maximal cardiovascular response. Injections of PGI_2 (0.05 - 50 nmol/kg) i.c.v. resulted in a depressor and cardiac accelerating response (dose dependent). However, the i.c.v. injections of PGI_2 were 25 times less potent (ED50) than i.v. injections of the same doses of PGI_2. Injections of PGI_2 into the DMN or PHN (0.05 nmol/kg, n=7) elicited only a short lasting (10 min) increase in heart rate, up to +55 ±4 beats/min, without effect on blood pressure. Plasma EPI was slightly elevated at the peak of the heart rate response, up to 140 ±35 pg/ml (versus 41 ±10 pg/ml at the control period, but bilateral adrenalectomy did not abolish the increase in heart rate induced by intrahypothalamic injections of PGI_2. These data suggest that PG's may modulate cardiovascular functions and sympathetic activity in specific brain nuclei. Various PG's may differ both quantitatively and qualitatively in their central actions.

263.13 USE OF TWO NON-CARDIOVASCULAR MODEL SYSTEMS IN THE ANALYSIS OF CNS AUTONOMIC ACTIONS OF CLONIDINE AND SOME ANALOGS OF CLONIDINE.

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The effects of intravenous administration of clonidine and five congeneric derivatives of clonidine were tested in anesthetized cats with regard to their relative ability to depress centrally and peripherally evoked electrodermal responses (EDR) as well as with regard to their ability to inhibit parasympathetic tone to the iris. With the exception of ST-91, all of the clonidine-like substances selectively reduced the amplitude of centrally (hypothalamic) evoked EDR in a dose-dependent fashion, with the order of sympatho-inhibitory potency being clonidine (ST-155) >ST-375>ST-606>ST-600>ST-608>>>ST-91. Of these agents only ST-91 exerted a peripheral inhibitory effect, indicating a CNS site of action for all of the other compounds. The same order of potency of these compounds was observed with regard to their mydriatic (parasympatho-inhibitory) effects. In some experiments the CNS inhibition of parasympathetic nerve activity was confirmed by means of direct recordings from the postganglionic ciliary nerves. In these experiments ST-91 exerted only a modest CNS effect and then only at dosage levels in excess of 300 μg/kg. This relative lack of CNS effect of ST-91 is likely due to its low lipoid solubility as it is at least as potent as clonidine in stimulating peripheral α-adrenoceptors.

Prior treatment with yohimbine hydrochloride (0.5 mg/kg, i.v.) antagonized both the CNS sympatho-inhibitory (depression of EDR) and parasympatho-inhibitory (mydriasis associated with inhibition of ciliary nerve activity) effects of all of these clonidine-like agents. The results of this study demonstrate that all of the analogs of clonidine tested (with the possible exception of ST-91) act like clonidine to depress tone and reactivity of both sympathetic and parasympathetic autonomic mechanisms, although all of the congeners of clonidine were less potent in their effects. These results also suggest that an action on a CNS adrenergic mechanism (probably via α_2-adrenoceptors) is involved in both sympathetic and parasympathetic control of these autonomic systems.

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264.1 RAPID RESETTING OF AORTIC AND CAROTID SINUS BARORECEPTORS. L.B. Bell*, J.L. Seagard, F.A. Hopp*, and J.P. Kampine. Depts. of Anesthesiology and Physiology, Med. Col. of Wis. and Wood VA Med. Ctr., Milwaukee, WI 53193

Previous studies have found that the arterial baroreceptors can adapt to changes in systemic blood pressure (BP) within hours to days, resetting both threshold and saturation pressures. A recent report has indicated that carotid sinus baroreceptors can reset as soon as 15 minutes. This study was performed to: 1) simultaneously record threshold pressures for both aortic and carotid sinus baroreceptors set at systemic BP over a physiological range; and 2) determine the ability of both sets of receptors to rapidly adapt to changes in BP within 5-20 minutes, as well as the pattern in which resetting occurs.

Mongrel dogs were anesthetized (35 mg/kg pentobarbital) and placed on positive pressure ventilation with oxygen. Simultaneous multifiber nerve recordings were made from the left carotid sinus and right aortic depressor nerves during each experimental procedure. Carotid sinus and aortic arch blood pressures were measured by cannulas inserted in the lingual and femoral arteries, respectively. Systemic BP was regulated by an isobaric pressurized reservoir system connected to the animal via the brachial arteries. This system was used to maintain constant peak systolic pressures of 80, 120, or 160 mmHg. Peak systolic BP were randomly set in each animal at these experimental pressures and thresholds were determined for both sets of receptors at 5, 10, 15, and 20 minutes following each pressure change.

The threshold pressures for either set of baroreceptors were not consistently higher than those for the other set at any of the experimental pressures. Both sets of baroreceptors demonstrated an ability to rapidly reset (within 5 min) to either an increase or decrease in BP. When BP was decreased, a rapid drop in threshold was observed at 5 min followed by a gradual increase during the next 15 min to a new threshold level below that established at the higher pressure. When BP was increased, threshold rapidly increased at 5 min and continued to gradually increase over the next 15 min. This resetting of threshold for both increases and decreases in BP appears to plateau between 15-20 minutes for both sets of baroreceptors. The magnitude of the change in threshold depended on the magnitude of the pressure change. This study has demonstrated that both aortic and carotid sinus baroreceptors begin to adapt to changes in BP within 5 min. The ability of these baroreceptors to adapt was independent of pressure within a physiologic range of 80-160 mmHg. (Supported by NIH Grants HL 16511, 06511, 05882, and VA).

264.2 REFLEX EFFECTS OF SPLENIC AFFERENTS ON RENAL AND CARDIAC SYMPATHETIC EFFERENT NERVE ACTIVITY. N.L. Herman*, D.R. Kostreva, and J.P. Kampine. Depts. of Anesthesiology and Physiology, Med. Col. of Wis. and VA Med. Ctr., Wood, WI 53193.

The reflex effects of splenic low-pressure baroreceptors on renal and cardiopulmonary sympathetic efferent nerve activity were studied in mongrel dogs (15-20 kg) anesthetized with sodium pentobarbital (35 mg/kg i.v.) and placed on positive pressure ventilation. Systemic blood pressure, splenic venous and splenic arterial pressure as well as a lead II electrocardiogram were all measured and recorded using a Grass polygraph. Splenic afferent nerve activity was recorded from the cut distal end of a branch of the splenic nerve. Renal and cardiopulmonary sympathetic efferent nerve activity were recorded from the cut central ends of the renal nerves and the anterior ansa subclavian nerve respectively. Both afferent and efferent nerve activities were amplified, filtered, displayed on an oscilloscope and recorded on magnetic tape, along with the other parameters that were measured. Splenic afferent nerve activity could be elicited by increasing splenic venous pressure by manual compression of the spleen, occlusion of a splenic vein or splenic contraction induced by injection of 100 ug of epinephrine into the splenic artery. Increases in splenic afferent nerve activity are linearly related to increases in splenic venous pressure but not splenic arterial pressure. Histological sections of the nerves from which afferent recordings were obtained demonstrated that all of the afferents were non-myelinated C-fibers. Electrical stimulation of the cut central end of the splenic nerves resulted in a marked reflex increase in both renal and cardiopulmonary sympathetic efferent nerve activity that remained elevated throughout the stimulation period. The reflex increase in cardiopulmonary sympathetic efferent nerve activity was associated with an increase in right ventricular contractile force measured with a Brodie-Walton strain gauge, an increase in heart rate (5-15 beats/min) and increases in blood pressure. The physiological effects of the reflex increases in renal sympathetic efferent nerve activity were not determined in this study. This study is the first to demonstrate both the existence of low pressure baroreceptors in the spleen and that these splenic afferents can reflexly alter cardiopulmonary sympathetic efferent nerve activity, heart rate, contractile force, blood pressure and renal sympathetic efferent nerve activity. (This study was supported by NIH Young Cardiovascular Investigator Grant HL 21042 and the VA Medical Research Service).

264.3 HEMODYNAMIC VARIABLES PREDICTING FIRING OF ATRIAL B-RECEPTORS. R. L. Stornetta* and D. G. Ward. Neuroscience Program, Univ. Virginia Sch. Med. Charlottesville, VA 22908.

It is generally accepted that atrial B-receptors respond to changes in atrial filling and pressure. However, the precise interrelations of the hemodynamic parameters responsible remain obscure. To define quantitatively the relationship of right atrial filling and mean right atrial pressure to the activity of atrial B-receptors, recordings of single fibers from the right cervical vagus were obtained from 8 cats anesthetized with chloralose/urethane. Recordings were made of 7 atrial fibers with B-activity, 2 aortic baroreceptors, 5 ventricular fibers and 6 respiratory fibers classified according to the criteria of Paintal. The activity of these fibers were examined in response to 10ml/kg hemorrhage for 3 min. Blood was reinfused after each trial. Firing rate, mean right atrial pressure (MRAP), magnitude of the right atrial "V" wave measured from its beginning to its peak ("V), mean arterial pressure(MAP) and arterial pulse pressure (APP) were each averaged for sequential 15 sec periods during each hemorrhage. All measurements were normalized as a percentage of pre-hemorrhage values. The normalized firing of each fiber was plotted against the normalized value of each hemodynamic variable. Multiple linear regressions of atrial B-receptor firing with mean right atrial pressure and magnitude of the right atrial "V" wave were calculated. Analogous calculations were made of baroreceptor firing with mean arterial pressure and arterial pulse pressure. The firing of the atrial B-receptors and the firing of the baroreceptors was each correlated statistically with each of the hemodynamic variables. However, the multiple linear regressions revealed that the firing of atrial B-receptors (F_a) was best predicted by;

$$\%F_a = -135.3 + 2.7(\%\text{"V"}) - 0.46(\%\text{MRAP}) \quad (R=0.97).$$

The firing of aortic baroreceptors (F_{br}) was best predicted by;

$$\%F_{br} = -266.1 + 0.76(\%\text{APP}) + 2.96(\%\text{MAP}) \quad (R=0.96).$$

The present data suggest strongly that the firing of atrial B-receptors during hemorrhage is best predicted by the magnitude of the "V" wave (weighting factor 2.7) in linear combination with the mean right atrial pressure (weighting factor -0.46). The weighting factors indicate that the "V" wave which estimates atrial filling is the dominant factor in predicting the firing of atrial B-receptors. Mean right atrial pressure is a minor factor. However, either hemodynamic variable alone inadequately predicts the neural response.
Supported by HL26349, RR05431 and HL00827.

264.4 DISTRIBUTED FUNCTIONS OF THE CENTRAL NERVOUS SYSTEM IN THE DYNAMIC CONTROL OF THE INTRAMUSCULAR VENOUS CAPACITANCE. F.J. Thompson, J.R. Wald*, G.T. Gobbel* and B.J. Yates*. Dept. of Neuroscience, University of Florida College of Medicine, Gainesville, FL 32610.

The functions of the venous system have recently been expanded to include a dynamic role in the regulation of circulation. We have been studying the sources of input from superficial veins (venous afferents), their projections, and connections in the feline CNS and contribute these findings to that concept.

Electrical stimulation of limb venous afferent produced activation of both flexor and extensor motoneurons; microstimulation of cortical neurons activated by venous afferent stimulation elicits EMG activity in the skeletal muscles in the same limb of origin of the venous afferent fibers, (Thompson et al. 1981). The present studies were done to examine the excitability of venous afferents by perfusion-distention of selected segments of forelimb and hindlimb veins, and to compare the distribution of central neural responses to those obtained by electrical stimulation.

Perfusion-distention of a segment of femoral-saphenous vein elicited potentials recorded from the dorsum of the lumbar spinal cord, with minimum perfusion pressures of 14 mm/Hg. Rostral-caudal mapping of the lumbo-sacral spinal cord revealed that maximal N_1 potentials were sharply restricted to the fifth and sixth lumbar cord segments, and overlapped precisely the distribution determined by electrial stimulation of the femoral venous afferent fibers. Perfusion-distention of a segment of cephalic vein evoked potentials in the pericruciate cerebral cortex, with minimum perfusion levels of 25 mm Hg. Short latency initially positive potentials characteristic of primary waveforms were recorded from the lateral sigmoid gyrus and were largest near the tip of the cruciate sulcus. The cortical distribution of the maximal perfusion evoked potentials overlapped the motor cortex distribution mapped by electrical stimulation of the cephalic venous afferents. Electrical and mechanical stimulation experiments have revealed topographically organized connections to motor neurons in both the spinal cord and in the cerebral cortex; the activated neurons are widely separated in the caudal-rostral extent of the central nervous system but are functionally inter-related. The authors propose that the venous afferents form a major contribution to the central integration and control of intramuscular venous capacitance through widely dispersed but functionally interconnected components of the somatomotor system. The organization of this system as a distributed system will be discussed.
Supported by NIH, RO1 HL 256190-02

264.5 EXERCISE TRAINING AFFECTS RESPONSES TO STRESSORS. E. M. Hull, S. H. Young* and M. G. Ziegler*. Univ. Tex. Med. Branch, Galveston, TX 77550.

It is well known that exercise training reduces a number of cardiovascular and hormonal responses to a fixed amount of exercise. Furthermore, situations requiring active psychological coping elicit B-adrenergically mediated cardiovascular responses similar to those of exercise. This study was undertaken to determine whether the exercise training effect would generalize to stressors which require active psychological coping and/or to passive psychological or physical stressors.

Subjects were 55 men and women between the ages of 21 and 64, who spent an average of at least 30 min per day either exercising vigorously or pursuing a sedentary hobby. Level of exercise training was defined as length of time they ran on a treadmill before exhaustion. Heart rate and blood pressure were recorded and blood was drawn from an indwelling catheter after a 20 min rest, during and 3 and 10 min after each of 4 stressors. The passive and active psychological stressors were a film of industrial accidents and the Stroop word-color task performed under distracting conditions. The passive and active physical stressors were putting a foot in ice water for 1 min and exercising on an automated treadmill to exhaustion. In addition, a psychological rating scale consisting of 7 pairs of mood adjectives was administered during baseline and after each stressor.

Diastolic blood pressure responses to the film and Stroop test were lower in exercise trained subjects 40 years of age or older than in untrained persons of the same age category. Diastolic responses to exercise were lower for the entire trained group. Exercise training was also associated with lower baseline systolic pressures for persons over 40 and with lower heart rates for the whole group at all times except during and after maximal exercise. Norepinephrine was lower in trained subjects after 9 min of exercise, but much higher after maximal exercise, reflecting the greater work done. Trained persons reported less depression and anger after the film than did untrained persons.

There was no preferential generalization of the training effect to the active psychological task. Thus, training affects more than the B-adrenergic system. The significant reduction in blood pressure responses only in trained subjects over 40 is compatible with other evidence that blood pressure is lowered by training only in older and/or hypertensive individuals. None of our subjects was hypertensive, but pressures were higher for older subjects at several times. Exercise training, by reducing labile responses to stressors, may help to avert the more dangerous and harder to treat stable hypertension.

Supported by a grant from the Moody Foundation.

264.6 ELECTRICAL STIMULATION OF THE AMYGDALA CENTRAL NUCLEUS IN THE AWAKE RABBIT: EFFECTS ON HEART RATE, RESPIRATION AND SOMATOMOTOR BEHAVIOR. Bruce S. Kapp, Craig D. Applegate*, Mark D. Underwood* and Carole L. McNall*. Dept. of Psychology, University of Vermont, Burlington, Vt. 05405. (SPON: F. Abraham).

We have shown that bradycardia in response to a conditioned threatening stimulus in the rabbit is attenuated by lesions of the amygdala central nucleus (ACE) and that electrical stimulation of the ACE elicits bradycardia in the anesthetized rabbit (Kapp et al., Physiol. Behav., 1979; Kapp et al., submitted). The present study was conducted to determine the extent to which ACE stimulation elicits bradycardia and concomitant somatomotor and respiratory responses in the awake rabbit.

Sixteen sites within the central nuclei of fourteen rabbits were examined under loosely restrained and unrestrained conditions using monopolar electrical stimulation (5.0 sec trains; 100 Hz; 0.5 msec pulse duration; 150 µm tip exposure). Under loosely restrained conditions and with current levels ranging from 10-100 µA, bradycardia was elicited from thirteen of these sites, tachycardia was elicited from one site, and negligible responses were elicited from two sites. The bradycardia was of short onset latency (< 1.0 sec) and was greatly attenuated or abolished by I.V. atropine methylnitrate. The median threshold current intensity to elicit a peak bradycardia response of ≥ 10% for the sites demonstrating bradycardia was 40 µA. At threshold current intensities necessary to produce ≥ 10% bradycardia, the most frequent respiratory response was one of increased rate and decreased amplitude (9 of 12), or of decreased amplitude with no change in rate (3 of 12). These respiratory changes were most prevalent during the first second of stimulation with variable responses occurring during the remainder of the stimulation period. Pupillodilatation, although not quantitatively measured, was frequently observed to occur at these threshold current intensities.

Of the thirteen sites from which bradycardia was elicited, stimulation at intensities necessary to produce ≥ 10% bradycardia invariably evoked movements of the mouth and tongue resembling chewing responses. Under unrestrained conditions stimulation at these current intensities produced an arrest of ongoing behavior to stimulus onset which varied from a momentary arrest to an arrest for the duration of the 5.0 second stimulation period.

The results suggest that stimulation of the ACE produces a variety of autonomic and somatomotor responses which may be mediated by direct ACE-brainstem projections in this species (Schwaber et al., Neuro. Lett., 1980). (Supported by USPHS Grants RO1 MH31811 and KO2 MH00118 to B.S.K.)

264.7 DESCENDING BRADYCARDIA PATHWAY(S) FROM PARABRACHIAL NUCLEUS IN RABBITS. D. Liskowsky*, H. Ellenberger*, J. Haselton*, N. Schneiderman, and R. Hamilton. Dept. of Psychology, Univ. of Miami, Coral Gables, Fl. 33124.

Electrical stimulation (10 sec; 100 pulses/sec; 0.25 msec pulse duration; 100-150 uA) elicited primary, short latency(<1.0 sec) bradycardia (N100 beats/min) from parabrachial nucleus (PBN) in ethyl carbamate anesthetized rabbits. Single pulse (0.4 pulses/sec) stimulation of PBN orthodromically activated barosensory-sensitive neurons in nucleus tractus solitarius (NTS) and cardioinhibitory vagal pre-ganglionic neurons in dorsal vagal nucleus (DVN) at a latency of 8.4 (±7.8) msec. However, because (1) extensive attempts to backfire barosensory-sensitive neurons in PBN by stimulating NTS or DVN were unsuccessful, (2) latencies and other characteristics of NTS and DVN neuronal responses to PBN suggested the presence of an oligosynaptic pathway, and (3) only sparse retrograde labeling was seen in PBN after injections of horseradish peroxidase (HRP) were made into dorsal medulla (NTS/DVN complex), attempts were made to identify alternate putative synapses in the central bradycardia pathway between PBN and cardioinhibitory motor neurons. Anterograde and retrograde labeling using HRP conjugated with wheat germ agglutinin revealed the presence of direct projections from PBN to NTS and from PBN to nucleus ambiguus (NA). Indirect projections from PBN to NA synapsed in either the A5 region or nucleus reticularis gigantocellularis (NRG). Direct projections were traced from NA to DVN, and reciprocal connections were observed between NTS and NA. (Direct projections between NTS and DVN have previously been observed using other neuroanatomical methods.) Extensive exploration of the caudal pons-rostral medulla indicated that train stimulation of the A5 region and ventral NRG elicited bradycardia responses that were similar to those elicited by stimulating PBN. These responses were eliminated by bilateral vagotomy, but not by artificial ventilation in decamethonium paralyzed animals. Bradycardia responses were also elicited at lower current intensity (∿30uA) by stimulating NTS, DVN, and NA. The results suggest that the bradycardia pathway(s) descending through PBN may have their outflow from DVN with intermediate synapse in NTS, and from NA either without intervening synapse or with intervening synapse in the A5 region or ventral NRG.

The study also showed that train electrical stimulation of the principle nucleus of V (Nvp) elicited bradycardia responses. The form and characteristics of these bradycardia responses were similar to the trigeminal depressor response described by Kumada, et al. (1979). Injection of HRP into Nvp showed that the descending trigeminal depressor response pathway is anatomically distinct from the descending PBN bradycardia pathway.

264.8 ANTERIOR HYPOTHALAMIC STIMULATION REVERSES OUABAIN-INDUCED ARRHYTHMIAS. C. Chinn* and B.H. Natelson, VA Medical Center and Dept. of Neurosciences, College of Medicine and Dentistry of New Jersey-New Jersey Medical School, East Orange NJ 07018.

Posterior hypothalamic stimulation at sub-threshold currents for arrhythmia is known to precipitate arrhythmias in the presence of otherwise sub-toxic doses of digitalis (Evans & Gillis, JPET, 195:577, 1975). The mechanism for this effect is thought to be due to increased sympathetic neural activity to the heart. Because it is known that anterior hypothalamic stimulation decreases heart rate and blood pressure due to concomitant sympatho-inhibition and parasympathetic activation in the cat, we thought that such stimulation might reverse digitalis-induced arrhythmias.

In the chloralose-anesthetized (70-80 mg/kg, IP) cat, cardiac arrhythmias were induced by bolus IV injections of ouabain (25 µg/kg every 15 min for 30 min, then 15 µg/kg every 15 min until the appearance of arrhythmias). Two min after the onset of a sustained arrhythmia, the left anterior hypothalamus was stimulated with a concentric stainless steel electrode (NE-100, Kopf) either continuously for 3-5 min (0.5 ms duration, 0.3-0.5 mA, 100 Hz) or for 2 min at 2 min intervals. In some animals, sympathetic discharge was recorded from a left renal nerve. Lead II ECG was monitored.

Ouabain produced various cardiac arrhythmias after a total dose of 65-100 µg/kg. Rhythm strips revealed runs of ventricular extrasystoles, supraventricular tachycardia or ventricular tachycardia. Sustained or intermittent anterior hypothalamic stimulation could stop ventricular extrasystoles or supraventricular tachycardia and produce normal sinus rhythm. Ventricular tachycardia could not be converted to sinus rhythm; but during stimulation, occasional normally conducted beats were seen. Anterior hypothalamic stimulation resulted in a transient decrease in integrated renal nerve activity which returned to normal within 20 sec. No consistent relation was apparent between renal nerve activity and onset or reversal of the arrhythmias.

These experiments indicate for the first time that activation of a brain locus can reverse digitalis-toxic arrhythmias. This effect is not related in time to diminution of sympathetic nerve activity. These observations suggest a possible role for the anterior hypothalamus in the control of heart rhythm as has been found earlier for heart rate and blood pressure.
(Supported by American Heart Association-New Jersey Affiliate and NIH grant #HL-24498)

264.9 THE FULMINANT HYPERTHERMIC-STRESS SYNDROME - POSSIBLE ROLE FOR NOREPINEPHRINE IN STRESS SUSCEPTIBLE PIGS. T.P. Davis*, C.W. Gehrke, Jr.*, and C.H. Williams*. (SPON: R.P. Gruener). Dept. of Pharmacology, Univ. of Arizona Health Sciences Center, Tucson, AZ. 85724 and Dept. of Biochemistry, Univ. of Missouri, Columbia, MO. 65211.

The idea that norepinephrine (NE) and other biogenic amines play a role in the development of the fulminant hyperthermia-stress syndrome has been presented on a theoretical basis and partially substantiated with catecholamine depletor, false transmitter, and alpha blocker studies. However, the crucial test of the NE hypothesis is to accurately quantitate key biogenic amines in blood plasma during the syndrome. To accomplish this we developed a sensitive, selective, pre-column derivatization method with HPLC (Davis, T.P., J. CHROM. 162, 293, 1979) to measure NE, dopamine (DA) and serotonin (5-HT) in plasma from normal and malignant hyperthermic (MH) pigs. Samples were carefully collected from control and stressed animals under halothane anesthesia. Using a simple extraction method involving pre-column derivatization with o-phthalaldehyde and ethyl acetate partitioning, the samples were chromatographed in less than 40 minutes. Norepinephrine was found to be elevated in the six hyperthermic pigs as the syndrome progressed, reaching levels of 2 to 15 ng/ml at the height of the syndrome, versus <0.5 ng/ml for the five controls. Body temperature and total peripheral resistance followed a similar pattern reaching 42.5°C and 4.3 PRU's, respectively. These experiments provide direct evidence for our hypothesis that a failure to metabolize excess NE may be one of the key metabolic defects in causing the pathophysiology of the malignant hyperthermia stress syndrome. The application of our chromatographic method in animal and human tests may provide a pattern of biogenic amine types and levels that could be diagnostic in identifying susceptible humans and carrier animals.

264.10 GASTRIC PATHOLOGY EFFECTS OF ELECTROLYTIC LESIONS OF THE DIENCEPHALON AND TELENCEPHALON IN THE RAT. J. R. Emslie* and N. I. Wiener. Department of Psychology, York University, Downsview, Ontario, M3J 1P3.

In a series of 72 rats, the pathological effects of electrolytic diencephalon and telencephalon lesions were assessed with respect to non-lesioned controls. Animals with ventromedial nucleus of the hypothalamus plus nucleus accumbens septi lesions had greatly reduced stomach pathology compared to those with ventromedial nucleus of the hypothalamus lesions alone. Similarly, the effect of lateral hypothalamic lesions was greatly reduced by the nucleus accumbens septi plus lateral hypothalamus combination. A homeostatic model is proposed which accounts for the observed brain/stomach correlations, allows predictions for novel lesion combinations and may generalize to other stress induced procedures.

264.11 THE CONTRIBUTION OF BILE TO GASTRIC MUCOSA DAMAGE IN RATS AFTER LATERAL HYPOTHALAMIC (LH) LESIONS. C. V. Grijalva, M. G. Tordoff, P. J. Geiselman and D. Novin. Dept. Psychol. and Brain Res. Inst., UCLA, Los Angeles, CA 90024.

There is experimental and clinical evidence that the reflux of bile into the stomach may play a role in gastric ulceration and gastritis. We conducted two experiments to investigate the contribution of bile to gastric mucosal damage in rats after anodal electrolytic lesions of the LH area.

In the first experiment we tested the possibility that LH lesions produce an immediate increase in biliary secretion. Twenty-two Long-Evans male rats were divided into two groups. All rats were deprived of food but not water overnight (16 h) and then they were anesthetized with urethane. The common bile duct was cannulated and biliary secretion was collected for 1 h prior to and every hour for 5 h following either LH lesions or control operations. There were no significant differences in the amount of biliary secretion between LH rats or controls either prior to surgery or during any of the five consecutive 1-h measures following surgery.

Although LH lesions did not alter bile flow, the possibility exists that bile may contribute to the occurrence of gastric mucosal damage after the brain lesion by refluxing into the stomach. Eighteen male rats were deprived of food but not water overnight (16 h), and then they were anesthetized with sodium pentobarbital (50 mg/kg, ip). In 9 rats the bile duct was ligated 1-cm from the duodenum. In the remaining 9 rats the bile duct was exposed but not ligated (sham ligated group). Following either bile duct ligation or sham ligation both groups received bilateral LH lesions. All animals were food and water deprived for an additional 24 h and then sacrificed with an overdose of sodium pentobarbital. The stomachs were examined for gross changes in the mucosa. The results showed that ligating the bile duct just prior to LH lesions significantly reduced the occurrence of gastric ulceration. Only one out of 9 rats in the ligated group displayed any obvious gastric pathology, whereas, 7 out of 9 rats in the sham-ligated group displayed varying degrees of glandular gastric erosions and hemorrhaging.

These results indicate that the reflux of bile into the stomach significantly contributes to the ulcerative process following LH lesions. The possibility exists that bile duct ligation reduces the propensity to ulcerate by eliminating bile salts from entering the stomach or by indirectly suppressing gastric acid secretion. (Supported by NIH grant NS07687).

264.12 MODULATION OF REGIONAL CEREBRAL BLOOD FLOW BY GAMMA-AMINOBUTYRIC ACID SYSTEMS. L. J. Bearden and R. M. Martin. Departments of Biomedical Engineering, Neurology, and the Neurosciences Program, University of Alabama in Birmingham, Birmingham, Al. 35294.

An understanding of the mechanisms involved in the control of the cerebral circulation has been the object of extensive experimental effort. However, relatively little attention has been given to the potential modulatory effects on the cerebral circulation of gamma-aminobutyric acid (GABA) systems; even though there are several lines of evidence which suggest such an involvement. Bicuculline, a potent GABA antagonist, has been shown to produce an increase in both cerebral arterial pressure and cerebral venous outflow. Both of these changes were observed approximately 6 seconds after bicuculline was administered (1.2 mg/kg, i.v.), and occurred simultaneously with the onset of the convulsive electrical effects of bicuculline (Meldrum and Nilsson, Brain 99:523-542 (1976)). It has also been demonstrated that there is a regional variability in the increase in cerebral blood flow which is induced by bicuculline (Horton, et al., Brain Research 192:399-412 (1980)), and that the increased rate of blood flow can be several times greater than normal, thus greatly exceeding the concomitant increase in cerebral oxygen consumption. Alternatively, it has also been reported recently that GABA itself and known GABA agonists produce a dose-dependent dilation of isolated segments of cerebral arteries in vitro. These observed dilatory effects could be blocked by bicuculline or picrotoxin (Edvinsson, et al., Brain Research Bulletin 5(2):335-340 (1980)). Therefore the manner of involvement of GABA-ergic systems in the regulation of cerebral blood flow remains questionable.

We have examined the effects of GABA-ergic compounds on the regional cerebral blood flow of rats by means of the hydrogen clearance technique. Adult male rats (250-350 g) were implanted with epoxylite insulated Pt/Ir macroelectrodes with the uninsulated tip of the electrodes located in the cortex, hippocampus, thalamus, or caudate-putamen of the brain. After recovery from surgery, control rates of blood flow were determined for each area of the brain. Animals were then given i.v. or icv. injections of GABA, bicuculline, picrotoxin, or muscimol, and regional blood flow rates redetermined as a function of time, dosage and type of drug. Results from these experiments support an involvement of GABA-ergic systems in the modulation of regional cerebral blood flow.

264.13 MAGNESIUM DEFICIENCY CAN INDUCE CEREBRAL ARTERIAL VASOSPASM. B.T. Altura* and B.M. Altura (SPON: J.B. Ranck, Jr.). Dept. of Physiol., SUNY Downstate Med. Ctr., Brooklyn, NY 11203

Recently, evidence has been brought forth which suggests that the concentration of external magnesium ions ($[Mg^{2+}]_o$) may be an important factor in the regulation of peripheral vascular tone and reactivity. To our knowledge, there are no direct observations on the influence of $[Mg^{2+}]_o$ on cerebral arterial tone. Studies were therefore undertaken on isolated canine cerebral (basilar-BA, middle cerebral-MCA), pulmonary (intralobular), coronary (left, right, circumflex and branches), splanchnic (mesenteric, hepatic, splenic, pancreatic) and renal arteries in the presence and absence of different $[Mg^{2+}]_o$. Lowering $[Mg^{2+}]_o$ below 1.2 mM produced spasms of BA, MCA and coronary, but not renal, splanchnic or pulmonary arteries. Withdrawal of $[Mg^{2+}]_o$ produced peak cerebral arterial vasospasms within 45-90 sec. In terms of % KCl maximal contractions, MCA $>$ BA $>$ coronary arteries. In contrast, elevation in $[Mg^{2+}]_o$ above 1.2 mM (e.g., 2.4-9.6 mM) reduced basal tone in MCA, BA and coronaries (but not renal, pulmonary or splanchnic), and in addition attenuated in a concentration-dependent manner the constrictor action of KCl, serotonin and other vasoactive neurohumoral agents. Lowering of $[Mg^{2+}]_o$ below 1.2 mM potentiated the constrictor action of these neurohumoral agents. In view of such findings, it is possible that some of the neurological derangements noted in Mg-deficient states may be a consequence of a cerebral hypomagnesemia which could result in local vasoconstriction and hypoxia. (Supported in part by the PHS Grants HL-18015 and DA-02339.)

264.14 BARORECEPTOR INPUT TO NEURONS IN THE MEDULLARY RAPHE NUCLEI OF THE CAT. C-T Yen and P.S. Blum, Department of Physiology, Thomas Jefferson University, Philadelphia, PA 19107.

Activation of baroreceptor afferents produces a reflex depression of blood pressure. Studies using electrical stimulation, neural recordings, and lesions support the hypothesis that neurons in the medullary raphe nuclei (nucleus raphe magnus, nucleus raphe pallidus, nucleus raphe obscurus; MRN) participate in this systemic baroreflex. We tested this hypothesis by examining the response properties and axonal projections of single neurons recorded within the MRN. Adult cats were anesthetized with alpha-chloralose, paralyzed with gallamine, and passively ventilated. Sinus nerve was isolated laterally and verified by a cardiac rhythm and a depressor effect following electrical stimulation. Sixty units in 13 animals were identified within the MRN that responded to electrical stimulation of the sinus nerve. The distribution of the initial spike latency for these 60 neurons ranged from 5 ms to more than 80 ms (median=25 ms). Thirty-five of these units also were tested for a response following 0.2-1.0 μg norepinephrine (i.v.). Fifteen MRN neurons had a response correlated with the pressor response induced by norepinephrine. The units responding both to sinus nerve stimulation and elevation of blood pressure were defined as baroreceptor-responsive. These units had the same distribution of latency to sinus nerve stimulation compared to other neurons but baroreceptor-responsive neurons could respond at a higher frequency of sinus nerve stimulation (median=8 Hz vs 1 Hz). In the same experiment, those units with axons projecting to the spinal cord were identified by an antidromic response to spinal cord stimulation at C5 and the quadrant of the descending axons were localized using a microstimulation technique. Four of 12 baroreceptor-responsive neurons had descending axons. Two of these axons were located in the lateral funiculus of the spinal cord (conduction velocity = 6 and 40 M/sec) and the other two were located in the ventral columns (conduction velocity = 6 and 38 M/sec). These data are consistent with the hypothesis that baroreceptor-responsive MRN neurons participate in the systemic baroreflex.

265 SYMPOSIUM

REGENERATION OF NERVE FIBERS IN THE MAMMALIAN CNS. A.J. Aguayo (Chairman; McGill Univ.), A.Bjorklund*(Univ.Lund), G.E. Schneider (MIT), S. Varon (UCLA).

Effective regeneration depends on survival of neurons, sprouting and elongation of axons and, eventually, the establishment of appropriate terminal connections. The panel will review experiments that provide new insights into conditions that influence these responses in the mammalian CNS.

A. Bjorklund: Studies using intracerebral neuronal transplants indicate that:a) embryonic CNS tissues from various parts of the neuraxis survive in the brain of adult hosts;b) axons from grafted neurons grow into the recipient while those from the host brain regenerate into the transplant; c) axons from monoaminergic and cholinergic grafted cells make highly specific connections in the hippocampal formation which mimic normal patterns of innervation; d) adrenergic growth into this formation is influenced by a neurotrophic factor released on septal deafferentation.

A.Aguayo: When nerve fibers are transected in the CNS of adult mammals there is no significant elongation of axons. However, the "substitution" of central by peripheral glia using Schwann cell containing grafts results in a marked growth (up to 3.5 cm in the rat) of axons from spinal, brain stem, basal ganglia and cortical neurons. Thus, although many mechanisms are probably involved, influences arising from glia may play an important role in the success or failure of axonal elongation after injury.

G.E. Schneider: The effects of CNS injury also differ according to age. Lesions in newborn hamsters can be followed by the growth of axons over distances greater than those observed in older animals. In addition, studies of regenerative responses in the immature CNS document compensatory changes in neuronal arborization and the functional effects of inappropriate connections.

S. Varon : Macromolecular factors controlling maintenance, growth and differentiation of nerve cells in the peripheral nervous system have been investigated in vitro. Similar techniques can now be applied to study growth requirements and regulation of central neurons while new, in vivo experimental models permit the investigation of humoral factors in regenerating nerves and possibly in the central nervous system.

266 WORKSHOP

IN VIVO ELECTROCHEMISTRY: PRINCIPLES AND APPLICATIONS. R.N. Adams (Chairman; Univ. of Kansas), R.F. Lane (Univ. of Oregon), R.M. Wightman (Univ. of Indiana), J. Justice (Emory Univ.), P. Knott (Marshall Univ.), G. Rebec (Univ. of Indiana), and P.M. Plotsky (Rhode Island Hosp., Providence).

The in vivo electrochemical methods employ very small graphite electrodes which voltammetrically monitor the concentrations of electro-oxidizable species present in brain extracellular fluid. Chronic measurements in unanesthetized, unrestrained animals are most frequently made. The endogenous compounds of major interest which can be determined are the catecholamines and their metabolites, and 5-hydroxytryptamine (5-HT) and its acid metabolite. Measurements may be carried out via potential scanning (voltammetry) or by measuring current at fixed potential (chronoamperometry), and the relative merits of each measurement mode are discussed.

In theory, each electroactive compound has a distinct oxidation potential, but in practice much overlapping occurs so that, for example, dopamine (DA) and norepinephrine (NE) cannot be distinguished in vivo by electrochemical techniques alone. Furthermore, catecholamines oxidize at about the same potential as ascorbic acid (AA) which is present in high concentration in CNS extracellular fluid. At present specificity of electrochemical response depends mainly on two experimental strategies: 1) stereotaxic placement of electrodes in brain regions where one neurotransmitter predominates, and 2) pharmacological manipulations which clearly differentiate between specific systems. Considerable progress can be expected, especially via surface modification of electrodes, in differentiating between AA and the catecholamines. However, it is unrealistic at present to suggest that direct electrochemical differentiation of DA vs. NE will be possible.

Despite the specific limitations cited above, the electrochemical technique can measure dynamic chemical changes in the CNS which are directly related to neurotransmitter functioning. Thus, the actions of various drugs, neuroleptics, etc. on catecholamines and 5-HT have been followed. Chemical changes which accompany behavioral manipulations are discussed by others in this workshop. Rather subtle sensory inputs such as vibrissae stimulation in rats result in measurable chemical changes in the brain. Simultaneous electrophysiological and electrochemical signals can be recorded and are discussed in this session. Using multibarrel pipets, Millar and coworkers have measured amounts of exogenous compounds injected iontophoretically while recording the activity of nearby neurons. Although much work is yet needed to improve the selectivity and quantitative response of the electrochemical methodology, there is good reason to believe that it can be developed into a new style of "chemical neurophysiology."

267.1 MECHANISM OF β-ENDORPHIN-REGULATED CALCIUM EVENTS IN SYNAPTOSOMES. S. Lin-Liu, W. R. Adey and E. M. Helm*. Research Service, VA Hospital, Loma Linda, CA 92357.

β-endorphin-regulated calcium events in synaptosomes were studied with a continuous perfusion technique. Synaptosomes, prepared from rat cerebri with a sucrose-Ficoll gradient and preloaded with $^{45}Ca^{2+}$, were applied to Millipore filters and perfused (1 ml/min) with a Ca^{2+}-free physiological solution at 31 C. Perfusate was collected at 1 min intervals for radioactive assay. By using the Ca^{2+}-free medium, Ca^{2+}-Ca^{2+} exchange was minimized but later activated by injecting (10 µl/min, 5-10 min) $CaCl_2$ solution with or without β-endorphin into the perfusion line. Upon injection, $^{45}Ca^{2+}$ efflux was stimulated, rose to peak in the first 3 min, then fell exponentially. This $CaCl_2$-stimulated $^{45}Ca^{2+}$ efflux was absent in mechanically ruptured synaptosomes. Also, controls injected with 0.6 M choline chloride, saline or β-endorphin alone did not show increased $^{45}Ca^{2+}$ efflux. The degree of stimulation was dependent on the concentration of $CaCl_2$ injected and reached a plateau with slow injection of 0.5 M $CaCl_2$ to a final concentration of 5 mM. The rising phase of the stimulation was probably due to activation of Ca^{2+}-Ca^{2+} exchange and the falling phase to a decrease in intracellular $^{45}Ca^{2+}$ concentration. When 500 nM β-endorphin was also included in the 5 mM $CaCl_2$, no difference in the rising phase was detected but the falling phase shifted downwards. This effect of β-endorphin can be explained by a higher Ca^{2+}-Ca^{2+} or Na^{+}-Ca^{2+} exchange rate during the falling phase. (Supported by Dept. of Energy Contract No. DE-AI01-79ET29078 and Southern California Edison Company.)

267.2 EFFECT OF OPIOID PEPTIDES AND MORPHINE ON THE RELEASE OF DOPAMINE AND SEROTONIN FOLLOWING ACTIVATION OF NICOTINIC CHOLINERGIC RECEPTOR IN RAT STRIATAL SLICES. Thomas C. Westfall and Heather Grant.* Department of Pharmacology, St. Louis University School of Medicine, St. Louis, MO 63104 U.S.A.

Activation of nicotinic cholinergic receptors in the rat striatum is known to increase the release of ^{3}H dopamine (DA) newly taken up or synthesized from tyrosine (Neuropharm. 13:693, 1974; Brain Res. 106:117, 1976). In the present studies a similar increase in the release of ^{3}H serotonin (^{3}H 5-HT) newly taken up by superfused striatal slices has also been observed. The percent stimulation induced overflow of ^{3}H 5-HT to 100, 300 and 500 µM of the nicotinic agonist, DMPP, or to 0.1 or 1.0 mM nicotine was 8.9, 13.9, 16.6, 4.0 and 8.5%, respectively. Similar concentrations of DMPP or nicotine resulted in a concentration dependent increase in the release of ^{3}H dopamine. In light of the recent report that morphine and opioid peptides might modulate the nicotine induced release of catecholamines from the adrenal medulla, we tested for such a possible interaction in the CNS. The effect of morphine, leucine enkephalin (leu-enkephalin) and methionine enkephalin (met-enkephalin) or their more stable derivatives D-ala-met-enkephalin and D-ala-leu-enkephalin on the DMPP or nicotine-induced release of ^{3}H dopamine and ^{3}H 5-HT from superfused slices was examined. Both leu-enkephalin and met-enkephalin produced a concentration dependent decrease in the release of ^{3}H 5-HT while met-enkephalin but not leu-enkephalin reduced the release of ^{3}H dopamine. Similar results were obtained with the stable derivatives of leu- and met-enkephalin. The specificity of these effects was demonstrated by the fact that neither met-enkephalin nor leu-enkephalin altered the release of these neurotransmitters when release was induced by potassium depolarization. Morphine decreased the release of both neurotransmitters but only at high concentrations (> 5 x 10^{-5}M). Due to the well-known presence of enkephalin neurons in the striatum these results suggest that one possible function of enkephalin neurons in the striatum is to modulate the release of dopamine and/or 5-HT following activation of nicotinic cholinergic receptors present on dopaminergic or serotonergic nerve terminals. (Supported in part by USPHS-NIDA 02668 and NINCDS 16215.)

267.3 BLOCKADE OF MORPHINE-INDUCED CHANGES IN STRIATAL SPIROPERIDOL BINDING BY CYCLO(LEU-GLY). J.Z. Fields, J.M. Lee* & R.F. Ritzmann, Dept. Pharmacol., Chicago Med. Sch., No. Chicago, IL. 60064 & Dept. Physiol. and Alcohol and Drug Abuse Research and Training Pgm., U. of Ill., Chicago, IL. 60612.

Neurohypophyseal hormones, their fragments and analogs appear to affect drug tolerance, physical dependence and memory. Both MIF (Pro-Leu-Gly-NH_2), the C-terminal fragment of oxytocin, and its analog, cyclo(Leu-Gly)(cLG) alter several specific biochemical and behavioral effects of chronic morphine administration. There is evidence suggesting that modulation of dopaminergic transmission is involved both in the effects of morphine and in the mechanism of action of these peptides.

To further study these drug interactions, morphine pellets (containing 75mg free base) were implanted (s.c.) 2 hrs. after an injection (s.c.) of cLG into male Swiss-Webster mice or Wistar rats. Pellets were removed 72 hrs. later, and physical dependence was assessed by the degree of hypothermia at 8 hrs. At 24 hrs. following pellet removal, animals were tested for their responses to the dopamine (DA) agonist apomorphine (APO). Measurements were made on APO-induced hypothermia, locomotion, and stereotypy. At 48 hrs. following pellet removal, (^{3}H)-spiroperidol (3H-SP) binding to DA receptors (DA-R) in homogenates of dissected striata was measured using 1uM d-(+)-butaclamol to define specific binding.

Chronic morphine treatment resulted in a statistically significant and high degree of physical dependence as measured by withdrawal hypothermia in mice (-1.3°C) and rats (-1.0°C). cLG completely inhibited the morphine-induced effect on this measure (hypothermia) of physical dependence. Simultaneously, morphine treatment resulted in increased behavioral responses to APO (in both species). The ED_{50} of APO was significantly shifted to the left in measurements of APO-induced hypothermia, locomotion and stereotypy. Paralleling the increase in stereotypy in the rat was a 50% increase in affinity (Veh Kd=20pM) of striatal DA-R for 3H-Sp binding. The number of these high affinity binding sites (26 fmol/mg tissue) did not change. Pre-treatment of the mice or rats with cLG(2mg/kg in mice and 8mg/kg in rats) prevented both the morphine-induced increases in behavioral responses to APO and the increased binding affinity of the DA-R.

These data suffest that some of the behavioral effects of morphine may be mediated by dopaminergic pathways and that the inhibition of these drug effects by neurohypophyseal peptides may be mediated by opposing neurochemical changes in these same dopaminergic systems. Supported by NIH grants (MH-33991, BSRG RR-5366).

267.4 ENDORPHIN AND CORTICOTROPIN REGULATE TRANSSYNAPTICALLY THE ACTIVITY OF SEPTO-HIPPOCAMPAL CHOLINERGIC NEURONS. L. J. Botticelli & R. J. Wurtman. M.I.T., Cambridge, MA 02139

The content of acetylcholine (ACh) in nerve terminals of the dorsal hippocampus was examined after intraventricular, intraseptal or intrahippocampal administration of a variety of endorphin/corticotropin neuropeptides. β-LPH, α-endorphin, γ-endorphin, α-MSH, β-MSH, ACTH(1-39) and ACTH(4-10) (10 ug each) did not affect ACh levels 30 min after injection into the lateral ventricle. As reported previously (Botticelli & Wurtman, Life Sci., 24: 1799 1979; Nature 289: 75, 1981) β-endorphin, administered intraventricularly (10 ug) or intraseptally (1 ug) increased hippocampal ACh levels (31% and 47%, respectively), while ACTH(1-24), injected similarly, decreased ACh levels in the hippocampus (26% and 41%, respectively). ACh concentrations remained unaffected after direct administration of β-endorphin or ACTH(1-24) (1 ug each) into the hippocampal formation.

Acute unilateral transection of the fimbria/superior fornix resulted in a time-related decrease in hippocampal ACh levels. Concentrations did not change 1 hr after transection, however levels decreased to 72% and 26% of control values 1 day and 1 wk after deafferentation, respectively. ACh levels in the contralateral hippocampus remained unaffected at all times tested. Fimbrial transection (performed 90 min before peptide administration) blocked fully both endorphin- and corticotropin-induced changes in hippocampal ACh after the neuropeptides were injected into either the lateral ventricle or septal nuclei.

Naloxone, which after subcutaneous (1 mg/kg) or intraventricular (100 ug) injection alone failed to change levels of hippocampal ACh, antagonized effects on hippocampal ACh levels produced by intraventricular or intraseptal endorphin or corticotropin.

Results suggest a site of endorphin/corticotropin-receptor interaction at the level of cholinergic cell bodies in the septal region for regulating, in part, the activity of septo-hippocampal cholinergic neurons. These observations recommend further the participation of opioid and related neuropeptides in the general synaptic organization of the mammalian limbic forebrain.

Supported in part by research grants awarded to RJW from the National Institute of Mental Health (MH 28783), the Ford Foundation and the Wallace Genetic Foundation. LJB was the recipient of National Institute on Drug Abuse Predoctoral Fellowship Award DA 05089.

267.5 EFFECT OF MORPHINE ON CHOLINE ACETYLTRASFERASE AND ACETYLCHOLINESTERASE ACTIVITIES IN SPECIFIC RAT BRAIN AREAS. J. O. Owasoyo,* K. Gipson* and C. A. Walker. Florida A&M University, School of Pharmacy, Tallahassee, FL 32307 and University of Ibadan, Veterinary Physiology and Pharmacy, Ibadan, Nigeria.

Specific drugs affecting the central nervous system have been shown to alter the acetylcholine (ACh) levels and acetylcholinesterase (AChE) activity in the rodent brain. Morphine has been found to increase the ACh level of rat whole brain following an IP dose of 16 mg/kg. In order to further elucidate this effect of morphine in the brain, we investigated simultaneously its effects on the activities of both the synthetic enzyme (ChAT) and hydrolytic enzyme (AChE) in specific rat brain areas.

Adult, male rats (200-250 g) adapted to a 12h light - 12h dark programmed illumination cycle were used in this study. Six control animals injected IP with physiological saline and six animals injected IP previously with 16 mg/kg morphine sulfate were sacrificed by decapitation at approximately 1200h. The brain from each animal was removed and quickly dissected on ice into cerebral cortex, cerebellum, hippocampus, hypothalamus, midbrain, pons, medulla oblongata and caudate nucleus and each brain part was homogenized (1% w/v) in ice-cold phosphate buffer containing 1% Triton X-100 (Sigma). AChE and ChAT activities in the homogenate were determined spectrophotmetrically as unmole substrate per gm of tissue and nanomoles COASH produced/min. respectively. The data was analyzed using the Student's T-Test. Results of this study show that, compared with control morphine sulfate significantly increased AChE activity in the cerebral cortex, hippocampus and caudate nucleus and also significantly increased ChAT activity in the cerebral cortex, hippocampus and cerebellum. No significant effect on either the AChE or ChAT activities of the other brain areas studied was observed. From our present findings, it appears that morphine sulfate selectively alters the synthesis and hydrolysis of ACh, the cholinergic neurotransmitter, in rat brain. This study was supported in part by the National Aeronautics and Space Administration (NASA).

267.6 EFFECTS OF PRENATAL EXPOSURE TO MORPHINE ON NEUROENDOCRINE FUNCTION IN DEVELOPING FEMALE RATS. J.P. Griffin*, D.M. Hux* and J. Rabii. Department of Physiology and the Bureau of Biological Research, Rutgers University, New Brunswick, N.J. 08903.

Since it has recently become apparent that endogenous opiate receptors mediate the effects of morphine and that the endogenous opiate ligands participate in the regulation of pituitary function, we have undertaken to examine the effects of prenatal exposure to morphine sulfate (MS) on the neuroendocrine function of the offspring. MS was injected (sc) between days 5 and 12 of pregnancy. Control animals received injections of normal saline. After birth, groups of rats 5, 15 and 25 days of age were sacrificed by terminal bleeding via the abdominal aorta (under light ether anesthesia) and plasma was kept frozen for luteinizing hormone (LH) and prolactin (PRL) radioimmunoassays. Plasma LH levels of the MS treated pups were not different from those of saline treated pups at 5 and 25 days of age. At day 15, however, the MS treated group had circulating LH levels that were 45% of the saline treated animals. In the case of PRL, the 15-day-old MS group had levels that were 57% of the controls, whereas the 25-day-old MS treated animals had plasma PRL levels similar to the 25-day-old controls. In the 5-day-old MS treated rats plasma PRL was significantly higher than that of the 5-day-old saline treated. We subsequently tested the feedback sensitivity of the hypothalamic-pituitary axis to estrogen in the pups born to MS injected dams. Groups of rats were ovariectomized at 15 days of age, under light ether anesthesia. One day after ovariectomy animals were either sacrificed or injected with estradiol benzoate in sesame oil (1 µg per 100 grams of body weight). Control animals were injected with sesame oil one day after ovariectomy. All injected rats were sacrificed 4 hours after injection. Oil injected rats did not show an inhibition of LH secretion in either ovariectomized pups born to MS treated dams or those born to saline treated controls. Estradiol benzoate, on the other hand, caused a marked reduction in plasma LH levels in ovariectomized control animals but not in ovariectomized MS treated rats. These results indicate a reduction in basal hormone release as well as a drop in steroid sensitivity of the animals born to MS treated dams. (Supported by NIH Grant DA0227-01.)

268.1 ELECTRICAL SIGNS OF A SHORT-LATENCY PATH FROM RETINA TO RAT HIPPOCAMPUS. E. F. Vastola. Dept. of Neurology, Washington Univ Sch. of Med., St. Louis, MO 63110.

Observations were performed in unanesthetized rats with rostral-pons transection, bi-polar 100μ electrodes with tip separation 0.5mm, conventional amplification and averaging ≤ 256 responses to photic or optic nerve stimulation. Electrolytic lesions 0.3-0.75mm were made to interrupt the pathway at various points and mark electrode position.

The response elicited in dorsal hippocampus has approximately the same latency, amplitude and time course as in striate cortex. After optic nerve stimulation the hippocampal response exhibits an early polyphasic deflection with the last peak at 10 msec followed by a larger bi-modal major deflection with peaks at 15 and 25 msec and later, slower, bi-phasic deflections at intervals of 125 msec. The polyphasic deflection except for a portion due to optic tract activity in the subjacent thalamus is maximal in the inferior part of the hippocampus but does not reverse polarity; the other deflections reverse above and below an isoelectric point in the hilum of the dentate gyrus, a distribution attributable to depolarization in the molecular layer, perhaps also cell bodies, of the dorsal and ventral leaves of that structure, the same distribution found for spontaneous activity in the 50/s range. The bi-modal major deflection is more sensitive to changes in optic nerve stimulus strength than the responses in striate cortex and more resistant to reduction in amplitude during repetitive stimulation, following frequencies up to 50/s. The pathway between retina and hippocampus can be interrupted by small lesions in the superficial tectum or posterior cingulum. There is probably a synaptic relay in the midbrain, possibly not in the cingulum. From the cingulum the pathway has fast and slow components in the lower and upper portions respectively associated with the first and second parts respectively of the bi-modal deflection. The pathway is not interrupted by lesions in fornix, septal nuclei, anterior cingulum, anterior medial thalamus or medial midbrain ventral to the superficial tectum.

These observations suggest that the hippocampus participates in visual processing at an early stage, i.e., one or two synapses beyond the eye. Visual deficits attributed to lesions in midbrain and striate cortex may in fact have been partially determined by associated involvement of the hippocampus. Visual functions of the hippocampus were probably developed at an early date in the evolution of mammalian brain. If this pathway continues to function in primates, as suggested by earlier observations (Brazier, M.A.B. Ann. NY Acad. Sci. 112:33-59, 1964), it seems a likely candidate for an important role in the phenomena of photogenic epilesy and, perhaps, the larger class of all petit mal seizure disorders.

268.2 AN HRP STUDY OF THE PROJECTION FROM VISUAL CORTICAL AREAS TO THE SUPERIOR COLLICULUS IN THE RAT. Jaime Olavarria* and Richard C. Van Sluyters (SPON: E. Marg). Group in Neurobiology and School of Optometry, University of California, Berkeley, CA 94720.

The posterior neocortex of the rat has been shown to contain at least seven visual areas, based on the results of physiological experiments revealing multiple retinotopic maps of the visual hemifield surrounding the striate cortex (Brain Res. 53:192, 197, 1973; 151:386, 1978), and anatomical studies demonstrating reciprocal connections between the striate cortex and each of these areas using degeneration, autoradiographic and horseradish peroxidase (HRP) techniques (Brain Res. 53:202, 1973; Olavarria & Montero, in press). In the present study we have traced the projection from the visual cortex to the superior colliculus (SC) to demonstrate the relationship between this projection and the physiologically defined visual cortical areas.

The SC of gray rats was stereotaxically injected with HRP (Boehringer Grade I, 0.02-0.04μl). Coronal sections (60μm) of the brains were processed for HRP histochemistry and the brains were later serially reconstructed to study the distribution of retrogradely labeled cortical cells. Labeled pyramidal cells were observed in layer V of striate cortex and in several well-defined cortical regions beyond striate cortex. The arrangement of labeled fields for striate and peristriate cortex corresponded closely to the previously reported subdivision of visual cortex into multiple visual areas. These peristriate fields have been named according to their position relative to striate cortex. Thus, area anteromedial (AM) lies medial to the anterior part of striate cortex; areas anterolateral (AL), lateromedial (LM), posterolateral (PL) and posterior (P) are arranged anteroposteriorly in the lateral peristriate cortex with area P in the occipital pole; and area laterolateral (LL) lies lateral to area LM. With topographically different injections into SC, the position of the labeled fields in the striate and peristriate areas varied in agreement with the previously reported retinotopic organization of these areas. The study of cases with superficial SC injections and of cases with additional involvement of deep SC suggests that posterior cortical afferents to superficial layers of SC come mainly from striate and peristriate visual areas, and that other cortical areas, located lateral and anterior to visual cortex and possibly related to other sensorial modalities, have a significant input to deep SC.

In conclusion, these experiments support the hypothesis that the rat's visual cortex is divided into multiple functional areas, and they suggest that the SC receives overlapping input from each of these visual areas.

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268.3 THE PROJECTION OF THE LATERAL GENICULATE BODY TO EXTRASTRIATE CORTEX IN THE OWL MONKEY AND SQUIRREL MONKEY. D. Fitzpatrick*, K. Itoh*, M. Conley* and I.T. Diamond. Dept. of Psychology, Duke University, Durham, NC 27706.

In the present study, we present evidence that certain cells of the lateral geniculate body of the monkey project outside of striate cortex. Injections of HRP and WGA-conjugated HRP were made into extrastriate cortical regions, from area 18 to the caudal portion of the temporal lobe including the middle temporal area (owl monkey) or the posterior bank of the superior temporal sulcus (squirrel monkey). In both species, labeled cells were found primarily ventral to the parvocellular layers including the interlaminar zones, the magnocellular layers and regions ventral to layer 1 (possibly including the "S" layer). The greatest number of labeled cells were found in the interlaminar zones, with fewer in the magnocellular layers and regions ventral to layer 1. Most of the labeled neurons were small in size (in owl monkey, mean = 12.6 μm), but there were differences in the cell sizes of labeled neurons depending on their location in the lateral geniculate body. For example, following injections into the temporal lobe of owl monkey, the labeled cells within the magno-cellular layers were small; on the other hand, labeled cells in the interlaminar zones were not only small but included some cells which were larger than those labeled in the magnocellular layers.

Since the zones in the lateral geniculate body which contain labeled cells following injections into extrastriate cortex are similar to the zones which project to the most superficial layers of striate cortex in Galago, it is tempting to speculate that the same cells are projecting both to the superficial layers of striate cortex and to extrastriate regions in the monkey. This pattern may be similar to the pattern of projections of the lateral geniculate body of the cat to striate and extrastriate regions: for example, small cells in the C laminae project to the entire visual field and also project to the most superficial layers of striate cortex. The present findings provide further support for the idea that similar cell types may exist in the lateral geniculate body of carnivore and primate lines, an underlying similarity which is obscured to some extent by differences in laminar organization.

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268.4 MULTIPLE MIDBRAIN INPUT ZONES WITHIN THE LATERAL PULVINAR OF MACAQUE MONKEYS WITH SPECIAL REFERENCE TO CERTAIN OF THEIR CORTICAL TARGETS. G. P. Standage and L. A. Benevento. Department of Anatomy, University of Illinois, College of Medicine, Chicago, IL. 60680.

Traditionally studies of the ascending inputs to the simian pulvinar complex have concentrated on the midbrain inputs to the inferior pulvinar. We have shown, however, that there are multiple midbrain inputs to all subdivisions of the pulvinar complex, i.e., lateral (PL), medial (PM), oral (PO) as well as inferior (PI) (e.g., Fig. 4 in JCN 160 (1975) 348; Fig. 5 in Brain Res. 127 (1977) 206). Autoradiographic (ARG) and horseradish peroxidase (HRP) tracing methods and single unit recording indicate that there are several input zones in PL, alone, which arise from the superior colliculus (SC) and, at least one from the pretectum (PT). The input zones are located chiefly at the borders of the subdivisions of the lateral pulvinar which were defined in earlier studies (e.g., Soc. Neurosci. Abst. 3 (1977) 573). Some of these zones can be described as follows: Zone 1 is located along the lateral edge of subdivisions PLβ and PLγ. Zone 2 is located in the lateral margin of PLα. Zone 3 overlaps the border between the medial pulvinar and lateral pulvinar. The PT projection zone is dorsal to and overlaps colliculus zones 1 and 3. In contrast to other reports we find that a significant portion of these inputs arise from retinorecipient regions of SC and PT. When HRP injections are made laterally in PL the superficial cell layers (upper stratum opticum and lower stratum griseum superficiale) of SC contain labelled cells. When HRP deposits are made progressively more medially in PL and PM, the deep layers of SC and retinorecipient subdivisions of PT (such as the sublentiform nucleus) contain well labelled cells. These results indicate that, in contrast to earlier notions, a significant amount of visual association cortex can be influenced by retinorecipient regions of the midbrain via pulvinar regions other than the inferior pulvinar. In contrast, focus 3 which arises from deep layers of SC is of particular interest as this region of the pulvinar complex projects ubiquitously to the portion of area 19 which forms the crowns of the dorsal and ventral preoccipital gyri, and includes visual area 4 of Zeki. Visual 4 is known for color processing but the present results would indicate that area 19, including visual 4, is involved in visuomotor functions as well. This latter position is further supported by our HRP data which show that visual 4 has extensive connections with the lower bank of the intraparietal sulcus (cf., Seltzer and Pandya, 1980). (Supported by NIH Grant EY 2940)

268.5 RECEPTIVE-FIELD PROPERTIES IN THE TECTORECIPIENT ZONE OF THE CAT'S LATERAL POSTERIOR NUCLEUS. Leo M. Chalupa, Michael J. Hughes, Robert W. Williams (SPON: R. P. Scobey). Department of Psychology. University of California, Davis CA 95616.

A major projection from the superior colliculus to the medial part of the lateral posterior nucleus (LPm) has been described in numerous anatomical studies. We have examined the visual receptive-field characteristics of single cells in the LPm of cats maintained on a 70% nitrous oxide and 30% oxygen mixture supplemented approximately every 8 hours with 20 mg/kg chloralose. In this preparation virtually all cells in LPm were responsive to visual stimulation. More than half of the neurons were binocular, about one-third responded only to stimulation of the contralateral eye, and the remainder responded to stimulation of the ipsilateral eye. A substantial proportion of the binocular cells demonstrated inter-ocular facilitation in that they responded poorly, if at all, when either eye was stimulated alone, while brisk responses were obtained with binocular activation. The size of the fields varied from 10 degrees^2 to 1000 degrees^2. Typically the cells with the largest fields were encountered in the most dorsal portion of LPm and many of these crossed into the ipsilateral hemifield by as much as 30 degrees. The centers of receptive-fields shifted from upper to lower portions of the visual field with increasing depth of penetration. Cells with the largest fields prefered large flashing spots giving transient on, on-off, or most commonly, off discharges. Neurons with smaller fields showed a preference for moving stimuli with a wide tolerance for variation in velocity. Somewhat less than half of the cells were directionally selective and these showed a preference for movement in the horizontal plane. About one fourth of the cells in LPm were orientation specific; a small number of these were also directional. The presence of orientation specific units in LPm suggests that there is intermingling of the cortical and tectal projections. Alternatively, this property may be organized intrinsically within LPm. These suggestions could be assessed by examining the effects of cryogenic blockade of the cortex or the superior colliculus on the response properties of LPm cells. (Supported by EY03491 from NEI)

268.6 A VISUAL AREA IN THE ANTERIOR ECTOSYLVIAN SULCUS OF THE CAT. C.R. Olson* and A.M. Graybiel, Dept. Psych., MIT, Cambridge, MA 02139.

We have examined the function and connections of a previously unrecognized zone of exclusive visual responsiveness in the cat's anterior ectosylvian sulcus. The ectosylvian visual area (EVA), as defined in microelectrode recording experiments, occupies approximately 20 mm^2 of sulcal cortex. Surrounded by auditory, somatosensory and nonresponsive zones, EVA is altogether isolated from the more caudal cortical district comprising all other known divisions of striate and extrastriate visual cortex.

Iontophoretic deposits of horseradish peroxidase and labeled amino acids were placed in the center of EVA under electrophysiological guidance. The resulting patterns of retrograde and anterograde labeling led to the following conclusions. (1) EVA is not directly connected to areas 17, 18 and 19. (2) EVA is related by a weak reciprocal pathway to the medial part of the Clare-Bishop complex (PMLS). (3) Strong reciprocal pathways link EVA to certain other extrastriate areas, viz. the lateral division of the Clare-Bishop complex (PLLS) and parts of the posterior suprasylvian sulcus and area 20. (4) EVA is connected reciprocally to a thalamic zone identifiable in cholinesterase-stained material as comprising both the AchE-dark medial tectorecipient part of the nucleus lateralis posterior (LPm) and the adjoining AchE-pale region which we term the nucleus lateralis medialis (LM).

Most neurons in EVA respond vigorously to visual stimulation of either eye. The most common preferred stimulus is a small spot moving rapidly in a particular direction. For a majority of cells the preferred direction contains a horizontal component pointing away from the visual-field midline. It is common for preferred direction to rotate gradually as responses are recorded at successive sites along tracks tangential to the cortical surface. The receptive fields of neurons in EVA range in area from fifty to a few hundred deg^2. They are distributed throughout the contralateral visual hemifield and may extend up to 20 deg into the ipsilateral hemifield. Although we know that receptive-field position tends to shift systematically with position in the cortex, we have not yet fully analyzed the complex pattern of retinotopy present in EVA.

The functions of the ectosylvian visual area must be far removed from those of areas 17, 18 and 19. EVA appears to be specialized for processing visual motion rather than form. Moreover, areas 17, 18 and 19 directly innervate neither EVA nor those extrastriate areas that project heavily onto it nor the thalamic region from which it receives input.

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268.7 SPATIAL PROPERTIES OF NEURONS IN THE CAT'S LATERAL SUPRASYLVIAN VISUAL CORTEX. Martin S. Gizzi, Ephraim Katz and J. Anthony Movshon. Department of Psychology, NYU, New York, NY 10003.

We have studied the selectivity of neurons in the lateral suprasylvian visual cortex (LS) for the orientation, direction of movement and spatial frequency of sine-wave gratings and checkerboards. Our recordings were mostly confined to the representation of the central visual fields in area PMLS.

Most LS cells respond as well to gratings as they do to lines or spots, although the suppressive surrounds present in some cells may require that the grating be confined to the excitatory zone of the receptive field. A comparison of direction selectivity for gratings and checkerboards reveal that LS neurons have a genuine sensitivity to the orientation of gratings, as well as their previously-reported direction selectivity. A substantial minority of LS cells have indefinite receptive fields when mapped with lines and spots; some of these give well-defined responses to gratings.

The range of orientation selectivity seen in LS is similar to that in V1 and V2, though broadly tuned cells are more common in LS. About half of LS neurons are direction selective, in that they give no excitatory response to optimally oriented gratings moving in one of the two possible directions. Another third of LS cells are directionally biased, responding more than twice as well to one direction as to the other. The remaining cells are bidirectional.

The optimum spatial frequencies of LS neurons cover a wide range, from below 0.1 to over 1 c/deg. Their bandwidths are somewhat broader than those seen in V1 and V2, ranging from about 1 octave to over 3 octaves. Thus LS cells respond both to the high spatial frequency range processed by V1 and the lower range processed by V2. Cells preferring high spatial frequencies (in the V1 range) tend to be more selective for orientation and spatial frequency than other cells.

Thus neurons in LS have spatial tuning properties generally similar to those seen in V1 and V2; there is little evidence that neural processing in LS cortex is designed to enhance the spatial selectivity elaborated earlier in the pathway. Rather, the cells' large receptive fields and prominent sensitivity to motion suggest a more general integrative function for LS.

268.8 WILL THE REAL MEYNERT CELL PLEASE STAND UP? M. Tigges, J. Tigges, and C. D. Sporborg*. Yerkes Regional Primate Res. Ctr. and Dept. of Anatomy, Emory Univ., Atlanta, GA 30322.

In 1867, Theodor Meynert described in layers 4 and 6 of his 8-layered human visual cortex "pyramids of extraordinary caliber". Because of their isolation by gaps up to 2 mm wide, he named them "Solitärzellen". With the advent of the Golgi method, Cajal, in 1899, described these solitary cells in his layer 7 with prominent features such as their extremely long ("from 0.3 mm to equal length of the apical dendrite"), thick, thorny, branching basal dendrites with exclusive horizontal orientation parallel and close to the white matter. The stout apical dendrite, after giving off side branches in layer 6, ascended unbranched to layer 1, where it ended in a tuft of horizontal branches. Recent descriptions of Meynert cells from Golgi material of area 17 of Macaca (Chan-Palay et al., J. Neurocytol., 3:631, 1974; Lund, J., J. comp. Neur., 147:455, 1973; Lund and Boothe, J. comp. Neur., 159:305, 1975) conflict with respect to soma size and distribution as well as to dendritic arborization pattern. In our Golgi material of area 17 of Macaca and Saimiri, we found at the junction between layers 5 and 6 a pyramid with a relatively large, triangular soma, usually wider than high, which conforms to Cajal's description in distribution and dendritic arborization pattern; the thick, spineous basal dendrites travelled up to 2 mm parallel to or in the white matter. At least one other type of pyramid with a large soma, but higher than wide and with much shorter basal dendrites of predominant vertical direction towards the white matter, was found regularly in layers 5 and 6 in our material. The Meynert cell is reported to project to area MT (Spatz, Brain Res., 92:450, 1975). After we injected HRP into area MT of Saimiri, numerous neurons, covering a wide range of soma sizes, including the largest ones, were labelled in layers 5 and 6 of area 17. Thus, the Meynert cell is only one type of cell projecting from area 17 to area MT. In our experiments, large HRP-filled cells were also found, among smaller labelled neurons, in the line of Gennari, probably in Meynert's layer 4. These large cells displayed no apical dendrite and, under the EM, revealed a larger number of axosomatic contacts than is the rule for pyramids, thus indicating their stellate nature. Polyak (The Vertebrate Visual System, Univ. of Chicago Press, Chicago, 1957) described a neuron of similar shape and location also as a solitary cell of Meynert. We propose to restrict this term to those relatively large and isolated cells at the junction between layers 5 and 6 of area 17 which correspond strictly to Cajal's classic description.

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268.9 RESPONSE PROPERTIES OF SINGLE NEURONS IN THE MIDDLE TEMPORAL VISUAL AREA (MT) OF ALERT MACAQUE MONKEYS. W. T. Newsome and R. H. Wurtz. Laboratory of Sensorimotor Research, National Eye Institute, NIH, Bethesda, MD 20205.

The response properties of neurons in the middle temporal visual area (MT) were studied in rhesus monkeys while they fixated a small spot of light. Of 151 cells examined, 67% were selective for the direction of motion of visual stimuli within the receptive field, 17% were biased for direction of motion, and 15% were not directional. For 72 cells for which quantitative data were obtained, indices of directionality were similar to those observed in anesthetized monkeys. Responses of MT cells were not dependent on the form of the stimulus; robust responses were generally elicited using small spots of light as well as larger slits and bars. Neuronal responses were frequently biased for a range of velocities. In 13 cells for which quantitative data were obtained, the preferred velocities ranged between 10°/sec and 150°/sec with the majority lying between 10°/sec and 40°/sec. In all these aspects, passive visual properties of MT neurons in the alert monkey are consistent with the results of previous studies in anesthetized animals.

Single cell responses were examined as monkeys made saccadic eye movements to stationary (n = 26) and moving (n = 12) spots of light within a neuron's receptive field. We have not observed any visual enhancement or discharge related to the eye movement itself when the visual stimulus was the target for a saccadic eye movement.

Neuronal responses were compared under two conditions of retinal stimulation: 1) when the stimulus was moved across the receptive field as the monkey fixated; and 2) when the monkey tracked a moving target and thereby moved the receptive field across a stationary stimulus. The large majority of MT cells responded similarly to equivalent retinal stimulation whether that stimulation was caused by moving stimuli during fixation or by stationary stimuli during smooth pursuit eye movements. However, preliminary evidence suggests that many direction sensitive neurons medial and anterior to MT respond during smooth pursuit eye movements in ways which cannot be fully explained by their passive visual properties observed during fixation.

268.10 ORGANIZATION OF DIRECTIONALLY SELECTIVE CELLS IN AREA MT OF MACAQUES. T.D. Albright*, R. Desimone* and C.G. Gross. Dept. Psychology, Princeton Univ., Princeton, NJ 08544 and Lab. Neuropsychology, NIMH, Bethesda, MD 20205

Area MT, also known as the "motion area of the STS", is located in the posterior portion of the superior temporal sulcus of macaques. MT is visuotopically organized and receives direct projections from striate cortex. Relative to other visual areas, MT has a high proportion of cells that are selective for the direction of stimulus movement but are relatively nonselective for stimulus form or color (Zeki, J. Physiol., 236:549, 1974). In this study we report on the spatial organization of directionally selective cells in MT. Single neurons were isolated every 50u along tangential, oblique or nearly normal penetrations. Projected spots or slits were moved on a tangent screen and the optimal direction and axis of stimulus movement determined for each cell. (Axis of movement is the orientation of the linear path defined by a moving stimulus and varies from 0° to 180°.)

Among 458 MT units recorded on 14 penetrations in three animals, 81% responded optimally to one direction of movement, 9% to two directions 180° apart (bidirectional) and 10% were not directionally selective. For all cells the optimal direction of stimulus movement was independent of whether the stimulus was a spot or a slit, although the maximal response was dependent upon the form of the stimulus for 40% of the cells. In long tangential or oblique penetrations through MT the prefered axis of movement of cells changed smoothly and systematically. Often the axis of movement would progress in a clockwise or counterclockwise direction for up to a few hundred microns and then reverse. By contrast, there were few long systematic progressions of optimal direction of movement, both because there were frequent 180° reversals in the optimal direction of movement and because of the presence of bidirectional cells.

The rate of change of optimal axis of movement was, on the average, greater along penetrations approximately parallel to the cortical surface than along penetrations more normal to the surface. This result suggests a vertical ("columnar") organization of axis of movement. An estimate of the column size is provided by the maximum rate of change of axis of movement, viz, approximately 180°/.5 mm of cortex.

The representation of axis of movement in MT is similar to that of orientation in striate cortex. For both, a full 180° cycle is represented in about 0.5mm of cortex. Furthermore, just as the ocular dominance system is superimposed on that for orientation in striate cortex, it is conceiveable that in MT there is some type of direction of movement system that is superimposed on that for axis of stimulus movement.

268.11 ABSENCE OF NARROWING OF THE SPECTRAL BANDWIDTH OF COLOR-SELECTIVE NEURONS FROM THE RETINA TO THE AREA V4. F.M. de Monasterio* and S.J. Schein (SPON: H. Wagner), Clinical Branch, National Eye Institute, NIH, Bethesda Md. 20205.

Because of the antagonistic signals that color-opponent cells receive from different cone mechanisms, these cells have spectral sensitivities narrower than the cone absorbance spectra. In addition, the peak sensitivities of these cells are spectrally displaced from those of the underlying cone mechanisms mediating their responses. Little is known regarding the degree of variation of spectral bandwidth at the different levels of the geniculo-striate pathway of the monkey visual system. If the spectral information were to be influenced at each successive level by color-opponent interactions, one could expect that the average spectral bandwidth of cortical cells would be narrower and their peak sensitivity spectrally more displaced than that of ganglion cells. Recent cortical work (Zeki, Nature 284:412-418, 1980) has suggested that this may be the case.

We describe the spectral response bandwidths of several types of color-opponent ganglion cells of the macaque retina, and we compare these results with published data from neurons of subsequent levels of the macaque visual pathway, including the extrastriate area termed V4. Our findings indicate that most color-opponent cells have specific "signatures" in plots of response bandwidth vs. wavelength of peak sensitivity. Such a specificity allows for an acceptable estimate of the type of cone input(s) mediating the wavelength-dependent responses of the neurons. Furthermore, the results show that there are no significant differences between the averaged spectral response bandwidth of color-opponent cells located as peripherally as retinal ganglion cells, and as centrally as V4 cells.

In association with recent results indicating that the relative concentration of color-selective cells in area V4 is not significantly different from that of antecedent cortical areas (Schein, Marrocco and de Monasterio, Neurosci. Abstr., 1980), our results indicate that current claims of a color specialization of this extrastriate area may have to be revised.

268.12 LIMITS OF VISUALLY RELATED CORTEX IN THE MONKEY'S PARIETAL AND TEMPORAL LOBES AS DELINEATED BY THE 2-DG TECHNIQUE. K. Macko, C. Kennedy, L. Sokoloff, and M. Mishkin. NIMH, Bethesda, MD 20205.

The parietal and temporal lobes of the monkey contain areas that are critical for higher cortical visual functions, but the full extent of these areas is still unknown. To delineate their borders we analyzed the brains of four rhesus monkeys, each with a combined unilateral optic tract section and forebrain commissurotomy, in which the 2-DG technique had been applied while they actively viewed visual patterns. The borders of the visually related areas were established by quantitative comparison of the metabolically inactive tissue in the "blind" hemisphere with the active tissue of the "seeing" hemisphere.

Nonvisual tissue first appears on the anterior lip of the intraparietal sulcus (ip) just rostral to the junction of the lunate sulcus and ip. As one proceeds rostrally, the visual-nonvisual (VN) border moves 1) ventromedially to the medial parieto-occipital sulcus (pom) and 2) ventrolaterally to about halfway down the anterior bank of ip. Near the posterior tip of the lateral fissure (la) the visually related tissue below these borders becomes separated into two parts, parietal and temporal, by nonvisual tissue appearing in the superior temporal gyrus.

Within the parietal lobe, the ventral VN border first appears in the fundus of la, and then moves out of la along the cortical surface to the posterior lip of ip. The dorsal VN border, which remains on the anterior bank of ip, gradually descends to the fundus. The two borders merge within ip approximately 5mm behind its anterior tip to form the rostral limit of visually related parietal cortex.

Within the temporal lobe, the dorsal VN border first appears at the fundus of la and moves quickly to the anterior lip and then halfway down the anterior bank of the superior temporal sulcus (ts). It remains at this depth through the posterior half of ts and then descends gradually to the fundus of ts. The ventral VN border, near pom posteriorly, moves gradually to the calcarine and then to the hippocampal fissure (h). It continues along the posterior half of h, moves laterally to the medial lip of the occipitotemporal sulcus, and then medially to the fundus of the rhinal sulcus. The dorsal and ventral borders merge on the ventral surface just in front of the anterior tip of ts to form the rostral limit of visually related temporal cortex.

Within visually related cortex metabolically different subareas could be delineated, as in the posterior bank of ip and the anterior bank of ts. Both VN and subareal borders were frequently sharp and consistent among animals. Preliminary analyses suggest that these borders separate architecturally different areas, lending new functional validity to architectonics.

269.1 CONNECTIONS OF THE "UNRESPONSIVE ZONE" IN THE GREY SQUIRREL (Sciurus carolinensis). H. J. Gould, III. Dept. of Anatomy, Louisiana State University Medical Center, New Orleans, LA 70119.

The sources of afferent connections to the special agranular, "unresponsive zone" (UZ) within the S I koniocortex of the grey squirrel were studied using the technique of retrograde transport of horseradish peroxidase (HRP). The borders of the UZ were determined electrophysiologically in four squirrels using the summary maps of Sur et al. ('78, J. Comp. Neurol., 179:425) as a guide. At the end of the recording session, HRP was injected through glass micropipets into the center of the UZ either by pressure or by application of an iontophoretic current. After a two day survival, the animals were perfused and the tissue was sectioned in the coronal plane.

As expected peroxidase labelled neurons were found within the contralateral anterior parietal cortex in a homotopically located agranular area. The cells were primarily medium-sized pyramidal cells and occupied layers III and V. Only a very few labelled cells were observed in the contralateral koniocortex. Ipsilaterally, peroxidase labelled neurons were found in the frontal, lateral parietal and anterior parietal areas of cortex and in a posterior portion of the ventral nuclear group of the thalamus. Cortical neurons in the frontal and lateral parietal areas were pyramidal-shaped and usually occupied layers III and V. In contrast, the interpretation of laminar distribution of labelled neurons in the anterior parietal cortex is equivocal because it was not possible to distinguish transported peroxidase from perikaryal filling within the limits of the spread of the injection. The cells, however, were found in all layers and did not extend beyond the borders of granular parietal cortex. In the thalamus, large multipolar neurons were densely filled in an area that lies just dorsal to the ventral posterior nucleus and corresponds in position to the central intralaminar nucleus (CIN) as defined in the oppossum and the hedgehog (Killackey and Ebner, '72, Brain, Behav. Evol., 6:141). The spread of the injection into adjacent granular cortex usually revealed in an additional small population of lightly labelled neurons in the medial to central portions of the ventral posterior nucleus. These results support the observations previously reported for the rat (Lin et al., '80, Soc. Neurosci. Abst. 6:62).

Although preliminary, the results suggest that a distinct group of thalamic neurons may directly affect interhemispheric connections between special agranular areas in the rodent cortex. These areas have been proposed as interhemispheric relays for adjacent acallosal regions of somesthetic cortex that contain representations of non-midline or highly lateralized portions of the body surface (Gould and Kaas, '81, J. Comp. Neurol., 196:489)

269.2 A SINGLE AND DOUBLE RETROGRADE STUDY ON THALAMIC PROJECTIONS TO SI AND SII IN CATS. R. Spreafico*, N.L. Hayes, and A. Rustioni (SPON: H. Krebs) Depts. of Anatomy and Physiology and the Neurobiology Program, The University of North Carolina at Chapel Hill, Chapel Hill, NC 27514, USA, and the Neurological Institute "C. Besta", Milan, Italy.

Single and double retrograde experiments were performed in cats to label thalamic neurons projecting to somatosensory cortical areas. In a first series of animals, HRP was injected in either SI or SII, and the distribution of labelled neurons was reconstructed in serial coronal and horizontal sections. After injections in SI limb representations, densely and lightly labelled neurons are consistently found throughout VPL and show some preferential pattern of organization. Outside VPL, moderate retrograde labelling is present in POm, even in cases in which the injection did not encroach upon area 5. After HRP injection in the anterior ectosylvian gyrus, it appears that as the injection is shifted from posterior to anterior, labelling of neurons in the thalamus shifts from the lateral portion of the posterior group (POl) and the caudal region of the medial portion of this group (POm) to involve progressively more rostral portions of this nucleus and also VP. SII-projecting neurons are confined primarily within the lateral portion (VPLl) and in posterior cap of VP, while in VPLm they are confined mainly in the periphery of this nuclear subdivision and are sparse within its core region.

In cats with injections of HRP in SI and ^{3}H-apo-HRP in SII of the same hemisphere, neurons projecting to SI, SII or to both areas are not distributed homogenously within the VP. Rather, their differential distribution define three divisions of the thalamic somatosensory relay. The region in which neurons projecting to SI are distributed among neurons projecting to SII and neurons projecting to both areas constitutes a shell of neurons within VPL. This inner shell is situated between a central core region of VPLm, which contains predominantly neurons projecting to SI, and a previously defined outer shell -- outside VPL -- which is characterized, as a whole, by widely divergent cortical connections. These three regions, distinguished from one another by their pattern of cortical projections may correspond to similar differential sites of afferent projections, such that each zone -- core, inner and outer shells -- would be dominated by a different ascending pathway.

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269.3 THALAMOCORTICAL SYNAPSES WITH CORTICOTHALAMIC NEURONS IN MOUSE SmI (POSTEROMEDIAL BARREL SUBFIELD) CORTEX. Edward L. White and Steven M. Hersch. Dept. of Anatomy, Boston U. Sch. Medicine, Boston, MA. 02118.

The application of combined anterograde-retrograde tracing techniques to the study of thalamocortical relations has shown that discrete regions of the cortex are reciprocally connected with corresponding regions of the thalamus (see White, E.L. Br. Res. Rev. 1: 275, 1979). Other evidence suggests thalamocortical relay cells are postsynaptic to corticothalamic afferents (e.g.Jones E.G. and Powell, T.P.S. Proc. Roy. Soc. B 172: 173,74) however, until now, the question of whether corticothalamic projection neurons are postsynaptic to the axon terminals of thalamocortical relay cells has been unresolved. An examination of this question has disclosed synapses between the spines of corticothalamic cells labeled by the retrograde transport of horseradish peroxidase (HRP) and the axon terminals of thalamocortical afferents identified by lesion induced degeneration. To do this, injections containing 40 % HRP were placed in the ventrobasal thalamus of young adult, male CD/1 mice. One day later, electrolytic lesions were made at the injection site, and about four days after this the animals were perfused with aldehydes. SmI cortex ipsilateral to the injection and lesion sites was tissue chopped and the sections then reacted for HRP using a modification (White et al., Neurosci. Let. 19: 149, 1980) of the diaminobenzidene-$CoCl_2$ method of Adams (1977). This procedure yields large numbers of corticothalamic projection neurons so well labeled with HRP reaction product that they resemble Golgi impregnated cells. The somata of these neurons average about 10 μm in diameter and occur in the upper half of layer VI and in the lower half of layer V; each has an apical dendrite which usually terminates within or just below layer IV, but which in some instances, extends nearly to the pial surface. Examination of long series of coronal thin sections cut through the layer III-V portions of the apical dendrites of several corticothalamic cells shows each to possess several spines which are postsynaptic to degenerating thalamocortical axon terminals. These findings provide unequivocal evidence of a direct thalamocortical projection to corticothalamic neurons and suggest a mechanism whereby the thalamus can monitor the effects of its input to the cortex with the shortest possible latency. Further, this pathway might serve as part of an excitatory feedback loop whose function is to exaggerate the differences in excitation levels between different thalamocortical circuits by lowering the threshold of excitation for select thalamocortical relay cells.

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269.4 SYNAPTIC RELATIONS OF GABAERGIC INTRINSIC NEURONS IN MONKEY SOMATIC SENSORY CORTEX. S.H.C. Hendry, C.R. Houser, E.G. Jones, and J.E. Vaughn Dept. Anatomy & Neurobiology, Washington University, School of Medicine, St. Louis, Mo. 63110 and Div. of Neurosciences, City of Hope Res. Inst., Duarte, California 91010.

Gamma-aminobutyric (GABA) is widely accepted as an inhibitory transmitter in the mammalian neocortex. GABAergic neurons of the monkey first somatic sensory cortex (SI) were identified immuno-cytochemically using antiserum to glutamic acid decarboxylase (GAD). Five cynomolgus monkeys were injected intracerebrally with colchicine 24-48 hours before perfusion with paraformaldehyde or mixed aldehydes; tissue from the pre- and postcentral gyri were processed for light or electron microscopy. GAD was localized by the indirect, PAP, method.

Labeled puncta which mostly represent GAD-positive axon terminals are concentrated in dense bands in layer IV and in the superficial part of layer I of all fields of SI. Labelled somata fall into two size ranges: small (7-12 μm) and large (15-25 μm). The smaller somata are present throughout the depth of the cortex but are concentrated in layer IV. Larger labeled somata and their dendrites are present exclusively in the deeper layers (layers III B-VI). No pyramidal cells are GAD-positive, indicating that the GABAergic cells of SI cortex are interneurons with local connections.

Somata and dendrites of GAD-positive cells receive many synaptic contacts, both with symmetric and asymmetric thickenings. The morphology of the somata and the processes of some GAD-positive neurons permits a correlation with certain forms of Golgi impregnated interneurons (Jones J. Comp. Neurol. 160: 205, 1975). Horizontally oriented, GAD-positive, myelinated axons present in the deeper cortical layers probably arise from the large GAD-positive somata which can be correlated with the basket cells. Beaded, unmyelinated GAD-positive axons which give rise to en passant terminals belong probably to one or more types of smaller GABAergic neurons.

GAD-positive synaptic terminals make only symmetric contacts. They are found in large numbers on the large GAD-positive somata and dendrites and to a lesser extent on the somata and dendrites of the small GAD-positive neurons. In addition, they synapse on the somata, dendrites and axon hillocks of pyramidal cells and on other unlabeled processes, possibly belonging to other forms of non-GABAergic neurons.

We are currently examining material from animals with thalamic lesions to determine whether the GABAergic interneurons receive degenerating thalamocortical axon terminals.

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269.5 THALAMIC CELLS OF DIFFERENT SIZES PROJECT TO DIFFERENT LAYERS OF THE SOMATIC CORTEX. G.R. Penny, K. Itoh and I.T. Diamond. Dept. of Psychology and Neurobiology Program, Duke University, Durham, NC 27706.

Parallel pathways of different cell sizes and fiber sizes have played a large part in the study of the somatic system since the pioneer work of Ranson, Erlanger, Gasser and Bishop. The dorsal column system projecting to the ventral posterior nucleus was viewed as an exclusively large fiber system, in contrast to the small fiber lateral column system. More recent evidence by Willis shows that the dorsal columns in fact contain fibers ranging in size from small unmyelinated to very large myelinated fibers. Certainly it has been clear that the ventral posterior nucleus contains a mixture of both very large and very small cells. This difference between the large and small cells could be dismissed according to the orthodox interpretation that most if not all of the small cells serve as interneurons. However, there is evidence in several species that very small cells in the lateral geniculate project to the striate cortex and to several other cortical areas and further that layer I seems to be the special target of such projections.

The present study was undertaken to determine whether the small cells in the ventral group of thalamic nuclei could be selectively labeled by HRP applications restricted to the superficial layers of the cortex. Small iontophoretic injections of HRP were placed in cortical areas 4, 3a and 3b of the cat. Labeled cells in the thalamus were drawn under a 63x or 100x objective and their cell areas were determined using an Apple computer and graphics tablet. In each of these cortical areas, injections of HRP into multiple layers labeled a wide range of cell sizes. Injections chiefly restricted to layer I labeled only small cells. Injections chiefly restricted to layers III and IV labeled medium and large neurons but few small cells. For example, after a layer I application in area 3b, labeled cells in the lateral division of the ventral posterior nucleus had a mean soma area of 119 μm^2. After an injection chiefly restricted to layer IV the mean soma area of the labeled cells was 322 μm^2. An injection involving most of the layers of the cortex in area 3b labeled a distribution of cells whose mean was 364 μm^2 and range was 100 to 1280 μm^2.

These results lead to the idea that there may be a simple principle underlying the organization of all thalamocortical projections. As a first approximation this principle concerns the difference between small cell (and presumably small fiber) systems terminating in layer I and large cell-large fiber systems that terminate in layers III and IV.

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269.6 A THALAMIC BASIS FOR THE PLACE AND MODALITY SPECIFIC COLUMNS OF MONKEY SOMATIC SENSORY CORTEX. E.G. Jones and D.P. Friedman Dept. of Anatomy and Neurobiology and McDonnell Center for the Study of Higher Brain Functions, Washington University, School of Medicine, St. Louis, Missouri 63110.

Previous anatomical studies (Jones et al. 1979 J. Comp. Neur. 183: 833.) have indicated that thalamic input to a small focus in monkey first somatic sensory area (SI) emanates from a narrow elongated "rod" of cells extending through the anteroposterior dimension of thalamic nuclei VPLc or VPM. A thalamic rod could be the basis for afferent inputs to the place and modality specific columns of the SI cortex. We have confirmed and extended this by a correlative anatomical and single unit study in cynomolgus monkeys (M. fascicularis).

Punctate injections of horseradish peroxidase ca. 1mm in diameter, made through micropipettes at points in area 3b from which neurons activated by localized natural peripheral stimuli were recorded, led to retrograde labeling of a single thalamic "rod" in the "cutaneous core" (Friedman and Jones, J. Neurophysiol. 1981 45: 59) of VPLc or VPM.

Focal injections of tritiated amino acids made through micropipettes at defined sites in the dorsal column nuclei led to anterograde labelling of lemniscal fibers in a sequence of clusters that are also aligned to form one or two narrow rods extending anteroposteriorly through VPLc.

Microelectrodes penetrating the cutaneous core of VPLc or VPM horizontaly from behind encounter long sequences of units whose modality properties and receptive field positions do not change for up to 800 µm anteroposteriorly. Microelectrodes traversing the nuclei vertically from dorsal to ventral or horizontally from anterolateral to posteromedial, encounter systematic but sudden jumps in modality properties and receptive field positions over distances of 50-100 µm.

Punctate injections of [^{3}H] amino acids made at defined sites in the cutaneous core of VPLc or VPM lead to anterograde labeling of thalamocortical terminations in one or two short strips in area 3b. Similar focal concentrations of labeled terminations can be detected in computer assisted grain counts of the uninterrupted lamina of thalamocortical labelling that ensues from large thalamic injections.

The results suggest that a place and modality specific column of the somatic sensory cortex receives its input from a narrow elongated rod of thalamic cells whose place and modality characteristics are imposed upon then by a bundled aggregation of incoming lemniscal fibers. Each thalamic rod forms a smaller division of the well known lamella organization of VPLc & VPM.

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269.7 SUBMODALITY AND ITS RELATIONSHIP TO SOMATOTOPY WITHIN AREA 3b OF THE CAT. R. W. Dykes and D. Sretavan*. (SPON: I. Bruce) Surgery, Neurology and Neurosurgery, and Physiology, McGill University, Montreal, Quebec, H3A 1A1.

The forearm portion of area 3b in cat somatosensory cortex was investigated. Experiments were performed in nembutal-anesthetized cats using tungsten electrodes to obtain multiunit recordings. Vertical penetrations were used to construct detailed maps of somatotopic and submodality organization. Tangential penetrations were then used in the same animal to examine the sequences of receptive fields encountered at 50 µm intervals across the cortical surface. Data from each experiment were analysed separately, providing the following results: (i) Regions of SA and RA submodalities were each arranged as bands varying between 0.5 and 1.5 mm wide. These bands branch and interdigitate with each other throughout this part of area 3b. (ii) In all animals, the ulnar forearm was most heavily represented. The radial aspect of the forearm, if encountered, was consistently under-represented and appeared to lie at the anterior and posterior borders of area 3b. (iii) There was a complete duplication of the forearm map within area 3b. For a given animal, if a point on the forearm was represented in one band it was also represented in the other; we were unable to detect any parts of the forearm represented in one submodality which were not also found in the other. As a result, the forearm representation in the RA and SA submodality bands is very similar. (iv) During horizontal penetrations, both in the anterior-posterior direction across area 3b and in the lateral-medial direction along area 3b, the progression of receptive fields appeared continuous when sampled at 50 µm intervals. Even when boundaries between the two submodality regions were traversed, abrupt shifts in receptive field location were not observed. We infer that there are similar maps of the forearm in both the SA and RA bands. Each of these interdigitating maps displays a smoothly changing gradient of receptive fields within itself and across the submodality boundaries. The nature of the submodality interdigitations may be determined by a requirement to maintain a continuous somatotopic gradient within area 3b.

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269.8 AN ELECTROPHYSIOLOGICAL AND ANATOMICAL INVESTIGATION OF THE ORGANIZATION OF THE SECOND SOMATOSENSORY AREA, S-II, IN OWL MONKEYS. C.G. Cusick*, J.T. Wall, D.J. Felleman, and J.H. Kaas. Psy. and Anat. Depts., Vanderbilt Univ., Nashville, TN 37240.

The goal of the present study was to describe the somatotopic organization of the second somatosensory area, S-II, using electrophysiological mapping methods and injections of anatomical tracers. Multi-unit recordings were made in 32-101 vertical penetrations in 8 owl monkeys anesthetized with ketamine HCl or urethane. In monkeys, the S-II representation lies buried in the lateral sulcus. Because the owl monkey has a smooth-surfaced brain, multiple recordings in single vertical penetrations can be used to relate the mapping data to 3 cortical levels: a superficial recording level, and 2 deep levels representing the upper and lower banks of the lateral sulcus. Receptive field locations determined at these 3 recording depths lead to the following general conclusions regarding the organization of S-II. (1) Most of S-II in owl monkeys lies under Areas 3a and 4 at the superficial recording level. Portions of the face representations in S-II lie under the Area 3b hand and lower face representation. (2) Recording sites located on the upper bank of the lateral sulcus reveal a convergence of the cutaneous representations of the head in Areas 3b, 1, and S-II. Areas 3b and 1 have a common border along the midline of the upper face. S-II appears to adjoin part of the lower face representation of Area 3b. In the region of the head top representation, S-II may have a common border with both Areas 3b and 1. (3) Both the trunk and forelimb representations usually lie near the fundus of the lateral sulcus, and the extent of their distribution on the upper and lower banks varies. (4) The forelimb and hand representation is located anterior to the trunk representation. (5) The hindlimb and foot representation is usually found posterior to the hand region on the lower bank of the lateral sulcus.

Additional observations derived from the recordings are that receptive fields in S-II are mainly contralateral and that receptive field sizes in S-II are generally larger than in Areas 3b and 1. Additional regions outside of S-II on the lower bank of the lateral sulcus respond to cutaneous or to pacinian input, and deep receptor input has been found on the upper bank. In some of the mapped cases in this study, injections of HRP were made into the hand digit, chin, and foot representations of Area 3b. The distribution of labelled neurons stained with the TMB method confirms the physiological mapping data regarding the overall somatotopic organization of S-II. The results indicate that the lateral sulcus contains representations of the contralateral body surface corresponding to Areas 3b, 1, and S-II as well as additional somatic representations. Supported by NIH Grant NS 16446.

269.9 PATTERNS OF 14C-2DEOXYGLUCOSE LABELING WITHIN THE LATERAL FISSURE OF THE MONKEY. S. Juliano, P. Hand, B. Whitsel. Dept. of Animal Biology, Sch. of Vet. Med., and Inst. of Neurol. Sci., U. of PA, Phila., PA 19104; and Dept. of Physiol., UNC, Chapel Hill, NC 27514.

The metabolic labeling pattern in the lateral fissure (LF) of fasicularis monkeys was investigated using the ^{14}C-2-deoxyglucose (2DG) technique coupled with precise somatic stimulation. Previous studies by this laboratory have shown that in monkey somatosensory cortex (SI) 2DG labeling occurs as elongated medio-laterally oriented strips. In this study, similar characteristics were observed in the LF, with some distinct differences.

Five unanestheized, paralyzed monkeys received a pulse injection of 2DG followed by a controlled somatic stimulation consisting of brush strokes at 27 cm/sec, flutter vibration at 15 hz,or joint movement of the elbow at 22°/sec. One animal received the joint movement stimulation of one arm and the flutter vibration stimulation on the index finger of the opposite hand. Otherwise, the animals were unilaterally stimulated on various parts of the forelimb ranging from the distal tip of the index finger to the proximal forearm.

Both banks of the contralateral LF and the insula were examined for 2DG labeling. In individual sections the labeling occurred as column-like patches, most often extending from lamina II-V and most densely labeled in laminae IV and IIIB, as in SI. The LF patches ranged in mean tangential width from 475 to 690 um, which is significantly wider than the patches measured in area 3b for similar stimulation. The LF patches were also more irregular in shape than those in SI.

Two-dimensional surface reconstructions demonstrated that the patches combined to form strips of label which occasionally branched and merged and were oriented along the antero-posterior axis of the fissure. When the labeling was compared to somatotopic maps of the second somatosensory area (SII) (Friedman, et al.,J.Comp.Neurol.192, 1980, and Whitsel, B., unpublished observations), an excellent correlation was found between the body region stimulated and the cortical area of representation predicted by SII somatotopic maps. Preliminary results from reconstructions of the ipsilateral LF and the bilaterally stimulated animal indicate that ipsilateral 2DG labeling patterns have features in common with the companion contralateral cortex, but of lesser extent and density. The observations of this study, combined with those of SI, indicate that the parcelling of the cortex into elongated strips may represent a basic functional unit of neocortical organization.

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269.10 KETAMINE ALTERS RESPONSE PROPERTIES OF S-I NEURONS IN MONKEY. G.H. Duncan*, D.A. Dreyer, T.M. McKenna and B.L. Whitsel. Dental Research Center and Departments of Neurobiology and Physiology, University of North Carolina, Chapel Hill, NC 27514.

The primary objective of the present study was to investigate the influence of the general anesthetic Ketamine on neural properties that are relevant to the study of S-I somatotopy. The effects of intramuscularly adminstered Ketamine on the spontaneous activity and evoked discharge of S-I neurons were studied in 10 monkeys (Macacca fasicularis). The majority of the data was obtained from 4 behaving monkeys trained to accept somatic stimuli delivered to the upper extremities. Additional studies were performed in 6 unanesthetized monkeys maintained under neuromuscular blockade. In both behaving and paralyzed animals, the response properties of cortical neurons were characterized, first while the monkey was in the unanesthetized state, then after injection of various doses of Ketamine, and finally after recovery from the effects of the anesthetic. No specific or general differences in the data derived from the two approaches could be demonstrated.

Spontaneously firing units frequently demonstrated dramatic Ketamine-induced changes in their pattern of discharge that appeared to be epileptogenic or "bursting" in character. In addition, both spontaneous and stimulus-evoked activity increased following low doses of Ketamine (less than 3 mg/kg), but typically decreased at higher doses. This drug-induced decrease in responsiveness was manifest as an elevation of the threshold of S-I neurons to mechanical stimulation of the skin, a reduction in the receptive field size, and a depression of the mean rate of discharge evoked by either punctate or surface-parallel moving stimuli. All effects on spontaneous and stimulus-evoked activity exhibited a dose and time dependency. These effects of Ketamine were observed for S-I neurons located in different cytoarchitectural areas, with different receptive field locations, and with stimuli applied to different locations within the receptive field.

The results of this study indicate that S-I is not spared the disruptive influence of even low doses of Ketamine. This general anesthetic has been found to alter significantly the size and configuration of the peripheral territories providing input to individual S-I neurons. Dosage and time course of recovery from Ketamine anesthesia are variables which influence the data from which maps of S-I topographical organization are prepared.

This work supported by grants DE 07018 and AD 356.

269.11 UNUSUAL RECEPTIVE FIELD AND RESPONSE PROPERTIES OF NEURONS IN THE UPPER LAYERS OF THE S-I CORTEX OF THE CAT. T.M. McKenna, A.R. Light, B.L. Whitsel. Dept. of Physiology and Dental Research Center, Univ. of North Carolina, Chapel Hill, NC, 27514

Extra- and intracellular recordings were obtained from S-I of unanesthetized cats with HRP or K citrate filled micropipettes. Within either lamina II or upper III a population of neurons was identified for which the preferred stimulus was a gentle brushing stimulus moved at low velocity (<1 cm/sec) over the skin. Stimuli moving faster than 10 cm/sec were inhibitory. In addition to preferring gentle, slowly moving stimuli, these neurons also exhibited decreased responsiveness to mechanical skin stimulation for protracted periods following repetitive adequate stimulation. Rapidly repeated high-velocity brushing stimuli frequently were observed to lead to complete and prolonged periods of total insensitivity to mechanical stimulation of the RF. Other unusual properties of these neurons were prominent stimulus-evoked after-discharge, and low rates of spontaneous activity which tended to increase in the absence of stimulation and to be suppressed subsequent to repetitive adequate stimulation. The RFs of some of these neurons consisted of discrete points of high sensitivity separated by insensitive zones which could not be mapped precisely. In penetrations in which such unusual neurons ("slow brush" or SB neurons) were encountered (usually 1 to 3 per radial penetration), further advance of the electrode into lower lamina III and IV (below 400 u) always sampled cutaneous neurons exhibiting RF and response properties similar to those reported in earlier studies of S-I. The latter preferred moving stimuli applied at velocities between 5-50 cm/sec, and had well-defined RF borders. To date, the three SB type S-I neurons which have been labelled intracellularly by iontophoretic injection of HRP were small pyramidal cells in either lamina II or upper III. Distinct from both the SB type neurons of the upper layers and the "typical" neurons of the middle cortical layers in S-I, was another class of upper layer neurons which possessed very large, bilateral RFs ("wide-RF" or WF neurons) that could be mapped using either gentle punctate or brushing stimuli moved across the skin at any of a wide range of velocities. To date, the one WF cell labelled intracellularly with HRP was a small pyramidal neuron in upper layer III. The data suggest that the upper and lower lamina of S-I cortical cell columns may contribute differentially to somesthesis.

(Supported by NIH grants DE 05282, DE 02668, and NS 10865)

269.12 REACTION-TIMES PROVIDE A TOOL FOR MEASURING THE PERCEIVED INTENSITY OF CUTANEOUS STIMULI IN MONKEYS. Esther P. Gardner and Janet M. Tast*, Dept. of Physiology & Biophysics, NYU School of Medicine, New York, N.Y. 10016.

To study the magnitude of tactile sensations produced by airpuffs of several intensities and spatial patterns, we trained two rhesus monkeys and five human subjects in a reaction-time (RT) task. The monkey or human subject initiated each trial by depressing a telegraph key. A 10 msec airpuff stimulus was delivered to the forearm skin at a random time 0.5-4 sec later, and the subject was required to release the key as soon as the stimulus was felt. The RT was defined as the time interval between airpuff and key release, and was measured in 121 sessions with monkeys and in 72 with humans. The number, spacing and peak force of the airpuffs were varied in different sessions.

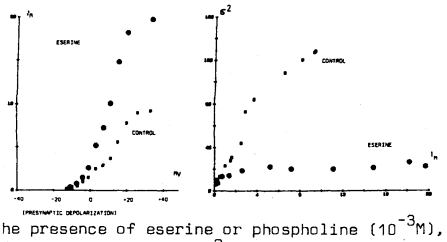
Mean RTs ranged from 228 to 287 msec in monkeys, and from 247 to 315 msec in humans, depending upon the intensity and number of airpuffs delivered to the skin. RTs were narrowly distributed around the mean, with standard deviations as small as 30 msec. Stronger airpuffs produced shorter mean RTs and increased stimulus detectability (d') over the range tested (400-1600 dyn peak force). Mean RTs could be significantly shortened ($p < 0.02$) and stimulus detectability improved by increasing the number of points stimulated from one to three. Three closely-spaced airpuffs (15 mm apart) evoked slightly shorter mean RTs and slightly larger values of d' than three widely spaced (30 mm) airpuffs in monkeys, but the differences were not statistically significant ($p > 0.05$). These changes in behavioral performance were due to increased d' rather than to alterations in the subjective criterion used to perform the task. Our findings extend previous magnitude estimation studies in humans, and demonstrate that the RT task provides a powerful tool for measuring relative magnitudes of sensory experiences in experimental animals.

Three simultaneously presented airpuffs have been shown to elicit higher numbers of impulses and to activate more neurons in somatosensory cortex of monkeys than single airpuffs of the same intensity. The present psychophysical observations suggest that this larger active cortical population is related to a parallel increase in sensation magnitude. Thus perception of a more intense and more easily detected sensation may result from increased number and degree of activity of cortical neurons.

(Supported by NIH Research Grant NS11862 and RCDA NS00142).

270.1 IS THERE A NON QUANTAL RELEASE COMPONENT AT A CHOLINERGIC NEURO-NEURONAL SYNAPSE ? M. Simonneau* & L. Tauc* (SPON : J. Bruner), Lab. Neurobiologie Cellulaire, C.N.R.S. 91190 Gif sur Yvette, France.

A leakage of acetylcholine (ACh) was revealed, at the neuromuscular junction, by ionophoretic application of curare, in the presence of an anticholinesterasic agent (antiAChE) (Katz & Miledi, 1977, Proc. Roy. Soc., Lond, 196:59-72). Determination of Ach quantal release parameters -amplitude and decay time (τ)- of miniature postsynaptic currents (MPSCs) was recently made possible in a cholinergic neuro-neuronal synapse in Aplysia (Simonneau & al., 1980, Proc. Natl. Acad. Sci., 77:1661-1665). Postsynaptic current fluctuations obtained by depolarization of the presynaptic neuron in the presence of tetrodotoxin were analyzed as the summation of individual MPSCs. The relationship between the variance (σ^2) and the mean (I_m) of the current was not linearly related for larger values of I_m. One possible explanation is that a voltage-dependent non quantal component contributed to I_m.



In the presence of eserine or phospholine (10^{-3}M), this non-linearity was enhanced ; σ^2 was lower than control and I_m was increased. This depression could be due to (i) a curare-like effect of antiAChE and/or (ii) a decrease in the response quantal content. This was tested by comparing the spike-induced response with ACh ionophoretically induced response. Iontophoretic injection of Carbachol (up to 40 nA) in the neuropile mimicked the effects of antiAChE on σ^2 and I_m. The quantal content was decreased but the size and τ of MPSCs did not change. These results show the presence of a non quantal component in the clamped postsynaptic current and its kinetics, especially in the presence of antiAChE suggest a voltage-dependance. The present results do not exclude the possibility that this component may be built up from ACh of the released quanta.

270.2 DIRECT EVIDENCE THAT Ca^{++} ACCUMULATION MEDIATES POST-TETANIC POTENTIATION IN APLYSIA. Robert Kretz*, Eli Shapiro, Eric R. Kandel (SPON: J. Koester). Center for Neurobiol. & Behavior, P & S, Columbia University, New York, N.Y. 10032.

We have examined the presynaptic ionic mechanisms underlying post-tetanic potentiation (PTP) in Aplysia by carrying out voltage-clamp experiments on the presynaptic neuron L10, while assaying transmitter release by recording the synaptic potential in its follower cells in the abdominal ganglion. 50 msec depolarizing command steps in high divalent cation seawater solution containing 30 µm TTX elicit stable PSPs in follower cells when presented at 0.2 Hz. Tetanic stimulation of L10 (100 command pulses at 4 Hz) elicits PTP of 200-800% that lasts up to 5 min. PTP is correlated with a slow outward current that we identified as a Ca^{++}-dependent K^+ current (Kretz, Shapiro and Kandel, 1980). This current provided an assay of changes in the free Ca^{++} level of the neuron (Gorman and Hermann, 1979) and its correlation with PTP provided indirect evidence for a residual Ca^{++} hypothesis of PTP (Rahamimoff, 1968).

To obtain direct evidence that intracellular Ca^{++} accumulation produced by tetanic stimulation is causally related to PTP we injected EGTA, a Ca^{++}-buffering agent, into L10 and examined its effect on the size and time course of both the PTP and the Ca^{++}-dependent K^+ current. Prior to the injection of EGTA, two control runs of PTP separated by 10 min were performed. Approximately 2.0 nL of 0.5 M EGTA in KOH at pH 7.5 was then pressure injected into cell L10 and PTP runs were followed over the next several hrs. As previously reported EGTA injections decreased the transient and steady-state outward currents, and increased the inward Ca^{++} current (Connor, 1979; Brehm, Eckert and Tillotson, 1980). In addition, EGTA affected the amplitude of the PSP and the time constant of PTP. Beginning 5 minutes after injection of EGTA control PSPs were progressively reduced so that 1-2 hrs after the injection pretetanic stimulation elicit only small or no PSPs. During and following the tetanus build-up of PSPs and PTP still occurred but the time constants of PTP were now changed. Normally, both PTP and the slow outward current have two matching time constants -- a fast one (τ=5-10 s) and a slow one (τ=50-150 s). Within 10 min after EGTA injections neither the outward current nor PTP show a fast time constant. Both the PSP and the outward current can now be fitted with single slow exponential functions. With further time after the injection this slow time constant decreases progressively.

These results indicate that increasing presynaptic capability for buffering Ca^{++} can alter the time course of PTP and directly support the idea that PTP involves the residual Ca^{++} that accumulates following a tetanus. This Ca^{++} accumulation results from saturation of the normal Ca^{++}-buffering capacity by the Ca^{++} influx accompanying the tetanus.

270.3 SENSITIZATION IN APLYSIA: SEROTONIN ELICITS A DECREASE IN SENSORY NEURON K^+ CURRENT NOT RELATED TO I_K EARLY OR $I_K Ca^{++}$. J.S. Camardo*, M. Klein*, and E. R. Kandel (SPON: L. Cote). Center for Neurobiology and Behavior, Depts. of Physiology and Psychiatry, Columbia University, P & S, New York, N.Y. 10032.

Sensitization of the gill-withdrawal reflex in Aplysia californica is mediated by presynaptic facilitation of transmitter release from sensory neurons which innervate the mantle. Increased transmitter release is correlated with an increase in the duration of the action potential, which results in increased Ca^{++} influx. Both the broadening of the spike and the enhancement of release can be elicited by connective stimulation, by application of serotonin (5HT), and with intracellular injection of cyclic AMP or cAMP-dependent protein kinase. Voltage clamp analysis has shown that the increase in duration of the action potential produced by 5HT is due to a decrease in outward K^+ current elicited by depolarizing voltage steps.

Three K^+ currents have been described in molluscan and other neurons, and we have found each of these currents in Aplysia sensory neurons: 1) Ca^{++}-dependent I_K; 2) Early I_K; 3) Delayed I_K. To determine which, if any, of these currents is decreased in sensitization, we studied the effect of 5HT on these currents in voltage-clamped sensory cells, and have found that 5HT exerts its action on a K^+ current which is neither the early I_K nor the Ca^{++}-dependent I_K.

The effect of 5HT on early I_K was studied in cells voltage-clamped at a hyperpolarized potential. Depolarizing voltage steps elicit the early I_K and slower outward currents. 5HT reduced the slower outward currents, but did not affect the early I_K, which subsequently was blocked by 4-aminopyridine.

The Ca^{++}-dependent K^+ current was studied in voltage clamped cells bathed in solutions in which the Ca^{++} current was blocked. The effect of 5HT persists in the presence of extracellular cobalt and nickel, and in low Ca^{++} solutions with EGTA. In addition, the 5HT response is not blocked by substitution of barium for calcium to reduce $I_K Ca^{++}$. Finally, intracellular injection of Ca^{++} elicits an outward current that is unaffected by 5HT.

Since reduction of the early I_K and the $I_K Ca^{++}$ does not account for the 5HT response, the biophysical phenomena which accompany sensitization and presynaptic facilitation in Aplysia seem to involve an action on delayed rectification or on a novel, serotonin-sensitive, K^+ current.

270.4 PROTEIN INHIBITOR OF THE CYCLIC AMP-DEPENDENT PROTEIN KINASE CAN BLOCK THE ONSET OF, AS WELL AS REVERSE THE ELECTROPHYSIOLOGICAL CORRELATES OF SENSITIZATION OF THE GILL-WITHDRAWAL IN APLYSIA. V. F. Castellucci, J. H. Schwartz, E. R. Kandel, A. Nairn*, and P. Greengard. Center for Neurobiology and Behavior, Columbia University, P & S, and Dept. Pharmacology, Yale University.

Sensitization of the gill-withdrawal reflex is due to presynaptic facilitation at the excitatory synapses made by sensory neurons on gill motor neurons. The facilitation is accompanied by an increase in the duration of the action potential in sensory cells; this increase is greatly enhanced in the presence of tetraethylammonium (TEA), resulting in an increase of Ca^{++} influx and a greater release of transmitter from sensory neurons (Klein and Kandel, 1978, 1980). There is evidence that serotonin is the facilitating transmitter and that the depression of the K^+ current produced by serotonin is mediated by cyclic AMP dependent protein phosphorylation (Brunelli et al.,1976; Paris et al., 1980). Pressure injection of the catalytic subunit of the cyclic AMP-dependent protein kinase into cell bodies of individual sensory neurons causes spike-broadening and increases in the release of transmitter and thereby mimics facilitation (Castellucci et al., 1980).

To test further the role of the cAMP-dependent protein kinase and of protein phosphorylation in sensitization we have attempted to prevent or reverse the development of electrophysiological correlates that accompany sensitization. In the presence of TEA we have pressure injected individual sensory cell bodies with the Walsh inhibitor, a specific and a stable protein inhibitor of the cAMP-dependent protein kinase while serotonin (10^{-6}M) was applied in the bathing solution to produce spike broadening. Pairs of cells were run in parallel (a non-injected cell and a cell to be injected with the protein inhibitor). The dramatic increase in spike broadening that accompanies facilitation was prevented or diminished by injection of the inhibitor. Moreover, injection of the inhibitor could reverse fully the developed spike broadening produced by a prior application of serotonin. No effect was obtained by injecting the vehicle alone. These findings strengthen the evidence for the involvement of protein phosphorylation in presynaptic facilitation, and suggest that the rate of decline of the memory does not reside in the persistence of a phosphorylated state in the absence of kinase activity but in a decline of the kinase itself. This decline is perhaps determined by the slow decay in the elevated level of cAMP produced by brief activation with serotonin (Cedar and Schwartz, 1972).

270.5 POSSIBLE INVOLVEMENT OF ACTIN IN THE RELEASE OF TRANSMITTER BY AN IDENTIFIED MOLLUSCAN SYNAPSE. E. Shapiro, D. A. Harris, and J. H. Schwartz. Ctr. for Neurobiol. and Behav., College of Physicians and Surgeons, Columbia U., New York, N.Y. 10032

Cell L10 is a cholinergic interneuron presynaptic to several follower cells in the Aplysia abdominal ganglion. This interneuron has been utilized as a model system for studying synaptic physiology and presynaptic ionic mechanisms of synaptic plasticity. To test the role of actin in transmitter release we have injected DNAse I, a protein that binds actin specifically and stoichiometrically and which causes depolymerization of actin filaments, into L10. DNAse has previously been shown to inhibit fast axonal transport of serotonergic vesicles when injected intracellularly in another Aplysia neuron (Goldberg, D. J., Harris, D. A., Lubit, B. W. and Schwartz, J. H., Proc. Natl. Acad. Sci. USA 77:7448-7452, 1980). Our standard experimental protocol involved recording from L10 and one of its follower cells in the isolated abdominal ganglion. L10 was stimulated at a constant frequency (1-5 Hz in different preparations) either continuously or as 2 min trains with 5-10 min intervals of rest. After recording stable baseline PSP's for up to 1 hr a concentrated solution of DNAse (40 ng/nl in 5 mM Na acetate, pH 5.0, with an activity of 0.08-0.11 Kunitz units/nl) was pressure-injected into the soma of cell L10. In successful injections, 1.5 - 2.5 nl of DNAse was injected, and the injection did not cause significant changes in input resistance, membrane potential or spike amplitude. In seven such experiments, the PSP's were reduced up to 50% following the injection. Inhibition occurred within 10 min and persisted for as long as the cell was monitored (up to 40 min after the injection). Preliminary examination of how the DNAse effect varies with stimulus frequency indicates that blockage of transmitter release is greater at lower stimulation frequencies, or with 5-10 min intervals of rest between trains of stimuli. Control injections with acetate buffer or with bovine serum albumin did not inhibit the PSP's. Preparations of the pancreatic nuclease may be contaminated with proteolytic enzymes. Proteolysis is unlikely to cause the inhibition in synaptic transmission observed, however, because injection of DNAse treated with the protease inhibitor phenylmethylsulfonyl fluoride, was equally effective. We are also testing other actin-binding proteins to strengthen the evidence for the involvement of actin in transmitter release, including brevin, a protein purified from rabbit serum which shortens actin filaments.

270.6 PROPERTIES OF TRANSMITTER RELEASE IN THE SQUID GIANT SYNAPSE VIA VOLTAGE CLAMP RECONSTRUCTED PRESYNAPTIC ACTION POTENTIALS. R. Llinás, M. Sugimori* and S. M. Simon*. Dept. of Physiol. & Biophys., New York Univ. Med. Ctr., New York 10016.

Previous studies of the transmission properties in squid giant synapse via presynaptic voltage clamping techniques allowed us to propose a model for transmission at that junction (Llinás et al.: Biophys. J. 33, 289-322, 1981). The model was derived from Ca^{++} currents obtained by presynaptic square voltage clamp steps after blockage of g_{Na} and g_K. From this model the time course and amplitude of presynaptic Ca^{++} current and the postsynaptic potentials generated by action potential-like depolarizations of different amplitudes and duration were numerically obtained. In order to test the validity of the model directly, potentials resembling presynaptic spikes were generated across the presynaptic terminal by feeding the command amplifier of the voltage clamp system with the presynaptic action potential recorded from the same synapse prior to the pharmacological blockage of Na^+ and K^+ conductances with TTX and TEA + 3-AmP respectively. Reconstruction of the presynaptic action potential via the voltage clamp was demonstrated to generate a potential profile almost indistinguishable from the original action potential at a recording site in the middle of the presynaptic terminal This artificial spike produced synaptic potentials of similar amplitude and latency as those obtained prior to spike blockage. The results indicated that the blockage of Na^+ and K^+ conductances did not affect in any measurable way the properties of synaptic transmission at this junction. Following a set of results obtained at different amplitudes and duration for the presynaptic spike-like voltage step, a similar set of depolarizations was repeated following the addition to the bathing fluid of 500 microMole CdCl, known to block presynaptic Ca^{++} current and synaptic transmission. Subtraction of the currents obtained before and after Cd^{++} administration gave us a direct measurement of the time course and amplitude for the Ca^{++} currents during these action potentials and their relationships to the amplitude of the postsynaptic responses. These results matched quite closely those obtained by the numerical solution of our equations confirming the validity of the model. Supported by USPHS grant NS13742 from NINCDS.

270.7 BURSTING, CA SPIKES, AND SPIKE BROADENING RECORDED INTRACELLULARLY FROM CRAB NEUROSECRETORY TERMINALS. I. Cooke and M. Nagano*. Békésy Lab. of Neurobiol., Univ. of Hawaii, Honolulu, HI 96822.

Peptide neurosecretory cells of mammals and molluscs characteristically fire impulse bursts. Bursting, both spontaneous and in response to brief electrical stimuli, is sometimes observed during intracellular recording from somata, axons, or terminals of the isolated X-organ-sinus gland neurosecretory system of crab (Cardisoma carnifex, Podopthalmus vigil) eyestalks. Most frequently observed are bursts lasting 10-20 sec with intraburst firing frequencies of 2-5/sec, recurring more or less regularly at 1-6/min. The impulses are superimposed on a depolarized plateau having an amplitude of up to 30 mV from resting potential (- 80 - - 60 mV). Occasionally, the plateau occurs without superimposed impulses. Such bursting was once recorded continuously from a terminal for 8 hr. Extracellular recording from the axon tract indicates that impulses originate near the soma. A brief (1 msec) shock applied to the axon tract illicits a burst in some units not exhibiting spontaneous bursting. If the impulses of a burst recorded in a terminal are viewed superimposed, their overshoot remains constant, but their duration progressively increases from 10 msec (at 1/2 amp.) to as much as 30 msec. By contrast, impulses recorded in an axon are of short duration (2-5 msec) and do not show spike broadening. Some, but not all, terminals of units not exhibiting bursting show spike broadening when tested by repetitive axonal stimulation. Axon impulses are reversibly blocked by perfusion of Na-free or TTX saline. Terminal impulses, however, can be elicited in these salines by depolarizing current passed through the recording electrode. The response in Na-free or TTX saline is reversibly blocked by addition of 0.1 mM Cd. Thus terminal, but not axonal, action potentials include a major component of Ca current. In the absence of evidence, morphological or physiological, for synaptic interaction between neurons in this isolated system, we infer that bursting is an endogenous property of some of the neuronal population. Bursting is a labile property, easily impaired by electrode-induced injury which leaves other responses unaffected. Thus, we cannot estimate how much of the population is capable of bursting. Light and EM studies show terminals of 6 morphological types, and at least 4 peptide hormones have been characterized. Hence, heterogeneity of electrical properties may be expected. In view of the demonstrated importance of Ca to peptide secretion in this and other systems, bursting, augmented voltage-dependent Ca channels in terminal membrane, and spike broadening may represent specializations for secretion.

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270.8 THE EFFECTS OF HYPERTONICITY AND ANION BLOCKERS ON QUANTAL TRANSMITTER RELEASE. W.D. Niles, Department of Physiology, University of Wisconsin, Madison, WI 53706

The effects of hypertonicity and the anion flux blockers Probenecid, DIDS, and pyridoxal phosphate were examined on quantal transmitter release at single excitatory synaptic sites on the crayfish leg opener muscle. Focal extracellular recording methods were used to measure the quantal content (m) and synaptic latencies of responses to nerve stimulation of 2Hz at 1.0-4.0°C.

Hypertonic solutions ranging from 537-1298 mosM/l made by adding either melezitose or NaCl to the normal Van Harreveld's solution of 430 mosM/l increased resting quantal release rates from .013 to .098/sec to .031 to .98/sec. Mean quantal contents were reduced from 0.1-2.5/impulse to 0.02 to 1.2/impulse, depending on the osmolarity. The mean synaptic latency increased with hypertonicity with no significant change in axon conduction velocity. After an impulse, the probability of any quantal release decreases from its peak value with time. The time constant of this decline (t) showed osmolarity-dependent increases; at one site t increased from 3.5 to 5.6 upon a change from 430 to 860 mosM even with a simultaneous increase in bath temperature from 1.0 to 4.0°C.

Probenecid and DIDS at > 200 μM blocked action potential initiation. These drugs in the range of 10-1000 μM and Pyridoxal phosphate in the 0.1-1.0 mM range reduced m with half-maximal inhibiting doses of 20 μM (DIDS), 100 μM (Probenecid) and 0.5 mM (Pyridoxal phosphate). No effects of these agents on the resting release rate or on the synaptic delays were detected

The facilitatory agent 5-HT at 10 μM increased the resting release rate by about 100-fold and increased m by a factor of 2-6 with a decrease in synaptic latencies. Hypertonic solutions reduced m and increased t at sites previously treated with 5-HT. However, 5-HT in 860 mosM/l saline elevated m without affecting t at sites previously equilibrated with 860 mosM/l saline.

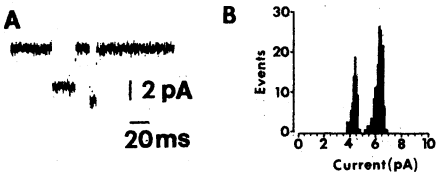
It is concluded that hypertonic solutions affect the time course of quantal release by action potentials. The ability of 5-HT to potentiate release at previously depressed synapses indicates that reduction in nerve terminal volume does not underlie this effect. Similarly, the inability of anion channel blockers to alter t suggests that impulse transmission failure alone does not account for these results. This dissimilarity of hypertonicity and drug effects suggests that they do not act on a common mechanism to reduce quantal release.

This work was supported by an NIH training grant to the Neurosciences Training Program, NIH grant NS13600, and the Alfred P. Sloan Foundation (to D.O. Smith).

270.9 ACh RECEPTOR CHANNEL POPULATIONS IN CULTURED XENOPUS MYOCYTE MEMBRANES ARE NON-HOMOGENOUS. R.B. Clark and P.R. Adams, Department of Physiology and Biophysics, University of Texas Medical Branch, Galveston, TX 77550.

The "infinite seal" patch clamp technique of Neher and Sakmann was used to measure single ACh receptor channel currents from embryonic Xenopus myocytes after 2 to 7 days in culture. Recordings from all cells always showed two different amplitudes of single channel events (e.g. Fig A), but the relative number of "small" and "large" currents varied considerably from one cell to another. Histograms of channel amplitudes showed two clearly separated peaks (Fig B). The ratio of mean "large" current amplitude to mean "small" current was relatively constant from one cell to another (1.39 ± 0.02, mean ± S.E., 7 cells) and was not significantly dependent on membrane potential. The current-voltage relation was approximately linear over the range +50 to -60 mV with respect to resting potential and the conductance of the channels from the slope of I-V plots was 42 ± 4 pS for the "large" and 33 ± 5 pS for the "small" channels (n = 5).

Channel open time had an approximately single exponential distribution at sites where one type of channel event predominated. At 5 sites where more than 90% of events were "small" channels, the mean open time was 3-8 ms (-50 to -100 mV from rest, 1μM ACh). At sites where the numbers of both types of channel events were comparable, the mean open time of "small" channels was 2-3 times greater than the mean open time of "large" channels recorded from the same patch at the same membrane potential. The differences in conductance and open time between these two channel populations appear analogous to the differences between junctional and extrajunctional ACh receptor channels of adult amphibian muscle membrane.



Supported by NIH grant NS-14920. R.B.C. is an M.D.A. Postdoctoral Fellow.

270.10 CHOLINE SENSITIVITY OF POSTSYNAPTIC RECEPTORS IN APLYSIA BUCCAL GANGLIA. R.L. Ruff*, R.L. White* and D. Gardner. Depts. of Physiology and Neurology, Cornell U. Med. Coll., NYC, NY 10021.

Choline has been identified as a partial cholinergic agonist in both classical (Dale, 1914) and recent (Krnjevic & Reinhardt, 1979) literature. Acetylcholine (ACh) is a neurotransmitter in several molluscan ganglia, responsible for many synaptic actions. We have investigated the effects and role of choline on identified neurons of the buccal ganglia of the mollusc Aplysia californica. We now report that several cholinoceptive neurons of the buccal ganglia respond to choline, raising the possibility that choline itself may normally mediate some synaptic conductance changes.

Choline and ACh were iontophoresed or pressure injected onto voltage-clamped neurons, and the resulting currents analyzed. Different groups of identified neurons produced consistent responses to each of the two agonists: 1) All conventional inhibitory follower cells (BL or BR3,6,8-11) responded to both ACh and choline (n=37), although some cellular regions responded only to ACh. The peak amplitude of the choline response was 10 to 100% of the peak of the ACh response (167 trials, 25 cells). Reversal potentials were similar: E_{ACh} = -78 ± 2 mV; $E_{Choline}$ = -77 ± 2 mV (SEM, n=12). The choline responses were reversibly blocked by 10^{-4} g/ml curare (n=4). Eserine potentiated the ACh response without affecting the choline response (n=11). 2) Excitatory follower cells (BL or BR13) or self-inhibitory interneurons (BL or BR4,5) responded to ACh but not to choline (156 trials, 23 cells). 3) In follower cells BL or BR7, cholinergic PSPs are diphasic, with excitatory and inhibitory components. With these cells clamped at -20 mV, postsynaptic currents (PSC) also were diphasic, with early inward and late outward components. The late component reversed at -67 ± 4 mV (n=7). Eserine (10^{-4} g/ml) selectively abolished the late component. With agonist iontophoresis, ACh always produced either diphasic responses or the early response alone, while choline produced only the late response (39 trials, 7 cells); $E_{Choline}$ = -68 ± 4 mV (n=7).

We suggest: 1) Buccal ganglia neurons are choline sensitive. 2) The existence of choline-insensitive ACh receptors implies that choline sensitivity is a non-trivial property. 3) Choline produced by hydrolysis of released ACh may be partially responsible for some postsynaptic conductance changes.

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270.11 DEGRADATION OF ACETYLCHOLINE RECEPTORS IN INNERVATED MYOTOMES OF XENOPUS LAEVIS MAINTAINED IN ORGAN CULTURE. P.M. Frair* and M.W. Cohen (SPON: R. Capek). Department of Physiology, McGill University, Montreal, Quebec.

Myotomal muscles of Xenopus embryos and tadpoles were isolated together with the spinal cord (SC) and were maintained in culture under sterile conditions for periods of about 1 day. The culture system permitted continued development of embryonic SC-myotome preparations. For example, at the end of the 1 day period in culture, preparations isolated from 1-day-old embryos exhibited increased localization of acetylcholine receptors (AChRs) at presumptive sites of innervation and the total number of AChRs in the preparations had increased to 80% of the value in intact animals. Synaptic activity was also recorded from the muscle cells at the end of the culture period.

Degradation of AChRs in these SC-myotome preparations was assessed by measuring the radioactivity remaining in the tissue at different times after pulse labelling with ^{125}I-α-bungarotoxin and extensive washing. In SC-myotome preparations obtained from stage 40-44 tadpoles (3-4 days old) the radioactivity remaining in the tissue declined slowly with time and was still 80-85% of the original value 24 hr after pulse labelling. This corresponds to an apparent half-life of about 3-4 days for the AChRs. Similar measurements were made on SC-myotome preparations obtained from stage 26-27 embryos (29-31 hr old), less than 12 hr after the onset of innervation. In these young preparations 72-77% of the radioactivity was still present in the tissue 24 hr after exposure to the iodinated toxin, corresponding to an apparent half-life of about 2-3 days for the AChRs. These findings suggest that the half-life of AChRs in innervated Xenopus myotomes increases with maturation. Radioautographs of the preparations are being processed in order to determine whether junctional and extrajunctional AChRs are degraded at different rates. (Supported by the Medical Research Council of Canada).

270.12 GATING AND BLOCKING OF ENDPLATE CHANNELS BY TRIMETHYLAMMONIUM DERIVATIVES. A. Auerbach*, M. Titmus* and J. del Castillo. Lab. Neurobiol., UPR Sch. Med., San Juan, P.R. 00901

The binding of ACh and other nicotinic agonists to endplate receptors causes the transient opening of ion channels across the muscle membrane. Channels opened by different agonists close at different rates. We have determined channel closing rates for two groups of trimethylammonium (TMA) derivatives. Synaptic regions of frog sartorius muscle fibers were voltage clamped. The agonists were applied electrophoretically or added to the bath. Channel closing rates were determined either from the decay times of membrane current relaxations in reponse to voltage jumps, or from spectral analyses of current noise. The first group of agonists include methyl (Me), ethyl (Et), pentyl (Pe), benzyl (Bz) and allyl (Al) TMA. MeTMA and EtTMA open channels which close at 3-4 times the rate of ACh channels. These compounds do not appear to act as channel blockers; conductance and voltage dependence of their channels are similar to those of ACh, and current relaxations were single exponentials with decay rates independent of the mean increase in current. PeTMA, BzTMA and AlTMA appear to both open and block channels; current relaxations show a rapid outward component followed by a slow inward one, while noise analyses yield conductance and voltage dependence estimates significantly lower than for ACh. The second group of agonists include carbachol (CCh), acetylthiocholine (AtCh), 4-ketopentylTMA (4kPeTMA), and cholinethiol (Chth). In agreement with previous results we find that CCh and AtCh, as MeTMA and EtTMA, open channels which close 3-4 times faster than those of ACh without acting as channel blockers. 4kPeTMA opens channels which close at similar rates as those of ACh but also can act as a channel blocker. The apparent channel closing rate for Chth is similar to that of MeTMA but in voltage jump experiments there is an additional slow outward component which reverses slowly if at all. These results suggest that both the ester group of ACh and the ketone group of4kPeTMA slow the channel closing rate (relative to MeTMA). In addition, the ester group prevents ACh from blocking endplate channels. Thus, the ester moeity plays an important role in determining the action of the natural transmitter. (USPHS NS-07464,NS-14938, & MDA)

270.13 MODULATION OF TRANSMITTER RELEASE BY ISOLATED RETZIUS CELLS IN CULTURE. J.G. Nicholls, P.A. Fuchs and L.P. Henderson. Dept. of Neurobiology, Stanford Univ. Sch. of Med., Stanford, CA 94305.

Chemical transmission develops between individual Retzius cells and P sensory neurons dissected from the CNS of the leech and maintained in culture. These connexions have been observed as early as 3 days, although the majority of the recordings were obtained from cell pairs which were 10 to 21 days old. Impulses in the Retzius cell evoke hyperpolarizing potentials in the P cell that can be reversed by hyperpolarization or intracellular injection of Cl. The synaptic potentials are blocked by raised levels of Mg and enhanced by high Ca in the culture medium. Both facilitation and depression occur depending on the frequency of firing. In addition, steady depolarization without impulse production in the Retzius cell can evoke synaptic potentials in the P cell. As at other synapses studied in the leech, Aplysia, squid and elsewhere, the synaptic potentials are markedly influenced by a) alterations in the amplitude of the presynaptic impulse and b) the steady value of the membrane potential from which the action potential arises: thus, hyperpolarization of the Retzius cell from -50 to -70 mV, produces a three-fold reduction in the size of the synaptic potential evoked by an action potential.

Preparations consisting of two identified cells in culture, with their somas in close contact, offer certain advantages for analyzing in detail membrane properties and release characteristics of presynaptic terminals.

271.1 LOCAL AND DISTANT DENERVATION AND REINNERVATION INDUCED CHANGES IN ACHE ACTIVITY OF FAST AND SLOW MUSCLES. K. E. Misulis* and W-D. Dettbarn. Dept. of Pharmacology and Jerry Lewis Neuromuscular Ctr., Vanderbilt Univ., Nashville, TN 37212.

With denervation and subsequent reinnervation of skeletal muscle there are changes in the activities of enzymes associated with neuromuscular transmission. The best studied changes are the loss of AChE activity in muscles of rat after denervation. While in reinnervated extensor digitorum longus (EDL) recovery of AChE exhibited a slow rise to control levels over 5 weeks, AChE recovery in the slow soleus (SOL) was characterized by a rapidly developing overshoot to over 200% of control levels during the initial 3 weeks of reinnervation, with a decay to normal in over 5 weeks. Since these changes were observed following crush of the sciatic nerve at the mid-thigh level, reinnervation of the slow SOL by "fast" nerve fibers may have been responsible for the recovery discrepancy. Fast muscles such as the EDL, as well as its nerve, have higher AChE activity than the slow SOL and the nerve fibers innervating it.

To examine this possibility we have denervated the SOL and EDL muscles of the rat 2 mm before the entrance of the innervating nerves into the muscles. This allowed for no reinnervation by nerve fibers destined for other muscles. The results of these experiments showed the same patterns of AChE loss and recovery observed for the EDL and SOL with high sciatic crush. That is, in the soleus there was the same overshoot of AChE activity to over 200% of control.

Since the rat SOL has a small population of fast fibers (32%) the overshoot could have been due to the effects of initial reinnervation by fast motor axons and sprouting. Therefore experiments were repeated using guinea pigs, which have exclusively slow fibers in SOL. In these experiments, as well, there was a similar overshoot of AChE activity in SOL but not in EDL.

The results of these experiments suggest that during the early phase of reinnervation the SOL reverts to a fast muscle type, and only with full functional activity develops the biochemical characteristics of a mature SOL. This is supported histologically by a reversible increase in the number of type II fibers high in ATPase activity during denervation and beginning reinnervation in the SOL.

CHANGE IN ACHE WITH DENERVATION AND REINNERVATION

AChE as % of control	1	2	3	4	5 weeks
Soleus	41	46	264	173	112
EDL	45	47	48	68	88

This work was supported in part by grants from NINCDS, NIEHS and MDAA.

271.2 CONTRACTILE PROPERTIES OF OVERLOADED RAT FAST MUSCLE. R.R. Roy*, I.D. Meadows*, K.M. Baldwin*, and V.R. Edgerton (SPON: R. Zernicke). Dept. of Kinesiology, UCLA, L.A. CA 90024.

Chronic overload of a fast skeletal muscle by removing its synergists produces hypertrophy and marked changes in its metabolic and biochemical properties. The purpose of this study was to determine if these morphologic and biochemical alterations were associated with changes in its contractile properties. After bilateral removal of the soleus and gastrocnemius, adult female rats were maintained post-surgically for 12-14 weeks. In situ isometric and isotonic contractile properties of overloaded plantaris (OP), normal plantaris (NP), and normal soleus (NS) muscles were tested at 33 ± 1°C. OP muscles were 97% heavier than the NP muscles and produced 43% and 46% higher twitch (P_t) and tetanic (P_o) tensions, respectively. However, NP muscles produced more tension per cross sectional area than the OP muscles (26.2 ± 1.37 vs 1.27 ± N/cm², P 0.001). Isometric twitch time-to-peak tension (TPT) and half-relaxation time (½RT) were significantly longer in the OP muscles (36.4 ± .79 vs 32.5 ± .60 ms and 23.9 ± .83 vs 18.4 ± .84 msec respectively). Mean V_{max} (mm sec^{-1}/1000 sarcomeres) was 34.1 for the NP and 18.1 for the OP (P< 0.001, see Fig. 1). The degree of conversion toward a normal slow soleus of the maximum velocity of shortening (V_{max}) was 74% compared to only 19 and 14% for TPT and ½RT. In addition, OP muscles produced a higher proportion of Po at a given stimulation frequency than the NP muscles and showed less fatigue than NP after 1, 2, 3 and 4 min of repetitive stimulation. These data demonstrate that although the maximum tension of OP muscle can be markedly enchanced it may not be in proportion to the increase in muscle mass.

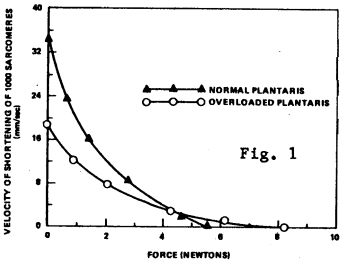

Fig. 1

271.3 DIRECT STIMULATION ACCELERATES SYNAPSE ELIMINATION IN NEONATAL RAT SOLEUS MUSCLE. W.J. Thompson, Department of Zoology, University of Texas, Austin, TX 78712.

Neonatal skeletal muscle fibers of the rat undergo a transient period of polyneuronal innervation before the adult state of single innervation is established at ca. 2 weeks of age. A number of reports have implicated use or disuse of the muscle and its nerve as an important determinant of the rate at which synapses are lost during this postnatal period (eg. O'Brien, et al., J. Physiol. 282:571). I have investigated the effects of chronic stimulation of the soleus muscle of neonatal rats.

Seven day old pups were taken from their mothers, placed in an incubator, and reared using an artifical diet delivered continuously through an intraoral cannula (Hall, Science 190:1313). Teflon insulated stainless steel wires were drawn underneath the skin and the uninsulated tips implanted into the muscles anterior and posterior to the right soleus. To avoid causing pain during stimulation, the spinal cords were transected at T11. Neither spinal transection nor artifical rearing produced alterations in the normal rate of synapse loss.

Stimuli were 1 msec, 4-6 mA pulses sufficient to produce ankle extension. When stimuli were presented in trains (1 sec duration, 100 Hz, 1 train/100 sec), within 2-3 days the stimulated muscle showed a reduction in ACh sensitivity, a markedly decreased twitch/tetanus ratio, an increased tetanic fusion frequency, and a decreased contraction time, all consistent with effective stimulation of the muscle. In addition, 50% of the muscle fibers were found to be singly innervated, a level of polyneuronal innervation found in animals 2-3 more days advanced in age. None of these changes were observed in the contralateral, unstimulated muscles. Almost all of the fibers in these control muscles were polyneuronally innervated. However, when stimuli were presented continuously at 1/sec (same # of stimuli as above), these changes were not observed in the stimulated muscles. Therefore, stimulation of the soleus muscle and its nerve accelerates synapse elimination and this acceleration shows a dependence on stimulation pattern similar to that for reduction of ACh sensitivity in denervated adult muscle (Lømo & Westgaard, J. Physiol. 252: 603).

(Supported by a grant-in-aid from the Muscular Dystrophy Association)

271.4 QUANTITATIVE HISTOCHEMICAL CHANGES IN MOUSE CORTICAL BARRELS AFTER WHISKER CLIPPING AND WHISKER REGROWTH. W.D. Dietrich, D. Durham, O.H. Lowry, and T.A. Woolsey. Depts. Pharm., and Anat. and Neurobiol., Washington U. Sch. Med., St. Louis, MO 63110.

The large whiskers on the face of mice project to contralateral barrels in a one-to-one fashion. Last year at these meetings we demonstrated that enzyme activities could be determined quantitatively in individual barrels with the use of microchemical techniques. We also showed that, by selectively damaging whisker follicles, the levels of enzymes in corresponding barrels could be significantly altered and could be followed as a function of time after whisker damage (Dietrich et al., '81, J. Neuroscience). For instance, 115 days after whisker damage, citrate synthase and malate dehydrogenase activity decreased to 65% and 70% of controls respectively, while glycogen phosphorylase increased to 132% of control. We interpret these changes as a metabolic plasticity in response to reduced activity in this central pathway after damage to peripheral afferents.

The present study was undertaken to see if similar enzymatic changes could be observed after removal of whisker hairs without damage to the neural sensory periphery. Further, we were interested in whether enzymatic changes, if any, could be reversed by reversing the deprivation, i.e. letting the whisker hairs grow back. Results are taken from a number of adult and newborn mice; and in each case, the whiskers of one side of the face were clipped every other day for 60 days with the use of a dissecting microscope. At this point, some animals were sacrificed, and in others the whiskers were allowed to grow back. Individual barrels were analyzed in hemispheres opposite clipped whiskers, with samples in opposite hemispheres serving as controls. In adult animals, sensory deprivation resulted in changes in enzyme levels of citrate synthase, malate dehydrogenase and glycogen phosphorylase, which when expressed as % of controls, were 67%, 75% and 133%, respectively. These changes were similar to those observed following peripheral sensory damage, indicating that sensory deprivation is sufficient to produce a metabolic plasticity. Animals in which whiskers were allowed to grow back had identical values for the enzyme citrate synthase in control and experimental barrels. The time course of the restoration of enzymatic levels is apparently faster than the changes observed during the deprivation.

Supported by NIH grants NS05227, NS07057, NS10244, and NS15070.

271.5 EFFECTS OF CHRONIC PARTIAL SENSORY DEPRIVATION: A (^{14}C)-DEOXY-GLUCOSE STUDY OF THE VIBRISSA-BARREL SYSTEM IN THE ADULT RAT. P.J. Hand and C.L. Hand* Department of Animal Biology, School of Veterinary Medicine and Institute of Neurological Sciences, University of Pennsylvania, Philadelphia, PA 19104.

Stimulation of mystacial vibrissa #3 of row C (C3) coupled with a (^{14}C)-deoxyglucose (2DG) injection produced candle-pin shaped metabolic labeling (vibrissal column C3) in layers I-VI of the contralateral first somatosensory barrel field (SI) of the adult rat (Hand et al., Neurosci. Abst.,'78). In subsequent adult studies, effects of vibrissae denervation (C3 whisker spared) on the "spared" C3 column was examined (Kossut et al., Neurosci. Abst.,'79). Though the labeled "spared" C3 column was similar in appearance to that of the control side, it was enlarged by 20% and local cerebral metabolic rates of glucose (LCMRG) reduced by 7% as measured in lamina IV. Lower layer V, however, exhibited an elevated LCMRG (18%). In addition, the activated region surrounding the C3 column was more extensive, particularly within layers I-IV.

In the present investigation the contribution of sensory disuse to the previously observed metabolic changes in the C3 vibrissal column following contralateral vibrissa follicle denervation was examined. All vibrissae excepting C3 were regularly clipped unilaterally for 3 months in 6 adult rats. The experiment consisted of an intravenous pulse injection of 50 uCi of 2DG followed by 45 minutes of precisely controlled rostro-caudal brush stroking (3-4 hz) of C3 vibrissa bilaterally (C3 stimulation on the unclipped side served as a control). LCMRG in layers I-VI were determined quantitatively. The spared C3 column was 27-51% larger (average 34%) than the control C3 column. The enlargement primarily involved neighboring barrels of row C and produced a less sharply delineated columnar image than that of the control side. Thionin stained sections revealed no enlargement of the C3 barrel itself. LCMRG in all six laminae were 11% less than those of the control C3 column. 2DG labeling in barrel rows B and D, immediately surrounding the spared C3 column, was 12% less than on the normal side. In conclusion, chronic, partial somatosensory disuse has produced alterations of 2DG labeling in a spared C3 vibrissal-barrel column in contralateral SI of the adult rat. The compensatory metabolic alterations produced by partial sensory disuse, though differing in detail, resemble functional changes resulting from partial vibrissa denervations in adult rats. Thus neocortical effects of denervations in this adult sensory system are primarily due to sensory disuse and not disruption of a trophic effect of receptor integrity as with neonatal vibrissa follicle ablations (Kossut et al., '79). (Supported by USPHS grant NS-14935)

271.6 SENSORY DEPRIVATION AFFECTS MITRAL CELL SYNAPSES BUT NOT SIZE OR NUMBER. T.E. Benson and J.W. Hinds. Department of Anatomy, Boston University School of Medicine, Boston, MA 02118.

Unilateral neonatal naris cauterization results in a functional deprivation of the ipsilateral olfactory bulb (OB) and a smaller volume of its laminae (excepting the olfactory nerve layer and ventricular/subependymal zone) as compared with the non-deprived (ND) OB (Benson & Hinds '80 Neurosci. Abstr., 6:637). Meisami ('78 Prog. Brain Res. 48:225) has reported a 26% reduction in the number of mitral cells (MC) due to unilateral deprivation in 25 day old rats. For 30 day old CD-1 mice we have determined the number of MC's, their nuclear and soma size, the number of nucleoli per nucleus, and lateral olfactory tract cross sectional areas from deprived (D) and ND OB's in coronal 1 μm plastic sections. For soma areas electron micrographs were used as well.

We have found no differences between the D and ND sides in the number of MC's (37,606 D, 38,355 ND; n=4 mice; P=0.5824), MC nuclear radius (5.28 μm D, 5.26 μm ND; n=4; P=0.8311) MC soma cross sectional area (193 μm^2 D, 198 μm^2 ND; n=6; P=0.6160), the number of nucleoli per nucleus (1.05 D, 0.98 ND; n=4; P=0.1953), or lateral olfactory tract cross sectional area (0.152 mm^2 D, 0.163 mm^2 ND; n=6; P=0.2652). Our MC totals derive from raw counts of all MC nuclei which were then corrected by the Floderus equation. We have used plastic sections because in our experience the identification of small MC's in thick paraffin sections was unreliable. We identified MC's by cytological criteria such as a pale nucleus and patterns of Nissl substance and not by "the special miter shape" which Meisami required for counting. These different procedures may explain the different results.

In contrast to these results, our electron microscopic, stereological, blind analysis of MC associated soma synapses has shown a markedly lower number per surface area (N_S) for both MC to granule cell (GC) and GC to MC synapses on the D side. For MC-GC synapses N_S=0.0812 μm^{-2} D, 0.1255 μm^{-2} ND (n=4; P=0.0005). For GC-MC synapses N_S=0.049 μm^{-2} D, 0.104 μm^{-2} ND (P=0.0034). Since soma area was the same for the D and ND side, there must be fewer synapses per MC soma on the D side.

A chi-square analysis of the MC-GC/GC-MC ratios showed that the D side was higher, as would be expected at an earlier developmental stage (Hinds & Hinds '76 J.C.N. 169:15).

Thus the functional deprivation seems to affect MC and GC synapses but not MC soma size or the number of these prenatally generated cells. One could hypothesize, in light of no difference in the lateral olfactory tract size, that soma size chiefly reflects axon development, which may not be affected by the deprivation. (Supported by USPHS Research grant AG-00001.)

271.7 **EFFECTS OF DEAFFERENTATION ON SPINOCERVICAL TRACT NEURONS IN THE CAT.** **A.G. Brown*, P.B. Brown, R.E.W. Fyffe* and Lillian M. Pubols. Dept. of Vet. Physiol. Univ. of Edinburgh, Edinburgh EH9 1QH, U.K.**

In 9 cats the L_6 and L_7 dorsal roots of one side were sectioned extradurally central to the dorsal root ganglia, under pentobarbitone anesthesia with strict aseptic precautions. Between 27 and 75 days later, under chloralose anesthesia and paralysis with gallamine triethiodide, spinocervical tract (SCT) neurons were recorded, extracellularly and intracellularly. Neurons were injected with horseradish peroxidase (HRP) for subsequent histological examination.

The results were unequivocal and similar for each cat irrespective of the survival time following deafferentation. A variety of responses to cutaneous stimulation were observed in deafferented segments. 1. In 17 cells recorded intracellularly, 5 had no receptive fields, 4 responded with either e.p.s.p's or impulses to brusque tapping, 4 responded with e.p.s.p's to hair movement or sustained pressure to part or all of their receptive field and 4 responded with apparently normal impulse discharges to hair movement. 2. In 20 cells recorded extracellularly, 6 had no receptive fields, 4 responded only to tapping and 10 had apparently normal responses.

There were no alterations in the normal somatotopy of the dorsal horn. Of 15 SCT neurons injected with HRP, 11 had appropriately located fields; the remaining 4 cells had no responses. There were no obvious morphological differences between the 15 stained SCT cells and those in normal cats.

It is concluded that after deafferentation SCT neurons lose part or all of their afferent input and that within 75 days of dorsal root section do not show any signs of new or inappropriate connexions.

Supported by the M.R.C.

P.B.B. and L.M.P. were U.S.P.H.S. Senior International Fellows.

271.8 EFFECTS OF NEONATAL MONOCULAR ENUCLEATION ON PROJECTIONS FROM THE SUPERIOR COLLICULUS TO THE LATERAL POSTERIOR NUCLEUS IN THE RAT. Richard T. Robertson and Scott M. Thompson*. Department of Anatomy, College of Medicine, University of California, Irvine, CA 92717.

The effects of neonatal monocular enucleation on secondary visual projections were studied using the retrograde transport of horseradish peroxidase (HRP). Hooded rat pups were monocularly enucleated 1-2 days after birth. After reaching adult size, the enucleated animals and normal controls received unilateral or symmetrical bilateral injections of HRP in the lateral posterior nucleus (LP) of the thalamus. Following 2 day survival times, frozen sections were processed for HRP histochemistry, using benzidene dihydrochloride as the chromagen. Only injections that were confined to LP were considered.

All injections resulted in retrograde labelling of cells in the pretectum, superficial layers of the superior colliculus, and striate and peristriate cortices. The numbers of labelled cells were compared between each of three experimental groups: Injections ipsilateral to the enucleation, contralateral to the enucleation, and in normal control rats. Although the total number of labelled neurons resulting from a given injection varied with the size of the injection, the relative proportions of labelled cells within each of the afferent regions (pretectum/tectum/cortex) remained generally constant for different cases within each experimental group.

The major finding was that the number of labelled cells in the superior colliculus (measured as the ratio of tectal cells to cortical cells) resulting from injectionsin LP contralateral to the enucleated eye (i.e., the partially denervated tectum) was significantly smaller (Mann-Whitney U = 6; $p < 0.01$) than the number of labelled tectal cells following injections in LP ipsilateral to the enucleated eye and was significantly smaller than the number of labelled tectal cells in normal controls (U = 4; $p < 0.01$). In contrast, the number of retrogradely labelled neurons in the pretectum (measured as the ratio of pretectal cells to cortical cells) showed no significant differences between the three experimental groups.

These results suggest a dramatic reduction in the development or maintenance of tectothalamic neurons following early eye removal. In addition, partial denervation appears to exert a differential effect on pretectal and tectal projection neurons.

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271.9 LATE SEGREGATION OF GENICULATE AFFERENTS TO THE CAT'S VISUAL CORTEX AFTER RECOVERY FROM BINOCULAR IMPULSE BLOCKADE. M.P. Stryker. Physiology, UCSF, San Francisco, CA 94143.

In the developing visual cortex of monkey (Hubel et al, Rakic, PRSB 278, 1977) and cat (LeVay et al, JCN 179, 1978), ocular dominance columns appear to form by a progressive segregation within layer IV of geniculate afferents serving the left and right eyes. In kittens younger than 2 weeks of age, physiological and several types of anatomical evidence suggest that afferents serving the two eyes make intermingled connections (LeVay &Stryker, Soc Neurosci Symp 4, 1979). By 6-8 weeks of age the segregation process appears from similar evidence to be complete or nearly so.

Last year at this meeting, I reported that blockade of all impulse discharge in the two optic nerves (by repeated intravitreal injections of tetrodotoxin) between 2 and 8 weeks of age prevents the segregation process. In such animals autoradiographic studies disclosed no periodic variation in labelling density within layer IV. Following brief recovery from the blockade, microelectrode recordings revealed nearly all cells within layer IV to be driven well by both eyes, suggesting that intermingled left and right eye afferents had maintained intermingled functional connections. In contrast to these effects of binocular impulse blockade, dark-rearing, binocular lid-suture, systemic infusion of TTX, or repeated intravitreal injections of vehicle solution all allowed the segregation process to occur.

The present study asked two questions. (1) At what time during normal development is the arrangement of the geniculocortical afferents no longer plastic? (2) If binocular impulse blockade is maintained up to this time, will the geniculocortical afferents then be able to segregate to form ocular dominance columns?

The first question was addressed by studying the effects of several months of monocular deprivation begun at progressively later ages. Deprivation beginning at or after 8 weeks of age produced no obvious effects on the cortical labelling pattern following injection of one eye, suggesting that the geniculocortical afferents were no longer plastic. To answer the second question, 5 cats were raised with binocular impulse blockade between 2 and approximately 8 weeks of age and were then allowed binocular visual experience for an additional 8-12 weeks. After this recovery period, microelectrode recordings revealed that many cortical neurons were monocularly driven, and autoradiography in every case showed apparently normal ocular dominance columns.

(Supported by EY-02874 and a Sloan Foundation Fellowship)

271.10 REORGANIZATION OF HAND REPRESENTATION WITHIN AREA 3b FOLLOWING DIGIT AMPUTATION IN OWL MONKEY. A. Schoppmann, R.J. Nelson, M.P. Stryker, M. Cynader, J. Zook, M.M. Merzenich. Coleman Lab and Physiology Dept, UCSF, San Francisco, CA 94143.

Earlier studies have revealed that after a zone of the Area 3b hand representation is deprived of its normal input by section of the median nerve, this zone is progressively and topographically reoccupied by other inputs. Immediately after nerve section, scattered responses from the dorsum of digits 1 and 2 are detectable in territory formerly responding only to the glabrous, now-denervated skin. After several weeks, this territory displays a complete and strictly topographic map of the digits' dorsum.

This study asked two questions: (1) Does reorganization of the cortical map occur when the rapidly-unmasked input from the dorsum is eliminated? (2) Does movement of a representation into a deprived zone of cortex require overlap of terminals?

Both questions were addressed by studying the reorganization resulting from amputation of digits. Amputation eliminates the easily unmasked input from the dorsum, and the cortical representations of the digits are, for the most part, discrete (that is, receptive fields of cortical neurons usually do not extend over the glabrous surfaces of adjacent digits except at the base). Digits 2 or 3 or both were amputated and the four digital nerve stumps ligated. Closely-spaced microelectrode maps (200-400 penetrations) of the Area 3b hand representation were made 2-5 months after, and in some cases before, amputation.

Adjacent glabrous digital and palmar surfaces completely occupied the former cortical representation of a single amputated digit. With amputation of two digits, occupation of the deprived zone of cortex was similar but usually incomplete. After reorganization, the deprived zone represented primarily the tips of adjacent, intact digits, and receptive fields were were commonly smaller than normal. Area 3b then contained a single orderly map of the remaining hand, suggesting that the representations of portions of the intact digits moved out of their normal territory into the deprived zone. Over much of the deprived zone, however, neurons were neither so strongly nor so unanimously driven as in normal cortex.

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271.11 EFFECTS OF MONAURAL OCCLUSION ON CELL SIZES OF BRAINSTEM AUDITORY NEURONS: AN EVALUATION OF CRITICAL PERIOD OF DEVELOPMENT. Albert S. Feng and Barbara A. Rogowski, Dept. of Physiol. & Biophys., Univ. of IL, Urbana, IL 61801.

We have earlier examined the normal developmental pattern (Rogowski and Feng, 1981) and effects of aural occlusion on morphological development (Feng and Rogowski, 1980) of bipolar medial superior olivary (MSO) neurons in the rat. Those results indicate that left-right dendritic dominance of this bipolar neuron population was altered following early monaural occlusion, but not binaural occlusion in favor of dendrites receiving normal inputs. The effect was thought to be a result of selective increase of dendritic resorption since dendrites and soma size reached the peaks of development early in life (PND 8-14) when the occlusion was implemented. In the current study, the effects of aural occlusion on soma sizes of bipolar MSO neurons and, large spherical cells in the anterior ventral cochlear nucleus (AVCN) consisting of the primary source of ascending afferents to MSO neurons, at various stages of development were investigated.

The experimental animals, comprising several groups of rats with more than 10 animals per group, were monaurally (M) occluded at PND's 12, 30 and 60 with silicone rubber cement and sacrificed 48 days later. Additionally, another group of PND 12 animals were raised with binaural (B) occlusion. Cell area and diameter of brainstem auditory neurons were drawn from Nissl stained frozen sections and processed by a computer. It was found that in almost all PND 12 M animals tested, the large spherical cells in the AVCN of the occluded side had significantly smaller cell areas than their counterparts on the opposite side as shown also by others (Webster and Webster, 1979; Coleman and O'Connor, 1979). On the other hand there was no significant difference in the average cell areas or diameters of these cells on the two sides of PND 12 B animals. In other monaurally occluded groups, significant difference was found in all but one PND 30 animals, whereas for PND 60 animals all but two showed no significant difference between the means of cell areas of these AVCN cells on the two sides. Data from MSO were less clear cut and will be discussed subsequently. This research is supported by a N.I.H. grant NS-14488 and a Biomedical Research Support grant awarded to the University of Illinois.

271.12 PROGRESSIVE INCREASES IN ENVIRONMENTAL COMPLEXITY PRODUCE MONOTONIC CHANGES IN BRAIN AND BEHAVIOR. Michael J. Renner, Mark R. Rosenzweig, Edward L. Bennett and Marie Alberti*. Dept. Psych. and Melvin Calvin Lab., UC-Berkeley, Berkeley, CA 94720.

Considerable previous research has shown that rearing in different environmental conditions alters cerebral and behavioral measures. Little has been done, however, to examine directly the extent to which a set of environments of ordered complexity will produce similarly ordered brain and behavioral measures.

Male Berkeley S_1 rats (n=40) were placed at weaning into one of four conditions: "Superenriched" (SEC), three large cages (ea. 75 X 75 cm.) connected by tunnels, containing toys and a maze, changed daily, through which the rats had to run for access to food and water; Enriched, one large cage with a variable complement of toys; Grouped rats, housed together in a large empty cage; and Impoverished, wherein rats were housed individually in small cages. After maintainance in these conditions for 69 days, rats were pretrained and tested over 20 days on the Hebb-Williams problems and then sacrificed. The SEC rats were tested to insure equal treatment of groups, but their behavioral data were excluded from the analyses due to their prior maze experience.

Total cortical weight correlated positively with ordinal rearing complexity ($r=+0.55$; $p<0.05$ for all reported correlations), as did the weight of the ventral cortex, including hippocampus and amygdala ($r=+0.45$), and the dorsal cortex ($r=+0.44$). Neither somesthetic cortex nor the combined cerebellum and medulla were significantly different in weight between the rearing conditions. The numbers of repeated errors on the Hebb-Williams problems were negatively related to complexity of the rearing environment ($r=-0.46$), as were running times ($r=-0.42$).

Relationships between brain and behavioral measures were larger than had previously been reported. The weights of occipital cortex samples, while not significantly different between the rearing conditions, correlated significantly with repeated errors ($r=-0.43$). Dorsal cortex sample weight was also correlated with repeated errors ($r=-0.37$). Somesthetic cortex weights, which generally show the least response to environmental differences, were not significantly correlated with any behavioral measure.

A multiple linear regression equation, calculated using non-invasive measures (body weight, ordinal complexity of the rearing environment, and repeated errors), accounted for 37.2% of the variability in total weight of the cortex in these rats, giving moderate predictive power. These results indicate that even simple linear modeling of the influence of the environment on brain measures can have heuristic value. Replication and expansion of this study are in progress.

272.1 THE AUDITORY BRAINSTEM RESPONSE (ABR) IN SEVERAL VERTEBRATE CLASSES: A PHYSIOLOGICAL FACET OF BRAIN EVOLUTION.
Jeffrey T. Corwin, Theodore H. Bullock, and Jeff Schweitzer*. Dept. Neurosci., Sch. of Med., and Neurobiol. Unit, Scripps Inst. Oceanogr., U.C.S.D., La Jolla, CA 92093.

ABR's are short latency microvolt potentials evoked by acoustic stimuli and recordable with remote electrodes and averaging. ABR's have been recorded in several mammalian species and are in wide clinical use. Yet virtually nothing has been known concerning far-field acoustic potentials in nonmammals.

Here we report on far-field ABR's recorded in eight species representing five of the six extant classes of nonmammalian vertebrates. ABR's were averaged from wire electrodes contacting cerebrospinal fluid near the medulla and telencephalon in rays, gars, surfperches, leopard frogs, clawed toads, painted turtles, ring doves, and adelie penguins. Like mammalian ABR's these appear at short latency as a series of sharp peaks followed by slow waves. This suggests that a substantial fraction of acoustic neurons remain well synchronized at brainstem levels in a range of species from fish to man. ABR's are similar for individuals within a species, but appear to differ between some classes. The bird's response most closely resembles the mammalian ABR, with at least five peaks in 10 ms. The elasmobranch's least resembles the mammal's, with only two peaks in 20 ms.

ABR's from a given species appear to have certain all or none features independent of the stimulus over ranges of intensity, frequency, rise time, and repetition rate. However, latency changes with these parameters, and both form and latency change when the brain is heated or cooled.

In correspondence with mammalian studies our manipulations of recording geometry, comparisons with microelectrode recordings, and transections of the brain indicate that the early peaks originate in the periphery, the middle peaks in the hindbrain, and the latest initial peaks in the midbrain or higher. Therefore, the different number of peaks in ABR's suggest physiological differences in ascending pathway complexity. These differences, together with the uniformity of ABR's reported for diverse mammals (Ridgway et al. P.N.A.S. in press), suggest that ABR's may reflect the broad characteristics of taxa higher than orders and not the specializations of individual species.

ABR's have been used here to evaluate auditory thresholds, dynamic ranges, frequency tuning, and phase sensitivity. In some cases we have recorded with surface electrodes; in several using chronic electrodes in awake animals. The technique is relatively simple and appears particularly promising for other neuroethological studies.

(Supported by NSF and NIH grants to T. H. Bullock.)

272.2 THE MAUTHNER CELL IS NOT THE ONLY NEURON THAT CAN INITIATE C-TYPE FAST-STARTS IN ADULT GOLDFISH.
R. C. Eaton, W. A. Lavender* & C. M. Wieland*. Dept. Biol., E.P.O., Univ. Colorado, Boulder, 80309

Goldfish Mauthner (M-) cells are a pair of large, identified reticulospinal neurons found at the level of the ear in the hindbrain. M-cells receive a major afferent input from the auditory system. Each M-axon crosses the midline and synapses on motoneurons innervating the contralateral trunk musculature.

Recent evidence shows a strict, one-to-one, correlation between firing of the M-cell and the onset of C-type fast-start, or "startle", responses following a sudden and unexpected stimulus.

Is the M-cell the only neuron that can initiate this behavior? To study this question, we electrolytically lesioned M-cells in adult goldfish by passing 7 μA for 20 s from a metal microelectrode penetrating the M-axon initial segment. For two animals, the lesioning electrode was chronically implanted at the site of the lesion and was used for recording to show that the M-cell did not recover. C-type fast-starts were elicited by dropping a 74-gm ball into an aquarium holding the fish. Responses were filmed at 500 frames/s.

The damaged M-cell did not fire during any behavioral responses. Responses initiated on the side contralateral to the lesioned M-cell (non-M responses) were not different in mechanical performance from those initiated contralateral to the intact cell. But, there was a significant increase in behavioral response latency associated with the non-M responses. The difference in means was 7 ms.

In two other animals, the M-cell was allowed to degenerate for one month after the lesion. A chronic electrode was then placed next to the intact M-cell, and M-initiated and non-M responses were compared. Again, no difference in performance was detected, and response latency for the non-M responses was significantly longer than for the M-initiated responses.

Responses in a fifth animal with both M-cells lesioned were also no different in mechanical performance from putative M-initiated responses seen in a control fish with a single M-cell lesion.

We conclude that the M-spike is causally related to the onset of C-type fast-starts but that the M-cell is not necessary for this behavior in goldfish. Because non-M C-starts were seen within a few hours after the M-cell lesion, we conclude that the non-M responses were not triggered by new neural circuits arising from loss of the M-cell. Such responses might be triggered by other reticulospinal neurons.

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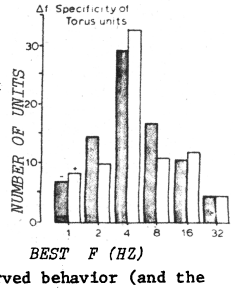
272.3 MAUTHNER FIBER REFLEX: DESENDING ACTIVITY MEDIATES "HABITUATION" OF THE RESPONSE TO MAUTHNER FIBER STIMULATION.
M.V.L. Bennett and J.W. Day, (Spon.: A.L. Harris), Div. of Cellular Neurobiology, Dept. of Neuroscience, Albert Einstein Col. of Med., Bx, N.Y.

As earlier reported, the tail flip response to intracellular stimulation of the Mauthner fibers (Mf) is depressed by repetition at 1/2 sec(Aljure et al., Brain Res. 188: 261, 1980). This depression is side specific for axial musculature, but bilateral for the pectoral fins. Since the two Mf's activate pectoral motoneurons via common interneurons transmitting electrotonically and responses of the interneurons are not depressed at these frequencies of stimulation, postsynaptic inhibition is implicated as responsible for the depression. Furthermore, paired stimulation which produces no response of axial musculature because of crossed inhibition in the spinal cord still leads to depresstion of the responses to either Mf. In deteriorated animals the depression can become greatly reduced, again implying an active process rather than fatigue or reduced effectiveness of transmission at an excitatory synapse.

It is possible to externally record Mf impulses from the caudal peduncle where they have an amplitude of about 0.1mV. The Mf, which are apparently the lowest threshold elements in the spinal cord, can then be specifically stimulated with external electrodes adjacent to the anterior spinal cord. Depression of responses to Mf stimulation has been studied in this manner and is the same as that observed with intracellular stimulation. Following high spinal section responses to Mf stimulation are absent for about ½ hour and then recover. At this point they are no longer depressed by stimulation at the lower frequencies that previously blocked them completely. The effect of spinal section confirms that desending activity, presumably inhibitory, is responsible for the depression of responses to repeated Mf stimulation. The underlying circuitry may operate in behavioral habituation or allow the animal to control the amplitude of the tail flip response in spite of the all-or-none nature of the desending command signal.

272.4 NEURONAL BASIS OF A SENSORY FILTER IN THE JAMMING AVOIDANCE RESPONSE:NO GRANDMOTHER CELLS IN SIGHT.
B.L. Partridge, W.F. Heiligenberg, J.A. Matsubara. Scripps Institution of Oceanography, La Jolla, CA. 92093.

The Jamming Avoidance Response (JAR), during which weakly electric fish modulate their electric organ discharge rate in response to an electric signal of nearly the same frequency is strongest for frequency differences (Δfs) between 3-8 Hz. We have searched for neural correlates of this behavioral specificity. Single unit recordings in the anterior lateral line ganglion (ALLG), the posterior lateral line lobe (PLLL) and the torus semicircularis (TS) of Eigenmannia virescens were made during electrical stimulation simulating jamming by a nearby conspecific.



Contrary to previously published reports (Scheich, 1974, 1977) we conclude that Δf specificity does not lie in a single class of receptors or higher-order units in the PLLL tuned to the most effective Δfs. No tuning is seen at the receptor level of the PLLL. Specificity seems to be a population effect first visible at the level of the torus semicircularis, with individual units responding most strongly to different Δfs, but with most units tuned to approximately + and -4Hz. By having cells tuned to a variety of Δfs but occuring in proportions corresponding to the observed behavior (and the degree to which Δfs impair electrolocation), animals would be better equipped to carry out other tasks such as detection of relative motion of objects in space and would also be better able to "read" complex stimuli corresponding to the more usual case of simultaneous jamming from several Conspecifics.

Two classes of TS units (the first described previously by Bastian and Heiligenberg, 1980) were observed which showed differential patterns of firing for positive and negative Δfs. These TS units, unlike their counterparts in the PLLL show different patterns of firing as a function of stimulus field geometry (eg. transverse or oblique to the body axis of the fish). Predictions these findings make about the wiring of TS units with respect to units in the PLLL are supported by preliminary efforts to localize the complex receptive fields of these units.

272.5 LABELLING OF FUNCTIONALLY IDENTIFIED NEURONS IN ELECTRIC FISH BY INTRACELLULAR INJECTION OF HRP. W.Heiligenberg and J.Dye * Neurobiology Unit, Scripps Institution of Oceanography and Department of Neurosciences, University of California at San Diego, La Jolla, Cal. 92093

Electric fish show a number of relatively simple natural behavioral responses which remain intact in neurophysiological preparations of the central nervous system. This greatly facilitates identifying the role of certain types of neurons in the control of these behaviors. In the particular case of the Jamming Avoidance Response (JAR) of the genus Eigenmannia, it has been demonstrated that this behavior is driven by specific modulations in amplitude and phase of electroreceptive afferences from different parts of the body surface. These two stimulus variables, amplitude and phase, are encoded by so called P- and T-type electroreceptors respectively. Primary afferents from these receptors project, in a somatotopic manner, to a laminated structure in the posterior lateral line lobe (PLLL) of the hind brain. Amplitude information and phase information are relayed to different classes of neurons which are located in separate layers of the PLLL.

By means of iontophoretic injection of HRP, we have labelled single primary afferents from electroreceptors. These units could be identified as being of the P- or T-type respectively, and the location of their receptor pore on the body surface was determined by applying localized electric stimuli via a small dipole. After survival of 2 to 4 hours, animals were perfused, their brains sectioned, and the sections processed by the Hanker-Yates reaction (as modified by Thomas Finger). In most instances, processes of primary afferents could be traced to their termination in the PLLL. The units were found generally to project to 3 different sites , with the most anterior termination in the lateral region of the PLLL, and with posterior terminations of the same unit in 2 distinct areas of the central region of the PLLL. Present data indicate the possible existence of 3 separate somatotopic maps in the PLLL. Their behavioral significance will be discussed.

272.6 SPAWNING IN GOLDFISH: PERIPHERAL AND SPINAL MECHANISMS OF SPERM RELEASE. L.S. Demski, J.G. Dulka* and P.J. Hornby*. School of Biological Sciences, Univ. of Kentucky, Lexington, KY 40506.

Experiments have been performed on goldfish (anesthetized in 2% urethane and then immobilized by 1.5 mg/kg of d-tubocurarine) in order to identify the motor system mediating sperm release (SR) evoked by electrical stimulation of the CNS (see Demski, Soc. Neurosci. Abstract 285, 1978). Several possible mechanisms have been ruled out as being of primary importance for SR. Control by a striated muscle urogenital sphincter (innervated by spinal nerves) is unlikely since SR can be elicited following paralyzing doses of d-tubocurarine and removal of all but the rostral 4 to 6 segments of the spinal cord. Mediation of SR by increased intra-abdominal pressure is also unlikely since the response persisted following the removal of large areas of the abdominal cavity wall and deflation of the gas bladder. In addition, intra-abdominal pressure increases could not be measured during evoked SR. A pressure gauge system sensitive to 1 cm or less of water was used. Pituitary and urophyseal hormones were also eliminated from consideration because SR was readily elicited after removal of these glands. In contrast, electrical stimulation in the vicinity of the sperm ducts (SD) does result in evoked SR and anatomical observations indicate that the SD has considerable smooth muscle interspersed between numerous ductules. Thus, contraction of the SD appears to be the primary motor mechanism controlling SR. SR was also evoked by IP injection of 10^{-2} M acetylcholine in the vicinity of the SD and this response was blocked by similar injection of 10^{-4} M atropine. SR evoked from the brain was also blocked by atropine injections (10^{-4} and 10^{-5} M). The results strongly suggest that SR is controlled by a muscarinic-cholinergic system. Spinal cord transection experiments revealed a critical area for SR in the rostral cord (segments 4-6 in a 16.5 cm fish-SL). SR could be evoked from the brain only when this area remained connected with the medulla and stimulation of isolated pieces of cord in only this area resulted in SR. SR in response to electrical stimulation of the subvertebral area following its isolation from surrounding structures and removal of the spinal cord indicates that the motor pathway descends from the rostral cord in a subvertebral position (near the midline) to the level of the caudal extent of the body cavity where it turns ventrally to reach the area of the SD. This subvertebral position is coincident with the location of the sympathetic trunks and it can be suggested that the SR is mediated by this automonic system. Supported in part by the Biomedical Research Support Grant RR 07114-09.

272.7 NEURAL CONTROL OF CHROMATOPHORES USED AS A SOCIAL SIGNAL. L. E. Muske*and R. D. Fernald (SPON R. F. Lane). Inst. of Neurosci., Univ. of Ore., Eugene, Ore. 97403.

Haplochromis burtoni, a colonial cichlid fish, relies on visual signals during social interactions. Important cues are provided by the dermal chromatophores, which produce color patterns characteristic of an individual's sex and social status. Territorial males have a distinctive dark vertical bar extending from the eye to the corner of the mouth, which can appear and disappear quickly, independently of the other markings. In some adult males the vertical eyebar is completely absent, although other territorial colors and behaviors are identical to barred animals. There are no intermediate morphs. The eyebar influences aggressive behavior in other males (C. Y. Leong, Z. Vergl. Physiol. 65: 29, 1969). Thus, the existence of barless morphs raises interesting questions about the function of this signal and about the mechanism by which it is controlled. This study was undertaken to investigate whether peripheral structures or mechanisms could be found to account for barred/barless polymorphism.

Histological examinations of eyebar pigment cells show distinct differences between the two morphs. Barless males have a lower density of melanophores. Only barred males have a dense network of irridescent pigment cells underneath the melanophores, which account for the intense, velvet-like blackness distinguishing the eyebar from other black stripes.

A small nerve containing post-ganglionic sympathetic fibers branches off from the maxillary (Vth cranial) nerve to innervate eyebar pigment cells. The effects of unilateral lesions of this nerve were assessed by comparing lesioned and intact eyebars in normally behaving animals. In barred males, the two sides are identical. In barless males, there is an "eyebar" on the lesioned side only, which is much less intense than a normal bar. Neither morph is able to turn "off" the eyebar thus produced.

Monopolar or biphasic stimulation of nerves controlling facial chromatophores produced rapid melanophore pigment aggregation (paling) in locations consistent with the lesion studies. Threshold curves reveal no differences in response, either between barred and barless males or between eyebar and other head melanophores, indicating that differential control of the eyebar is mediated at a site central to the chromatic-motor nerve.

This evidence that the eyebar contains unique pigment cells and is neurally controlled by the CNS supports behavioral observations that the eyebar is an important element in the repertoire of social signals. Supported by NIH 5T32 GM07257.

272.8 VISUAL FIELD AND RETINAL PROJECTIONS IN THE AFRICAN CICHLID FISH, HAPLOCHROMIS BURTONI. Russell D. Fernald*. Institute of Neuroscience, University of Oregon, Eugene, Oregon 97403.

The African cichlid fish, Haplochromis burtoni, has a highly evolved social system in which behavioral interactions are mediated by bright chromatic and spatial patterns on the fishes' bodies. The temporal margin of the retinal contains cell densities 2-4 times greater than other retinal regions for all retinal cell types except rods. To understand how this retinal specialization might affect visual performance, the size of the visual field has been analyzed.

To determine the extent of the visual field, a surveyor's transit was modified allowing rotation of a fish about the center of the lens. By rotating around two orthogonal axes, the limits of the visual space could be measured along equidistant meridians around the margin of the eye.

The visual field is eccentric in shape towards the nasal pole. The long axis of eccentricity coincides with the nasotemporal axis of the eye. This nasal extension of the visual field results in considerable binocular visual space in front of the animal. The visual field extent remains approximately constant as the animal grows larger, even though the eye increases greatly in size. The relationship of the visual field to the retinal field has been examined and correspondence demonstrated. The implications of the eccentric visual field for central neural connections will be discussed.

(Supported by USPH grant EY-02284.)

272.9 VISUAL AND ELECTROSENSORY RESPONSES IN THE OPTIC TECTUM OF A WEAKLY ELECTRIC FISH. J. Bastian. Dept. of Zool., Univ. of Okla., Norman, OK 73019.

Single unit responses were recorded from the optic tectum of the electric fish Apteronotus albifrons. Many of the cells encountered could be driven by visual as well as electrosensory stimulation. Simple visual stimuli, such as strobe flashes, were usually effective however simple electrical stimuli, such as amplitude modulation (AM) of the animal's electric organ discharge (EOD), which affect the entire fish simultaneously were usually ineffective. The majority of the cells were sensitive to more natural electrical stimuli such as small conducting or non-conducting objects moving within a few cm of the fish. Such objects evoke responses when the animal is in total darkness eliminating the possibility of visual stimulation. The responses changed form or even disappeared when identically shaped non-conducting objects were substituted for conductors. This eliminates the possibility that mechanoreceptive inputs are solely responsible for the responses since the mechanical effects are independent of object conductivity. These cells frequently had no spontaneous activity, responses occurred only when the stimulus moved through the cell's receptive field. Changing object conductivity usually shifted the position of this response relative to the fish. Responses were also influenced by movement direction. In the extreme cases movement opposite the most effective direction produced no response.

The stimulus objects contained a small light source and could provide a visual as well as electrosensory stimulus. The size of the visual stimulus was large, about 23^{o} of visual angle, and was usually moved 1 cm lateral to the fish. Simultaneous visual and electrosensory stimulation usually changed the intensity or the area over which a response occurred compared to electrical stimulation alone. Diffuse illumination of the animal's eyes, in a way that did not allow the fish to see the moving object, also altered the responses but in a different fashion.

The electrosensory response of most of the tectal cells seen was sensitive to jamming. A 4 Hz continuous AM of the animal's EOD, 2 mV/cm in amplitude, was applied to the fish while objects were moved as before. This jamming stimulus reduced the response to moving objects without visual input by as much as 50% of the normal value. These cells gave no response to the jamming stimulus alone. Addition of the moving visual stimulus frequently restored the jammed response to near normal values. The opposite effect was also seen, in some cases the addition of the visual stimulus further reduced the electrosensory response in the presence of jamming.

Supported by NIH grant NS-12337

272.10 SIGNIFICANT DIFFERENCES IN VOLUME OF SONG CONTROL NUCLEI IS ASSOCIATED WITH VARIANCE IN SONG REPERTOIRE IN A FREE RANGING SONG BIRD. R. Canady*, D. Kroodsma*, and F. Nottebohm (Spon: D.R. Griffin). Rockefeller U., N.Y., NY 10021,and Dept. of Zool., U. of Mass., Amherst, MA 01003.

In the adult canary a positive correlation exists between the number of songs an individual produces (repertoire) and the volume of two telencephalic nuclei known to be involved in song production; hyperstriatum ventrale, pars caudale (HVc) and nucleus robustus archistriatalis (RA)(Nottebohm et al, Br. Res. in press). In an effort to determine how general this phenomenon may be and to look at a possible extreme example of it, a free ranging species of song bird with marked repertoire variance was studied.

The Long Billed Marsh Wren (Cistothorus palustris) is a species in which song repertoire size occurs as a seemingly continuous cline, increasing east to west, across North America. Populations on the west coast have repertoires that average 2-3 times that of those on the east coast with little or no overlap in distribution. An eastern population located on the Hudson river near Tivoli, New York and a western population located in the Grisely Island national park near San Fransisco, California, both with males in full song and at very similar stages in breeding cycle, were analyzed with respect to repertoire size and the volume of 4 well defined, discrete brain nuclei; HVc, RA, nucleus rotundus, (Rot), and spiriformis medialis (SpM). SpM and Rot are not involved in song production and were used as controls.

For confirmation of the west-east repertoire difference up to 600 songs were recorded from each of 7 western males and 15 eastern males. Recorded and unrecorded males were captured, perfused with 10% formalin in 0.9% saline, the brains removed and processed for nuclei volume estimations (Nottebohm, op. cit.). 28 brains and 14 repertoire estimates are included in this report.

The samples differed in several respects. Western birds were slightly lighter (av. 5% $p<.01$) with brains showing a trend in the same direction (av. 4% $p<.1$). The control nuclei showed the same trend, in volume (Rot av. 12% $p<.01$, SpM NS). The difference in volume seen in Rot and the difference in weight seen in whole animal and brain were in the opposite direction of the repertoire size difference (west > east) and the difference in volume of HVc and RA. HVc and RA were 33% ($p<.01$) and 37% ($p<.01$) larger, respectively, in the western population than in the eastern population. Correcting for brain wt. differences by dividing each HVc by its control nuclei gives a west-east difference of 50% (west > east, $p<.001$).

To our knowledge this is the first demonstration of a difference in brain nucleus volume associated with a behavioral variable within a species of free ranging song bird.

272.11 A NEURAL MECHANISM FOR ECHO-DETECTION AND ECHO-RANGING IN FM BATS. S.A. Kick* (SPON: J. Simmons). Institute of Neuroscience, Univ. of Oregon, Eugene, OR 97403.

The threshold for the detection of echoes by the sonar receiver of Eptesicus fuscus corresponds to the distance at which a sonar target returns an echo sound-pressure in the region of 0 dB SPL. Echo-detection at this low an amplitude is consistent with a neural mechanism for recognition of echoes that is organized around the temporal synchronization of neural discharges in auditory-nerve fibers. It is probable that weak echoes are initially represented in VIIIth nerve fibers by a cluster of spike activity associated with the echo's time-of-occurrence, as plotted in a pulse-echo inter-stimulus- interval (ISI) histogram. As echo amplitude increases above the detection threshold, the small peak of spike activity associated with the echo's time-of-occurrence in the ISI histogram would also increase, providing progressively better estimates of target range. On a neural level, the processes underlying echo-detection and the perception of target-range information must be closely intertwined. The representation of information about sonar echoes by the cross-correlation function between pairs of emissions and echoes, which has been shown to govern target-range perception by bats, is similar to the representation of nerve-spike timing by ISI histograms, suggesting that a single neural mechanism may be responsible for several aspects of the performance of echolocating bats.

272.12 PHYSIOLOGICAL AND BEHAVIORAL CHARACTERISTICS OF DENTATE GRANULE CELLS. G. Rose*, D. Diamond* and G. Lynch (SPON: J. Masserano). Dept. of Psychobiology, Univ. of Calif., Irvine, CA 92717.

The efferents of the granule cells of the dentate gyrus are generally considered to serve as the major pathway for information transfer into the hippocampal formation. Thus, knowledge of the conditions under which the granule cells are active should provide important clues as to the behavioral function(s) of the hippocampus itself. Sixty-three single neurons were recorded from the granule cell layer of 10 Sprague-Dawley albino rats which had been prepared for recording by cementing the base for a microdrive system to the skull overlying the dorsal hippocampus. A bipolar stimulating electrode was placed in either the entorhinal cortex or angular bundle to allow activation of the perforant path input to the hippocampus.

The neurons were divided into three classes using the duration of the extracellularly recorded action potential as the criterion. Durations of unfiltered action potentials were: Class I - 0.20-0.25 ms; Class II - 0.30-0.40 ms; Class III - 0.60-1.0 ms. Neurons of Classes I and II were always observed to fire single action potentials, while Class III cells fired both single and complex spikes. Fifty-two of the 63 cells (83%) recorded from the granule cell layer were identified as Class I neurons. Forty-six of these cells were orthodromically activated by perforant path stimulation at a mean latency of 3.4 ms (range 1.7-5.0 ms). Four Class II neurons were observed, 3 of which were activated by electrical stimulation of the perforant path. Seven Class III neurons were recorded, none of which could be discharged by perforant stimulation, even at high stimulus intensities.

Behavioral correlates for all neurons were observed during unstructured activity and while the animals acquired and performed a simple appetitive discrimination task. The most obvious behavioral correlate of Class I and Class II neurons was movement, although Class II neurons also showed increased firing under conditions of high arousal. Most Class III cells exhibited no obvious behavioral correlate, but three neurons had the characteristics of "place cells" as described by others. No new behavioral correlates for the neurons of any class were seen as a function of conditioning.

The principal conclusion from this work is that the majority of dentate granule cells are Class I neurons. These cells share the physiological and behavioral characteristics of the "theta" cells of Ranck (Exp. Neurol., 41: 462-531).

(Supported by NSF Grant BNS-17370 to G.L.)

273.1 DEVELOPMENTAL INCREASE IN ADRENAL MESSENGER RNA CODING FOR PHENYLETHANOLAMINE-N-METHYLTRANSFERASE (PNMT) E.Sabban,* M. Goldstein, M.C. Bohn and I.B. Black, Neurochemistry Laboratories, Dept. of Psychiatry, N.Y.U. Med.Center New York, N.Y. 10016 and Dept. of Neurology, Cornell Univ. Med. Coll., New York, N.Y. 10021.

We have been studying expression and development of the adrenergic phenotype by following the ontogeny of PNMT, the epinephrine-forming enzyme. To determine whether the increase in PNMT catalytic activity during development of the rat adrenal is paralleled by comparable changes in mRNA coding for the enzyme, total poly(A) RNA was isolated from newborn and adult rat adrenals. Their translation was monitored in a cell-free system. Translation was assessed by measuring the incorporation of ^{35}S-methionine into total protein.

Pilot experiments measuring translation of bovine adrenal medullary poly(A) RNA demonstrated that translation was linear for 60 min. at 25°C. Optimal concentrations for Mg^{++} and K^{+} were 1.6 mM and 132 mM respectively, and RNA concentrations were defined for each experiment. The newly synthesized polypeptides were separated by gradient SDS gel electrophoresis and the dried gels were processed for fluorography and exposed to x-ray film. The amount of PNMT translated was estimated by indirect immunoprecipitation with rabbit anti-rat PNMT antiserum followed by SDS gel electrophoresis. Although total translational activity and the ^{35}S-polypeptide profiles were similar in neonate and adult, several polypeptides appeared greatly enriched in the translation of adult mRNA. Immunoprecipitation of equal amounts of ^{35}S-methionine - labelled translation products showed that the density of the PNMT band (MW = 38,000) was strikingly greater in adult than in neonate. Densitometer scans and scintillation counting of eluted bands revealed that there was at least a 10-fold increase in incorporation into PNMT translational product using mRNA from adult adrenal. Catalytic activity of PNMT per adrenal was approximately 40-fold higher in adult. These results suggest that the developmental increase in PNMT activity may be largely attributable to increases in specific mRNA. This work was supported by NIH grants NS 06400, NIMH 02717, NS 10259, HD 12108 and NS 06801.

273.2 BIOCHEMICAL DEVELOPMENT OF THE NUCLEUS LOCUS COERULEUS (l.c.) IN VIVO AND IN CULTURE. C.F. Dreyfus and I.B. Black. Div.of Developmental Neurology, Cornell University Medical College, New York, New York 10021.

Although extensive work is beginning to elucidate phenotypic expression and ontogeny in the peripheral nervous system, the molecular mechanisms underlying brain development are largely unknown. To approach this problem, we have initiated parallel in vivo and in vitro studies of the mouse l.c. The activity of tyrosine hydroxylase (TH), the rate-limiting enzyme in catecholamine biosynthesis, was used to monitor development of this brain nucleus.

TH activity was initially detectable at 13 days of gestation (E13) and increased approximately 20 - fold by E-18, immediately prior to birth. From birth to adulthood TH activity increased an additional 10 - fold.

To determine whether comparable development can occur in vitro, explants of embryonic brainstem were placed in organotypic culture as previously described (Dreyfus et al., Brain Res. 161 '78, Dreyfus et al., Brain Res. 194 '80). Explantation of E-12 brainstems, initially devoid of TH activity,as measured by a sensitive catalytic assay,resulted in the de novo appearance of enzyme activity after approximately 1 week in culture. Activity subsequently increased approximately 5 - fold, reaching plateau values by 14 days in vitro, and persisting to at least 21 days. Explantation at E-18, after TH has already appeared, also resulted in an increase in activity, but 3 - week plateau values were significantly higher than those in E-12 explants. Our studies suggest that the development of discrete brain nuclei may be quantitatively monitored in vivo and in vitro. Moreover, it may be possible to define the factors governing initial phenotypic expression and development of specific transmitter phenotypic characters in central structures. (Supported by NIH grant NS 14990 and NSF grant BNS 80 - 24081).

273.3

WITHDRAWN

273.4 IMMUNOCHEMICAL STUDIES OF PERIPHERAL NEUROGENESIS USING MONOCLONAL ANTIBODIES. G. Ciment*, G. Spady* and J. Weston*. (SPON: C. Kimmel). Dept. Biology, Univ. Oregon, Eugene, OR 97403.

The embryonic neural crest appears to segregate several distinct cell lineages during normal development. These lineages ultimately produce all peripheral nervous system neuronal and glial cells, as well as endocrine, paracrine, skeletal and pigment elements. In order to deduce the order of segregation of various lineages, and the intermediate cell types in each lineage, we have developed a battery of monoclonal antibodies which bind to antigens found on subpopulations of neurons within embryonic sensory ganglia. One such antibody (E/C8) is of a class that binds to the cell bodies and fibres of some neurons. Using direct and indirect immunoperoxidase staining methods with this antibody, we have examined cultures, tissue "squashes" and tissue sections containing various crest-derived cells. This antibody mediates staining of a subpopulation of cultured, apparently undifferentiated neural crest cells. In vivo, a subpopulation of cells in nascent (4 day) sensory ganglia and in 7 day sympathetic ganglia stains. The subcellular localization of staining indicates that the antigen is present on the initial segment of the neurite as well as a patch of the adjacent cell body. As development of the sensory ganglia proceeds (days 8 through 13), the amount of neuronal staining increases and becomes more extensive. In contrast, staining of sympathetic neurons becomes more restricted and eventually disappears from the soma altogether. Ultrastructural analyses of the staining patterns on the various crest-derived intermediate cell types should elucidate the role of such antigens in the normal development of peripheral neurons.

273.5 BIOCHEMICAL MATURATION OF EMBRYONIC DOPAMINE NEURONS IN AGGREGATING COCULTURES OF MURINE MESENCEPHALIC TEGMENTUM AND NEOSTRIATUM. Connie Kotake*, Philip Hoffmann*, and Alfred Heller. Dept. Pharmacol. & Physiol., Univ. of Chicago, Chicago, IL 60637.

Dopamine (DA) neurons from the rostral mesencephalic tegmentum (RMT) of embryonic mouse brain were dissociated and allowed to aggregate in vitro with dissociated cells from the neostriatum (NS). Dopamine cells of RMT form a dense axonal plexus only in the presence of target cells of NS (Hemmendinger et al., PNAS 78: 1264, 1981). This plexus is morphologically similar to that seen in vivo. In order to further characterize the differentiation of DA neurons in this system, we examined the development of a number of indices of dopaminergic function, including DA histofluorescence, tyrosine hydroxylase (TH) activity, endogenous DA content, DA accumulation and its sensitivity to either reserpine or 6-hydroxydopamine (6-HDA) in RMT-NS coaggregates cultured for 3-21 days. Fluorescent cell bodies and patches of punctate pericellular fluorescence due to endogenous DA were visualized by the Falck-Hillarp method in 14 day cultures. This pattern of fluorescence was more prominent in 21 day cultures and resembled that seen in intact mouse striatum. Fluorescence was not observed in 3 and 7 day cultures unless they were exposed to exogenous DA (1 μM; 10 min). The estimated Vmax of TH increased by 2-fold between 3 and 14 days in culture to 5.0 pmoles DOPA formed/min/mg protein and remained elevated in 21 day cultures (4.6 pmoles/min/mg protein). Endogenous DA in RMT-NS aggregates increased progressively from 0.56 ng/mg protein at 3 days to 22.6 ng/mg protein at 21 days. The accumulation and retention of exogenous DA (1 μM; 10 min) followed a developmental course similar to that observed for endogenous DA content. At 21 days in culture, the aggregates accumulated 80 ng DA/mg protein; 8-10 times greater than that found in 3 day cultures. Aggregates of various ages were exposed to reserpine (100 nM;24 hr). Such aggregates at 14 and 21 days accumulate 96% less exogenous DA than vehicle-treated aggregates. In younger cultures, reserpine was less effective, producing a 16% decline in DA accumulation in 3 day cultures, and a 48% decline in 7 day cultures. Aggregates cultured for 3,7,14, or 21 days and exposed to 6-HDA (10^{-4}M; 1 hr) exhibited reductions in DA accumulation of 21%, 52%, 92%, and 95%, respectively, 24 hr after 6-HDA exposure. These cultures, therefore, show age-dependent development of mechanisms for accumulation, synthesis and storage of DA. Thus, RMT-NS aggregates display characteristics of anatomical, biochemical and pharmacological development qualitatively similar to those seen in intact brain. This system provides a useful in vitro model for investigation of factors which influence the ontogeny of brain dopaminergic systems. (U.S. PHS MH 28942 and NS 10714)

273.6 GLOBOSIDE AS A POSSIBLE SURFACE MEMBRANE RECEPTOR FOR THE LECTIN SOYBEAN AGGLUTININ IN CULTURED SYMPATHETIC NEURONS. Anne Zurn* and Paul H. Patterson, Dept. of Neurobiology, Harvard Medical School, Boston, MA 02115.

The type of transmitter produced by sympathetic neurons taken from neonatal rats and grown in dissociated cell culture can be experimentally controlled. If the neurons are depolarized or made active, they can be maintained in their original noradrenergic state. However, diffusible or surface-bound factors from heart cells can induce the neurons to become cholinergic. In addition to the difference in transmitter, neurons grown under these two conditions also display differences in their surface membranes. One method used to detect such a difference was an electron microscopic analysis of the binding of the lectin soy bean agglutinin (SBA) labeled with colloidal gold. The density of binding is five-fold higher on noradrenergic axons than on cholinergic axons (M. Schwab & S. Landis, Devel. Biol., in press).

Several methods are being employed to identify the molecule(s) acting as a receptor for SBA. When neuronal cultures are dissolved in SDS and run on SDS-PAGE, specific binding of ^{125}I-SBA was found only in an area just ahead of the dye front, which suggested the lectin was binding to lipids and not to proteins. When neuronal glycolipids were extracted, separated into neutral and acidic fractions, and electrophoresed in this system, only the neutral glycolipid fraction labeled specifically. To determine the molecule(s) to which SBA binds in living neurons, cultures were incubated with SBA for 30 min at 20°C and then treated with galactose oxidase and NaB^3H_4. After extraction and fractionation, the labeled lipids were analyzed by TLC. In the presence of SBA, the labeling of the neutral glycolipid, globoside (cer-Glc-Gal-Gal-GalNAc), was decreased by 50%. Maximal inhibition was seen with 100μg/ml SBA, while little inhibition occurred at 10μg/ml. Thus globoside may be a receptor for SBA in extracts and in living neurons.

To see whether there are any differences in the glycolipid composition of noradrenergic and cholinergic neurons, cultures were metabolically labeled with ^{3}H-galactose or surface-labeled with galactose oxidase and NaB^3H_4, extracted, and analyzed by TLC. Quantitative differences are seen in both the ganglioside and neutral glycolipid profiles of these two types of sympathetic neurons. The results of these preliminary lipid composition studies therefore support the suggestion generated by the SBA binding experiments that the glycolipids of cholinergic and noradrenergic neuronal membranes are different. (Supported by the NINCDS, the Rita Allen Foundation, and the Swiss National Fund).

273.7 PREDOMINANTLY CLEAR VESICLES OF CHOLINERGIC SYMPATHETIC NEURONS IN CULTURE BECOME DENSE-CORED FOLLOWING DEPOLARIZATION AND RELOADING WITH NOREPINEPHRINE: PRELIMINARY OBSERVATIONS. M.I. Johnson and D. Higgins*. Dept. Anatomy & Neurobiology, Washington University School of Medicine, St. Louis, MO 63110.

Adrenergic sympathetic neurons of perinatal rats, under certain culture conditions, acquire cholinergic function, including choline acetyltransferase activity and hexamethonium sensitive synaptic interaction. Whereas early in culture the majority of the synaptic vesicles in the varicosities of these neurons are dense-cored, later, when cholinergic properties can be demonstrated, the vesicles are predominantly clear (Johnson, et al. Nature 262:308-310 1976; S. Landis PNAS 73:4220-4224 1976). The following study asked whether the vesicle population in these cholinergic sympathetic neurons is fixed or whether the vesicle population could be made to become dense-cored by depolarization and incubation in norepinephrine. Single or small numbers (3-7) of superior cervical ganglion neurons were grown in the absence of nonneuronal cells on small (1-2 mm) collagen islands under culture conditions promoting cholinergic function. At 2-3 weeks, electrophysiological methods were used to determine which neurons released acetylcholine; only those islands on which all neurons could be identified as cholinergic were used in the electron microscopic studies. Three treatment groups were used prior to $KMnO_4$ fixation. 1) NE (10 μm) with no choline 2) NE (10 μM) with 30 μM choline 3) K^+ (80 mM) followed by NE (10 μM) with no choline. The vesicles of those neurons treated with NE and either no or 30 μM choline were predominantly clear (94 and 87%). All varicosities had less than 30% dense-cored vesicles. In contrast the vesicles of the neurons depolarized with 80 mM K^+ and incubated in NE (no choline) were predominantly dense-cored (86%). All of the varicosities had >80% dense cores. The vesicle morphology of functionally cholinergic sympathetic neurons therefore can be dramatically altered by depolarization and reloading with exogenous NE. Further studies are directed at the questions: 1) is the population of vesicles involved in cholinergic transmission the same as those with dense cores following depolarization and incubation in NE and 2) can similar reloading occur via an endogenous pool of NE? (Supported by NIH Grants NS 14416 and NS 09809.)

273.8 DEVELOPMENT OF BENZODIAZEPINE AND GABA BINDING IN INBRED MICE: RELATION TO AUDIOGENIC SEIZURES. C. Kellogg and P. Syapin*. Dept. of Psychology, Univ. of Rochester, Rochester, NY 14627; Dept. of Neurology, Univ. of Southern Calif. Sch. of Med., Los Angeles, CA 90033.

Mice of the DBA/2J (DBA) strain have a genetically determined sensitivity to audiogenic seizures (AGS) showing maximal sensitivity around 21 days of age. The seizure response to high intensity sound dissipates with maturation and by 42 days of age the mice are no longer sensitive. Mice of the C57BL/6 (C57) strain are not AGS sensitive at any age. Considering the general inhibitory nature of GABA - containing neurons and the anticonvulsive effect of benzodiazepine compounds, the development of binding sites for these substances was evaluated in the two strains.

Benzodiazepine binding was studied at 21 and 42 days of age using ^{3}H-diazepam (DZ) as the ligand and was carried out using a well washed membrane fraction. Brains were dissected into 5 regions. Scatchard analysis using 6 concentrations of ^{3}H-DZ indicated no difference in the dissociation constant (KD) as a function of strain, age, or brain region (KD=8.11±.26nM). Maximal binding also did not vary as a function of strain or age. However, GABA stimulation of ^{3}H-DZ binding (2-2.4nM) differed between strains. At 21 days, the DBA mice demonstrated significantly greater stimulation than the C57 mice. By 42 days, due to an increase in GABA stimulation with age noted in the C57 mice, a significant strain difference was no longer apparent. Hence GABA stimulation of ^{3}H-DZ binding matures earlier in mice of the seizure sensitive strain and is adult-like during the period of maximal seizure sensitivity.

Analysis of GABA receptors has been initiated using ^{3}H-muscimol as ligand. Total binding has been evaluated in 5 brain regions from mice of both strains at 21, 28, 42, and 60 days of age using a ^{3}H-muscimol concentration of 10nM (with whole brain KD varying from 6.54 to 9.4nM). At 21 days of age, total binding is significantly greater in most brain regions of DBA mice as compared to the C57 strain. However, with maturation, total binding increased markedly in the C57 strain reaching, in some regions, levels considerably higher than in the DBA mice. In the DBA mice, the only region showing an increase in binding with age was the cerebellum. In the AGS mice, therefore, there appears to be an earlier maturation of certain aspects of GABA function, perhaps reflecting some compensatory response to a primary deficit responsible for inducing seizure sensitivity.

273.9 MONOAMINE OXIDASE ACTIVITY IN EARLY QUAIL EMBRYOS AND RAT NEURON CULTURES WITH DIFFERENT TRANSMITTER PHENOTYPES. J.E. Pintar*[1], G.D. Maxwell[2], K.J. Sweadner[3], P.H. Patterson[3], and X.O. Breakefield[1]. [1]Dept. Human Genetics, Yale Med. Sch., New Haven, CT 06510; [2]Dept. Anatomy, U. Conn. Health Center, Farmington, CT 06032; [3]Dept. Neurobiology, Harvard Med. Sch., Boston, MA 02115.

Monoamine oxidase (MAO) is primarily responsible for the degradation of catecholamines and other biogenic amines. Two types of MAO activity (MAO-A and MAO-B) are characterized by different substrate specificites and inhibitor sensitivities, and are mediated by molecules with different covalent structures. We have begun to examine the level, type, and functional significance of MAO activity in embryonic quail and in cultures of rat neurons with different phenotypes. Whole quail embryos (12 hours-4 days incubation; hatching at day 16) were isolated and MAO activity was determined against tryptamine, a common substrate for MAO A and MAO B activity. MAO activity was highest at the earliest stages examined (12-24 hrs incubation); these activity levels (>300 pmol/min/mg protein) are higher than many adult tissues and most cultured cell lines. By 2½ days incubation, MAO specific activity had decreased to 20% of the previous levels. Clorgyline concentrations that selectively inhibit MAO-A but not MAO-B were used to estimate the relative amounts of these two types of activity. At all stages examined, 20-50% MAO activity was retained under conditions that inhibit MAO-A. These results indicate not only that high MAO activity is present at early embryonic stages but also that both MAO A and B molecules are being synthesized. Daily clorgyline injections into eggs (on days 0-3 incubation; 10 μM clorgyline final concentration in ovo) inhibited >90% MAO-A activity, but not MAO-B, as determined by assays on day 4 embryos. This ability to inhibit MAO-A should make it feasible to establish the functional significance of this activity in early embryos.

To investigate the amount and type of MAO activity in specific neuronal cell types, neuron cultures that synthesize primarily either noradrenaline or acetylcholine were derived from newborn rat superior cervical ganglia and subsequently assayed for MAO activity. Levels of MAO-A activity in adrenergic populations were 5-fold higher than in cholinergic populations, while MAO-B activity levels were similar. These data support the ideas that high MAO A activity is associated with adrenergic neurons and that the expression of the adrenergic phenotype involves regulating both synthetic and degradative enzymes used in catecholamine metabolism.

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273.10 ONTOGENY OF MONOAMINE METABOLITES IN BRAIN AND CEREBROSPINAL FLUID IN NORMAL AND 6-HYDROXYDOPAMINE TREATED RAT PUPS. B.A. Shaywitz, G.M. Anderson*, J.G. Young and D.J. Cohen*. Lab. Devel. Psychobiol. & Neurochem., Yale U. Sch. of Med, New Haven, CT 06510

Within the last decade, experiments in a number of laboratories have provided strong evidence to support the notion that the concentrations of monoamine metabolites in cerebrospinal fluid (CSF) may be utilized to assess the functional state of their parent monoamines within brain. However, both technical and ethical limitations in human studies have precluded careful examination of many of the assumptions about the relationship between both the CSF and brain concentrations of the metabolites as well as that between the metabolites and their parent amines in brain.

We utilized recently described assays (Anderson et al, J. Chromatog., 1980) and specially developed techniques for the collection of CSF from the cisterna magna of developing rat pups to examine the concentrations during the first month of postnatal life of homovanillic acid (HVA), 5-hydroxyindoleacetic acid (5-HIAA), tryptophan (TRP), and tyrosine (TYR) in CSF and dopamine (DA), serotonin (5-HT), and norepinephrine (NE) in brain in normal developing rat pups and littermates treated at 5 days of age with desmethylimipramine (DMI) and 6-hydroxydopamine (6-OHDA). Concentrations of all the parent monoamines increased by a factor of two between 12 and 26 days in normal pups. In 6-OHDA pups, DA was reduced immediately and remained at low levels throughout, averaging 21.5% of controls at 26 days. NE and 5HT were not significantly affected by the DMI/6-OHDA regimen. Brain concentrations of HVA were highest at 12 days then declined, while 5-HIAA and TRP remained stable at 12, 19, and 22 days in both normal and treated pups. CSF HVA declined between 19 and 26 days in normal pups while 5-HIAA and TRP remained stable. Pups treated with DMI/6-OHDA as neonates exhibited a significant reduction in CSF HVA to concentrations 30% of controls at 19 and 26 days ($p<.001$), but no change was observed for 5-HIAA or TRP. As expected, significant correlations emerged for CSF HVA-brain DA ($r=.65$, $p<.02$) and for brain HVA-DA ($r=.76$, $p<.001$), as well as for CSF HVA-5-HIAA ($r=.65$, $p<.01$), but surprisingly there were no significant correlations between CSF 5-HIAA and either brain 5HT or 5-HIAA nor did we observe any relationship between CSF TRP and 5-HIAA.

Our findings indicate that it is now possible to reliably determine concentrations of monoamine metabolites in CSF samples obtained from developing rat pups. They further suggest that while CSF HVA is a reasonable estimate of brain DA, correlations between CSF 5-HIAA and its parent amine (5HT) in brain and 5-HIAA in brain are disappointing. Future experiments should provide further insights into the relationship between CSF monoamine metabolites and central monoaminergic mechanisms.

273.11 APUD CELLS OF MOUSE EMBRYONIC PANCREAS ORIGINATE FROM CATECHOLAMINE PRECURSORS. G. Teitelman, T.H. Joh, and D.J. Reis. Lab. of Neurobiology. Cornell Univ. Med. Coll. New York, NY 10021.

A population of cells containing the enzymes tyrosine hydroxylase (TH) and dopa-decarboxylase (DDC) but not dopamine-B-hydroxylase (DBH) nor phenylethanolamine-N-methyltransferase (PNMT) and hence probably dopaminergic (DA),is found in the pancreas of mouse embryos at the 11th day of development (E 11). The presence of TH in embryonal pancreatic cells is transient: such cells containing TH are not observed after E 15 (Teitelman, et al. (Proc. Nat. Acad. Sci. 1981, in press). Thus, the embryonic pancreatic DA cells are part of a wider group of transient catecholaminergic (TC) cells which populate the gut, kidney and spinal cord during development of mice and rats (Teitelman, et al., Dev. Biol., 1981 in press). The fate of the TC cells is unknown. Conceivably, they might die or, alternatively, interconvert into other cells harboring other neuromodulators. In this study we sought to determine whether the pancreatic TC cells transform into peptidergic cells of the endocrine pancreas.

Mouse embryos or pancreases removed from adult mice were processed for immunocytochemical localization of insulin, glucagon, somatostatin, TH and DDC by the PAP method. Embryonal TH cells appear on E 11 and then decline to disappear by E 15. In adults TH reappears in a few cells (Adult DA cells) differing in location and morphology from the embryonal ones. DDC is first observed on E 13. Cells containing DDC increase in number during development. In adults DDC is contained in almost all islet cells. At E 12 glucagon appears in cells of the pancreatic lobe while insulin appear at E 14.5 along newly formed pancreatic tubules. Somatostatin is first observed at E 16 in a few scattered cells. The number of cells containing glucagon, insulin and somatostatin increase during prenatal development and on into maturity. By use of a method for simultaneously detecting two antigens (Teitelman et al, Proc. Natl. Acad. Sci., 1981 in press) both TH and glucagon were visualized in the same cell on E 12 and 13. Double labelled cells comprised 10% of all stained cells. At E 14.5, some of the cells stained for TH also contained insulin. However, at the time somatostatin appears no embryonal cells containing TH remain. The adult DA cells do not contain glucagon nor insulin. We conclude that two cell types of the APUD series, i.e. the glucagon and insulin cells of pancreas, arise from tranformation, in situ, of cells that transiently express a dopaminergic phenotype. These results raise the prospect that other peptidergic cells of the APUD series in periphery and possibly brain may have aminergic precursors.

273.12 EFFECT OF PHENOTHIAZINES ON NEUROENDOCRINE REGULATION OF GROWTH. T. T. Soncrant*, C. L. Sowek* and G. P. Redmond. Depts. of Pharmacol., Med. Technology and Pediatrics, Univ. of Vermont College of Medicine, Burlington, VT 05405.

Growth hormone (GH) secretion is under stimulatory control of noradrenergic and possibly dopaminergic systems in the hypothalamus. Phenothiazines block both of these receptors. Previous work has demonstrated that a single dose of perphenazine (PER), a potent phenothiazine of the piperazine class, blocks spontaneous GH secretion and that chronic administration retards growth. PER treated rats were 29% lighter than controls after 29 daily treatments. Concurrent GH supplementation decreases the growth deficit produced by PER, suggesting that inhibition of GH secretion is at least one of the mechanisms by which PER retards growth. We wished to determine: 1) whether tolerance to the GH-suppressing action of PER developed during chronic treatment, and 2) whether phenothiazines of other classes had similar GH-suppressing activity.

Spontaneous GH secretion was assessed by means of a non-stressful frequent-sampling method previously described (Redmond, Neuroendocrinol. 30:243, 1980). Plasma samples were taken every 15 min for 3 h prior to and 3 h following drug or saline administration; GH levels were measured by RIA. To detect development of tolerance to GH suppression after chronic PER treatment, rats were treated with PER 5 mg/kg, or saline, daily for 29 days. Both chronic PER and chronic saline animals exhibited GH suppression when given PER on day 31 (see table). GH suppressing activity of other classes of phenothiazines was investigated using chlorpromazine (CPZ), an aliphatic phenothiazine, and mesoridazine (MES), a piperidine phenothiazine. Single doses of CPZ, 10 mg/kg, and MES, 2.5 mg/kg, significantly inhibited spontaneous GH secretion:

		MEAN GH LEVEL (ng/ml)		
DRUG	N=	BEFORE DRUG	AFTER DRUG	P<
Saline	14	45 ± 5	61 ± 9	N.S.
CPZ (1 dose)	12	72 ± 12	34 ± 2	0.01
MES (1 dose)	6	35 ± 12	12 ± 1	0.05
PER (chronic)	7	53 ± 9	14 ± 1	0.01

Conclusions: 1) Tolerance to the GH-suppressing activity of PER does not develop with prolonged administration. 2) Since CPZ and MES suppress spontaneous GH secretion as previously demonstrated for PER, this appears to be a property of all three classes of phenothiazines. 3) It is likely that GH suppression by phenothiazines is a mechanism of their growth retarding action. 4) The possibility that other NE and DA blocking drugs may alter growth should be considered.

274.1 GABAERGIC RECURRENT INHIBITION IN NEOSTRIATAL SLICE PREPARATION. J.W. Lighthall and S.T. Kitai. Dept. of Anatomy, Mich. State Univ., E. Lansing, MI 48824.

A short duration recurrent inhibition is demonstrated in a slice preparation from rat neostriatum. Inhibition is observed following both local stimulation of neostriatum and as a result of direct activation of a single neuron recorded intracellularly. Parasagittal slices of rat neostriatum (caudate-putamen), 350-400 μm thick, were obtained and maintained using methods previously described (Lighthall et al., Brain Res., in press, 1981). Intracellular analysis shows the initial response recorded following local bipolar stimulation of neostriatum to be a monosynaptic excitatory postsynaptic potential (EPSP). Although long lasting inhibitory effects of local stimulation could not be observed, monosynaptic inhibitory postsynaptic potentials (IPSPs) 18-43 msec in duration followed EPSPs in 17% of 74 recorded neurons. In paired shock experiments, when a test EPSP is preceded by a conditioning stimulus of equal duration and amplitude, reduction of the test EPSP amplitude occurs over interstimulus intervals (ISIs) of 4-27 msec. Inhibition of test EPSPs occurred in all neurons tested. Test orthodromic action potentials (APs) are also inhibited by a conditioning stimulus. APs triggered by a depolarizing pulse (1-5 msec; 0.2-2.5 nA) were used to condition a test EPSP evoked by local stimulation. Reduction of test EPSP amplitude, indicative of recurrent inhibition (Park, M.R., et al., Brain Res., 194:359-369, 1980) occurred at ISIs less than 25 msec. Test orthodromic APs are also inhibited by a direct depolarizing conditioning stimulus. Twin near-threshold depolarizing pulses of sufficient amplitude to trigger APs were delivered at ISIs of 5-25 msec. No inhibition of the second AP was observed over these ISIs. This suggests that AP currents are not responsible for the shunting of orthodromic test EPSPs and APs. Inhibition observed in double shock experiments was antagonized in a dose-dependent manner following addition of known GABA antagonists bicuculline methiodide (10^{-5}-10^{-4}M), and picrotoxin (10^{-5}-10^{-4}M) to the bathing medium. Inhibition was also blocked by application of penicillin-G (2000 units/ml) to the bathing medium. Potentiation of test EPSPs occurred over ISIs of 4-35 msec. EPSPs conditioned by a depolarizing pulse were potentiated rather than inhibited in bathing medium containing $5x10^{-5}$M bicuculline methiodide. This finding supports previous in vivo studies (Park et al., 1980) which demonstrated neostriatal recurrent inhibition to be mediated by GABA. Inhibition of test EPSPs returned following wash in normal bathing medium. Neurons exhibiting recurrent inhibition were injected with horseradish peroxidase (HRP) and identified as medium spiny neurons. Supported by USPHS Grant NS 14866 (to STK).

274.2 LONG LASTING INHIBITION OF NEOSTRIATAL NEURONS FOLLOWING AFFERENT STIMULATION RESULTS FROM DISFACILITATION VIA CORTICO-STRIATAL AND THALAMOSTRIATAL FIBERS. C.J. Wilson*, H.T. Chang and S.T. Kitai. Dept. of Anatomy, Mich. State Univ.,E.Lansing, MI. 48824.

Stimulation of neostriatal afferents from substantia nigra, thalamus and cerebral cortex produces a complex sequence of excitation and inhibition in neostriatal neurons. The inhibitory portion of this response lasts 100-300 msec and can be seen both as a hyperpolarization of neostriatal neurons or as a reduction of amplitude of EPSPs produced by stimuli conditioned by previous stimulation of neostriatal afferents. Intracellular recordings from neostriatal neurons were compared in intact rats, rats subject to unilateral acute or chronic decortication, or acute decortication combined with unilateral knife cuts through thalamus at the level of the rostral pole of the parafascicular nucleus. Stimuli were applied to ipsilateral cerebral cortex, thalamus, substantia nigra or descending cortical axons at the level of the pons. In acute or chronic decorticate rats, EPSPs evoked by stimulation of intact striatal afferent fibers were not followed by hyperpolarizations. Monosynaptic EPSPs evoked from pons, substantia nigra or thalamus in acute or chronic decorticate rats showed no decrease in amplitude with paired shocks at interstimulus intervals from 30 to 150 msec, a range in which pronounced attenuation of test responses is obtained in intact animals. Polysynaptic EPSP components evoked from pons, substantia nigra or thalamus in acute animals were considerably attenuated in paired stimulus tests over this range of intervals. These polysynaptic responses were abolished by thalamic transection, after which no evidence of long lasting inhibition could be demonstrated. Intracellular recordings from identified brainstem-projecting neurons in the cerebral cortex of intact rats exhibited EPSP-IPSP sequences with the same time course following stimulation at these same sites. These neurons, which have been previously shown to contribute to the corticostriatal projection, exhibited the same responses to paired stimuli as those seen in neostriatal neurons in the intact animal. These findings indicate that prolonged hyperpolarization in neostriatal neurons arises primarily from a temporary cessation of a tonic excitatory corticostriatal input. Attenuation of test responses in paired stimulus experiments likewise affects only polysynaptic components of excitatory responses in neostriatal neurons, and results from inhibition in cortical and thalamic circuits responsible for these responses. Supported by USPHS Grants 17294 (to CJW) and 14866 (to STK).

274.3 LOSS OF NEUROCHEMICAL RESPONSES TO ACUTE AND CHRONIC HALOPERIDOL IN THE INTACT CAUDATE-PUTAMEN OF RATS WITH UNILATERAL LESIONS OF NIGROSTRIATAL DOPAMINE PATHWAYS. K. Gale and H. Bernstein*. Department of Pharmacology, Georgetown University Schools of Medicine & Dentistry, Washington, DC 20007.

The intact caudate-putamen (CP) contralateral to a unilateral 6-hydroxydopamine (6-OHDA) lesion placed in the medial forebrain bundle (resulting in > 95% decrease of tyrosine hydroxylase in the ipsilateral CP), was examined for neurochemical changes in response to haloperidol. Two effects of haloperidol were evaluated: 1) activation of tyrosine hydroxylase (TH), measured as a decrease in the Km for pteridine cofactor, following acute injection of haloperidol (1.0mg/kg i.p. 45 min before killing) and 2) increase in the activity of glutamic acid decarboxylase (GAD) (1.0 mg/kg s.c. daily for 6 weeks). In the acute studies, rats were lesioned with 6-OHDA and then challenged with haloperidol 3, 7 or 21 days postoperatively. Rats which were not lesioned, vehicle-lesioned, or lesioned with 6-OHDA 3 days before, showed a significant decrease (2-3 fold) in the Km of striatal TH for cofactor in response to haloperidol; no change in Vmax of TH was observed. In contrast, in rats which were lesioned with 6-OHDA 7 or 21 days before, haloperidol did not cause a change in the affinity of TH for cofactor, as compared with striatal TH from rats which had not received haloperidol. The TH of the CP contralateral to the 6-OHDA lesions was not significantly different (with respect to Km and Vmax) from that obtained from non-lesioned controls. In the chronic studies, rats were lesioned with 6-OHDA and subsequently (one week later) started on daily haloperidol treatment. Four days following cessation of chronic treatment, rats were killed for measurement of GAD. Chronic haloperidol administration in rats which were not lesioned or vehicle-lesioned caused a significant increase (140%-160% control) in GAD activity in the CP from both hemispheres. In contrast, after chronic haloperidol treatment, rats with unilateral 6-OHDA lesions showed no increase in GAD in the CP of the intact hemisphere when compared with 6-OHDA lesioned and non-lesioned controls (chronic saline injections). In the ipsilateral CP, 6-OHDA lesions caused a significant increase (150% of control) in GAD activity which was not further altered by chronic haloperidol treatment.

These data suggest that unilateral 6-OHDA-induced degeneration of dopamine neurons causes changes to occur in the contralateral hemisphere which render the nigrostriatal circuitry in that hemisphere resistant to acute and chronic actions of dopamine-receptor blockade.

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274.4 DOPAMINE ISLANDS AND ACETYLCHOLINESTERASE PATCHES ARE COEXTENSIVE IN THE STRIATUM OF THE FETAL CAT. Ann M. Graybiel and Clifton W. Ragsdale,Jr.* (SPON: W.A. Richards). Department Psychology & Brain Science, MIT, Cambridge, MA 02139.

The first clear indication of compartmental organization in the mammalian striatum was the finding that during development certain incoming dopaminergic (DA) fibers form clumps visible as islands of intense fluorescence in Falck-Hillarp preparations. A high degree of patterning has since been shown also in the mature striatum, where cortical and thalamic afferents, striatal projection cells and some neuropeptides are disposed in mosaics aligned with a network of cholinesterase-poor zones called striosomes. Though several similarly striking inhomogeneities have been described for the immature striatum in addition to the DA islands--including a transient appearance of patches rich in acetylcholinesterase (AchE) activity--information is lacking on the degree of correspondence among these compartmental orderings. To test for one such possible linkage we have compared the distributions of DA and AchE in the striatum of fetal cats by applying the histofluorescence and thiocholine methods to sections from the same brains.

Unfixed 16 μm sections from 3 fetuses (E49-E57) were prepared for catecholamine histofluorescence by de la Torre's modification of the glyoxylic acid method and adjoining sections were processed for AchE activity by the procedure of Geneser-Jensen and Blackstad. Patches of intense fluorescence 200-600 μm wide were scattered through the caudoputamen in each brain, as were discrete AchE-rich patches of comparable size and shape. The two sets of patches appeared to be in close register when serially adjoining sections were compared. The alignment of the DA and AchE patches was directly established by a sequential protocol in which fluorescence sections were photographed, then processed for AchE and rephotographed. The AchE patches in the caudoputamen matched the DA islands precisely, and a correspondence between AchE and DA extended into the ventral striatum-nucleus accumbens district. By contrast, there was no clear alignment of striatal neurons stained for AchE with either the DA or the AchE patches. In both cells and patches the thiocholine reaction product appeared specific for AchE as it was sensitive to BW284c51 and was demonstrable after brief elution of catecholamines with 2 N HCl. These findings are consistent with Butcher and Hodge's speculation (1976) that DA and AchE coexist in the system of dopaminergic island-fibers and suggest that these fibers are not distributed in register with AchE-containing neurons intrinsic to the striatum.

(Supported by the Scottish Rite Foundation)

274.5 THE FORMATION OF NEURONAL CLUSTERS IN THE EMBRYONIC PRIMATE NEOSTRIATUM. Scott Brand. Dept. of Anatomy, Uniformed Services Univ. of the Health Sci., Bethesda, MD 20014

An intrinsic cytoarchitectural pattern of cell clustering in the neostriatum has been demonstrated both directly and indirectly in rodents, cats and primates using techniques as varied as opiate receptor localization, acetylcholinesterase histochemistry, autoradiographic analysis of neostriatal afferents and ^{3}H-thymidine analysis of time of neuron origin. At this date there is no information concerning how and when the cell clusters form. To investigate the problem of how such clusters developed, a ^{3}H-thymidine autoradiographic study of four fetal rhesus monkeys was undertaken. Rhesus monkeys were used because of their slow rate of fetal development which allows a pulse of ^{3}H-thymidine to label a small percent of the neostriatum's neuronal population, thus increasing the resolution of the technique. Four pregnant rhesus monkeys were injected on the 40th day after conception (E40) with ^{3}H-thymidine. The total gestation period for rhesus monkeys is 165 days, of which neostriatal neurons are generated between E36 and E80 (Brand and Rakic, Neuroscience, 4:767, 1979). The fetuses were removed via Caesarean delivery and sacrificed at various times after injection, specifically, E44, E48, E65 and E80. The ^{3}H-thymidine labeled cells in the animal sacrificed at E44 were predominately located within the ganglionic eminence (ventricular ridges) in both the ventricular and subventricular layers with only a few cells having migrated into the caudate nucleus. No labeled cells were present in the putamen. On E48, labeled cells were present in both the caudate nucleus and putamen. The labeled cells at first appeared to be ubiquitously distributed throughout the neostriatum. Upon closer inspection there were isolated areas in both the caudate nucleus and putamen of the E48 specimen where labeled cells were not present suggesting the beginning of cluster formation. At this age less than 3% of the labeled cells were found in the ganglionic eminence. In the E65 specimen clusters of labeled cells were seen throughout the neostriatum. The E80 specimen showed similar clusters to the E65 specimen, but the clusters were located farther apart possibly due to the increased growth of neostriatum. Several conclusions can be derived based on the present data: (1) clusters are formed after the neurons have migrated and reached both the caudate nucleus and putamen and (2) clusters begin to form prior to the beginning of synaptogenesis (first neostriatal synapses are formed at E65, Brand and Rakic, Anat. Rec., 193:490, 1979). Supported by N.I.H. grant NS16905.

274.6 ULTRASTRUCTURE OF IMMUNOCYTOCHEMICALLY IDENTIFIED SEROTONINERGIC AXONS IN THE NEOSTRIATUM AND PALLIDUM OF MONKEYS. Pedro Pasik, Tauba Pasik, Jorge Pecci Saavedra and Gay R. Holstein*. Dept. Neurol., Mount Sinai Sch. Med., CUNY, New York, N.Y. 10029.

Monkeys (M. fascicularis) were briefly perfused with 4% pure formaldehyde and 0.25% gluteraldehyde in 0.12M phosphate buffer. Some animals received an intracerebral 3 µl injection of 2.5% colchicine solution, 16 hr prior to perfusion. Others were given Pargyline (75 mg/kg) and tryptophane (100 mg/kg) i.p., three and one hour, respectively, before sacrifice. Vibratome 30 µm sections were incubated with rabbit antisera raised against a serotonin-BSA conjugate, and further processed with Sternberger's PAP technique. No immunostain was detected in sections incubated in the same antisera preabsorbed with serotonin-BSA, or coprecipitated with the conjugate plus rabbit anti-BSA.

Light microscopy of the caudate nucleus, putamen and both segments of the globus pallidus reveals the presence of immunoreactive axons, branching profusely into 0.1-0.2 µm diameter fibers with irregularly spaced varicosities up to 0.7 µm in size. Electron microscopy of serial sections shows profiles filled with dense, mostly round particles, of sizes within the range of synaptic vesicles. Occasionally, 26-30 nm electron lucent vesicles are seen in the midst of the denser material; these probably represent cross-sections of vesicles with the label attached to the outer surface. Reaction product is also observed studding outer mitochondrial membranes. The high density of the deposits contrasts with the finely granular reaction product present in perikaryon and dendrites of neurons in the raphe nuclei of the same animals. In the neostriatum, the labeled profiles are near dendrites of Spiny I type neurons, and in some instances are clearly presynaptic to dendritic spines or, less frequently, dendritic trunks. These synapses exhibit a markedly thick postsynaptic membrane. In the pallidum, immunoreactivity is present in profiles of similar morphology to those of the striatum. Sometimes, one such element is seen as a single fiber within a bundle of thin unlabeled axons, probably of striatal origin. Some of the immunostained axons are finely myelinated. Only rarely, a synapse is formed between a labeled profile and a dendritic trunk receiving other unlabeled terminals. The diameter of such dendritic elements suggests that they are distal segments of the dendrites of large pallidal cells.

The results offer a positive identification of serotoninergic terminals, at least in the neostriatum. The marked asymmetry of the synapses supports the physiologically defined excitatory nature of these endings. It is not clear whether the serotoninergic fibers in the pallidum provide similar innervation to this structure or represent passing axons en route to the striatum.

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274.7 POSTNATAL DEVELOPMENT OF THE KITTEN SUBSTANTIA NIGRA: A COMPARATIVE GOLGI AND ELECTRON MICROSCOPY STUDY. P.E. PHELPS an A.M. Adinolfi, Dept. of Anatomy, UCLA Medical School, Los Angeles, Calif. 90024.

Rapid Golgi impregnation and electron microscopy were used to describe the morphological maturation of substantia nigra neurons from newborn to 55 days old kittens. Observations were made from 33 animals grouped at 1-3, 7-10, 18-24, and 40-55 days of age. In Golgi preparations, nigral neurons were selected from the pars compacta, reticulata and lateralis. Cell bodies vary in size and range in shape from round or oval to triangular or fusiform. Somatic appendages are found on cells in the younger age groups but are rare in the oldest group. Three to five primary dendrites taper gradually from the cell body and branch infrequently. Neurons located centrally in pars reticulata have dendrites which course rostrocaudally in sagittal sections and ventrolaterally and dorsomedially in coronal sections. Near the ventral border of the nigra, pars reticulata dendrites course parallel to the crus cerebri. Near the dorsal surface, dendrites of pars compacta neurons run parallel to the medial lemniscus but send their longest dendrites ventrally into pars reticulata.

Dendritic shafts at 1-3 days and 7-10 days exhibit prominent varicosities and filiform processes along their shafts and growth cones at their tips. Branching commonly occurs at large varicosities. Ultrastructurally, these dendritic varicosities contain a fine floccular matrix, mitochondria, and a few membranous and tubular elements. The constricted portions of the dendrites are packed with parallel arrays of neurotubules. The filopodia are long and thin and contain an electron dense matrix, glycogen, and a few vesicles. Synaptic contacts occur on the dendrites and filiform processes but much of these surfaces are covered with astroglial processes. The dendrites at 18-24 days are longer thicker, and have more regular contours proximally. Varicosities and filopodia are still present towards the dendritic ends. Most dendrites have many more spine-like appendages in this age group than at younger or older ages. We suggest that these neurons are in a spiny stage of maturation. At 40-55 days, dendrites are longer and most have smooth surfaces which are covered by synaptic contacts. Astroglial processes, apposed to dendritic surfaces in younger kittens, now envelop the axodendritic complexes. Most synapses at all ages form symmetrical contacts and the axon terminals contain an increasing number of mitochondria and pleomorphic vesicles. These have been classified as type I and are neostriatal in origin.

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274.8 **ACETYLCHOLINESTERASE-CONTAINING PROJECTION FROM THE BASAL FOREBRAIN TO THE SUBSTANTIA NIGRA IN THE RAT.** Nancy J. Woolf(1) and Larry L. Butcher(1,2) Brain Research Institute(1) and Department of Psychology(2) University of California, Los Angeles, CA 90024.

Evans Blue dye (EB) was injected into the pars compacta of the substantia nigra and retrorubral area. After the brains were sectioned and the tissue examined for retrogradely transported label, slides were counterstained for acetylcholinesterase (AChE). This procedure led to the visualization of EB dye in AChE-containing cells throughout the basal forebrain. Retrogradely labelled neurons were found in the ventral striatum, ventral pallidum, nuclei of the vertical and horizontal limbs of the diagonal band, lateral and medial proptic nuclei, medial septum and nucleus ansa lenticularis. These neurons had light, medium and dark AChE staining charateristics.

Our delineation of the ventral striatum and pallidum is similar to that described by Heimer (in Limbic Mechanisms: The Continuing Evolution of the Limbic System Concept, Eds. Livingston and Hornykiewicz, 1978). Heimer described the ventral striatum in the rat as the subcommissural caudate-putmen that extends by cell bridges to the olfactory tubercle. We, however, define ventral striatum as the ventralmost caudate-putamen extending above and below the posterior limb of the anterior commissure, but not part of the olfactory tubercle. This delineation is based upon the presence of small intensely AChE stained and large lightly AChE stained EB labelled neurons, which are atypical of neurons found in the caudate-putamen.

Since some basal forebrain cells projecting to the midbrain stain intensely for AChE, it is possible that this pathway is cholinergic. This AChE-conaining projection to the midbrain does not originate in the magnocellular basal nucleus, known to give rise to a cholinergic projection to the neocortex (Wenk et al., Brain Res. Rev., 1980, 2, 295-316). Biochemical data suggesting a cholinergic nigral afferent do exist, however. (Butcher, L. L., Ed., Cholinergic-monoaminergic interactions in the Brain). [Support: USPHS NS-10928 to L.L.B.]

274.9 DOPAMINERGIC SYSTEMS CONTROL METABOLISM IN LATERAL HABENULAR NUCLEUS INDIRECTLY VIA THE ENTOPEDUNCULARIS. G. F. Wooten, Dept. Neurology, Washington Univ. Sch. Med. St. Louis, MO 63110

Administration of dopamine (DA) agonists decreased and antagonists increased glucose utilization (GU) in the lateral habenular nucleus (LHN) (Brain Res. 194:117-124, 1980). Six-hydroxydopamine lesions of substantia nigra resulted in an increase in GU in the ipsilateral LHN, an effect reversed by apomorphine but not amphetamine administration (Neurosci. Abstr. 6: 124.2, 1980; J. Neurosci. 1:285-291, 1981). The LHN receives its major source of afferents from the entopeduncularis (EP), but recent studies have also demonstrated the existence of direct projections to LHN from DA neurons in the mesencephalon. To determine whether DA neurotransmission controls GU in LHN by a direct action or alternatively via polysynaptic pathways to LHN via EP, we have made selective lesions in EP with kainic acid and studied the effects of these lesions on GU in the LHN by ^{14}C-2 deoxyglucose autoradiography.

Glucose Utilization in Lateral Habenular Nucleus
(O.D. in LHN/O.D. in mean white matter)

Condition	Left side	Right side
Naive Control	3.7±0.2	3.6±0.2
Apomorphine (1 mg/kg S.C.)	2.9±0.2	2.8±0.3
Fluphenazine (1 mg/kg S.C.)	6.6±0.3	6.9±0.2
Entopeduncularis lesion (on left side)	2.9±0.1	3.8±0.2
Entopeduncularis lesion (Left) + Fluphenazine 1 mg/kg (S.C.)	3.1±0.2	6.1±0.3
Entopeduncularis lesion (Left) + Apomorphine 1 mg/kg S.C.	2.7±0.1	2.8±0.2

Thus entopeduncularis lesions prevent the fluphenazine-induced increase in GU in the LHN. Further, apomorphine administration does not affect GU in the LHN ipsilateral to an EP lesion. These results suggest that dopamine neurotransmission controls GU in LHN indirectly via a polysynaptic pathway requiring integrity of the entopeduncularis.

274.10 SUBSTANTIA NIGRA PROJECTIONS TO THE MESENCEPHALIC LOCOMOTOR REGION. Garcia-Rill, E., Skinner, R. D. and Smith, M.*, Dept. of Anatomy, University of Arkansas, Little Rock, AR 72205.

Recent studies from our laboratories described a small projection from the entopeduncular nucleus (EN) to the Mesencephalic Locomotor Region (MLR) in the cat. Extracellularly recorded EN neurons were activated antidromically from the same posterior mesencephalic site which when stimulated after performing a precollicular-postmamillary transection, elicited locomotion on a treadmill. In parallel anatomical experiments injections of fluorescent dyes into the area of the MLR induced retrograde cell labeling in EN as well as in the substantia nigra (SN).

The present study was undertaken to determine electrophysiologically if there are direct projections from SN to the physiologically-defined MLR. Under short-acting barbiturate anesthesia, the carotid arteries were ligated and the cortex overlying the midbrain removed by suction. A precollicular-postmamillary transection was made, the animal's weight was supported in a hammock and the limbs lowered onto a moving treadmill. A stimulating electrode was stereotactically lowered into the area of the MLR until the lowest threshold for locomotion (and EMG) was obtained (usually 20-50μA, 60Hz, 1 ms duration pulses). The animal was then locally anesthetized and paralyzed for single unit extracellular recording. Cells in the SN were studied for anti- and orthodromic responses to activation of the MLR electrode. Locations of recorded neurons were identified using Fast Green dye injections through the recording electrode.

Our results revealed that less than 10% of SN neurons were antidromically activated from the MLR (mean latency 1.9 ± 0.8 ms). These neurons were located in the posterior part of SN, mostly in the dorsal pars reticulata, both medially and laterally. A few neurons in the retrorubral nucleus also appear to send projections to the MLR. These findings are supported by our previous anatomical experiments. A large number of SN neurons also responded orthodromically in a bimodal distribution according to latency. Close to 16% of SN neurons responded orthodromically at short latency (2.5 ± 0.7 ms), and about 22% of SN cells responded at a longer latency (4.9 ± 1.1 ms).

These findings provide evidence for a small projection from SN to the physiologically-identified MLR. A similar projection from EN also has been described. The convergence of two major outputs of the basal ganglia at the level of the MLR suggests that these projections may trigger and/or modulate sequences of movement involving locomotion. Absence of activity in these pathways may lead to an inability to initiate these sequences of movement (akinesia).

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274.11 A REEVALUATION OF THE ROLE OF SUPERIOR COLLICULUS IN TURNING BEHAVIOUR. G. Di Chiara, M. Morelli*, A. Imperato* and M.L. Porceddu*. Institute of Pharmacology, University of Cagliari, Cagliari, Italy.

Much debate does exist over the role of superior colliculus (SC) in turning behaviour. In order to clarify this issue, unilateral kainate lesions were made by infusing 0.25 μg of kainate at two different anterior planes 0.8 mm apart in the lateral or in the medial aspects of the deep collicular layers (DLSC), in the dorsal mesencephalic reticular formation (MRF), or in the lateral periaqueductal grey (PAG), both in normal rats and in rats made unilaterally supersensitive to DA-receptor agonists by unilateral infusion of 6OHDA in the rostral substantia nigra. The effect of kainate lesions on spontaneous as well as on apomorphine-induced motor behaviour was studied. In normal rats, unilateral kainate lesions of lateral DLSC or dorsal MRF resulted in a short-lasting spontaneous ipsiversive turning and in a persistent ipsiversive circling in response to peripheral apomorphine. In 6OHDA rats, kainate lesions of lateral DLSC or of dorsal MRF ipsilateral to 6OHDA denervation reduced or even reversed the contralateral circling normally elicited in these rats by peripheral apomorphine. Lesions of dorsal MRF were always more effective than lesions of lateral DLSC in producing these changes. Kainate lesions restricted to medial DLSC or to the PAG failed to elicit motor asymmetries in normal rats or to significantly modify the intensity of contralateral turning in 6OHDA rats. These results clearly indicate that the lateral DLSC play an important role in turning behaviour. Previous negative conclusions on the role of SC in turning probably derive from inadequate localization and extent of these lesions as well as from the use of bilateral SC lesions. These results also indicate that the dorsal MRF and not the PAG is responsabile for the turning effects obtained by lesions of the subcollicular tegmentum. On the basis of these data we propose that the deep collicular layers and the dorsal MRF form a complex of primary importance as an out-put station for the expression of striatal function.

275.1 PERIPHERAL CAERULEIN, LIKE CCK, ACTS IN THE ABDOMEN AND NOT IN THE BRAIN TO PRODUCE SATIETY IN RATS. C. Jerome*, P. Kulkosky*, K. Simansky and G.P. Smith. Dept. of Psychiatry, Cornell Univ. Med. Coll., The New York Hospital, White Plains, NY 10605.

Peripheral administration of CCK produces satiety in rats by acting at a vagally innervated abdominal site (Smith et al.,1979). Peripheral administration of caerulein, a decapeptide that is structurally similar to CCK, also elicits satiety in rats. We blocked the satiety effect of caerulein by subdiaphragmatic vagotomy (Simansky et al., 1980). This suggests that caerulein, like CCK, acts at a vagally innervated abdominal site.

Stern et al. (1976), however, suggested that caerulein acted directly on the brain because (1) low doses of caerulein administered intraventricularly inhibited food intake; and (2) bilateral ventromedial hypothalamic (VMH) lesions abolished the satiety effect of one dose of caerulein administered peripherally. Given the structural similarities of caerulein and CCK, the possibility that they act at different sites of action is paradoxical. Since the paradox depends on the results of Stern et al., we attempted to replicate them. In the first experiment, caerulein (.01 to 4.0 µg/10 µl CSF) or 10 µl CSF was administered unilaterally to 5 rats through chronic lateral ventricular cannulas. No dose of caerulein inhibited 30-min food intake after 18-h food deprivation:

	Caerulein (µg)				
CSF	.01	0.1	1.0	2.0	4.0
16.0±2.6*	18.4±2.4	19.8±3.5	16.6±2.2	16.0±3.5	18.4±2.6

*Mean ± S.E. (ml/30 min)

In the second experiment, caerulein (.25 to 2.0 µg/kg) was administered peripherally (ip) to 8 rats with bilateral VMH lesions and to 8 surgical control rats. The body weight of the VMH rats was maintained within 20% of preoperative body weight by restricting food intake. All doses of caerulein produced equal inhibitions of 30-min food intake after 17-h food deprivation in VMH and control rats:

	% Inhibition (±S.E.)			
Caerulein (µg/kg, ip)	0.25	0.5	1.0	2.0
VMH	7.3±12.1	35.2±10.0	50.4±13.4	79.1±4.9
Control	16.2±10.0	24.6±8.9	38.6±6.1	64.1±13.8

Since we cannot replicate the results of Stern et al., we conclude that there is no paradox. Caerulein, like CCK, acts at a vagally innervated abdominal site in the rat.

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275.2 GASTRIN RELEASING PEPTIDE (GRP) REDUCES FOOD INTAKE IN RATS. Leslie J. Stein*, David B. West*, and Stephen C. Woods. Dept. of Psychology, University of Washington, Seattle, WA. 98195.

Bombesin (BBS),a tetradecapeptide originally isolated from frog skin, has been shown to be physiologically active in mammalian systems. Intracranial administration of nanogram quantities of BBS to rats results in alterations of glucoregulation (Brown et al.,Life Sci., 1977, 21, 1729) and thermoregulation (Brown et al., Science, 1976, 196, 998). Furthermore, peripheral injections of BBS elicit satiety in rats (Gibbs et al., Nature, 1979, 282, 208).

Recently, a 27-amino acid peptide, termed gastrin releasing peptide (GRP), has been isolated from porcine gut (MacDonald et al., Biochem. Biomed. Res. Commun., 1979, 90, 227). GRP and BBS share a common C-terminal decapeptide, and GRP has been reported to reproduce the disruptions of thermoregulation and glucoregulation induced by injections of BBS (Brown et al., Life Sci., 1980, 27, 125). We now report that GRP also mimics the satiety-producing effects of BBS.

Long-Evans rats were deprived of food every day for six hours. After two weeks on this regimen, they were injected intraperitoneally (ip) with either saline or GRP at the end of the deprivation period and then allowed access to a liquid diet (Ensure, Ross Labs) for thirty minutes. Chow was then returned to the rats until the next morning. Injection of 4 µg/kg of GRP resulted in a 16.6 ± 7.1% suppression of liquid food intake compared to saline control days ($p<.02$); 8 µg/kg of GRP reduced food intake by 35.0 ± 5.0% ($p<.001$). Neither dose had any effect on chow intake over the remainder of the day. The percent suppression of food intake induced by ip injections of GRP was comparable to that we have observed with approximately equimolar doses of BBS when given either ip or subcutaneously.

These results lend additional support to the hypotheses that 1) GRP may be a mammalian counterpart of BBS, and 2) BBS/GRP has a role as an endogenous satiety hormone in mammalian systems.

Supported by NIH grant AM-17844. We thank J. Rivier of The Salk Institute for supplying the GRP and BBS.

275.3 LITORIN SUPPRESSES FOOD INTAKE IN RATS. P.J. Kulkosky*, D.J. Fauser*, and J. Gibbs. (SPON: P.R. McHugh). Cornell University Medical College and Edward W. Bourne Behavioral Research Laboratory, The New York Hospital, White Plains, NY 10605.

Litorin (LIT) is a bombesin-like nonapeptide recently isolated from the skin of the frog Litoria aurea (Anastasi, Erspamer, & Endean, 1975). The C-terminal nonapeptide of tetradecapeptide bombesin (BBS) differs from LIT by a single substitution at the penultimate position. BBS has recently been shown to specifically suppress deprivation-induced, tail-pinch-induced, and sham feeding in rats after peripheral injection. We examined the effects of peripheral injections of LIT on ingestion and other behaviors in rats to determine if LIT acts analogously to BBS in feeding suppression.

Six rats were first adapted to an 18-hr food deprivation schedule with 30-min access to 25% liquid diet (EC 116, GIBCO), preceded by an intraperitoneal 0.15M NaCl injection. Intraperitoneal injections of LIT and BBS were then given across a dose range of 0.5-128.0 µg/kg. Significant suppressions of food intake were observed after BBS injections at all doses $\geq$ 1.0 µg/kg, and with LIT at all doses $\geq$ 4.0 µg/kg. Despite threshold differences, feeding inhibition by these peptides did not differ significantly at any dose, with a maximal effect of about 50% at all doses $\geq$ 16.0 µg/kg. However, BBS was more effective than LIT on a molar basis. In addition, both peptides similarly suppressed 3-hr-deprivation-induced liquid diet intake in a second group of rats (n=11).

An observational time-sampling behavioral analysis of demonstrated reliability (Gibbs et al., 1980) revealed that only feeding behaviors were significantly decreased following 8.0 µg/kg LIT, relative to saline control: grooming, standing, resting and other behaviors did not differ significantly from saline. Thus, the characteristic sequence of behaviors which follow normal meal termination appeared intact after LIT injection.

We conclude that litorin, which has previously been shown to share several visceral actions with its analog bombesin after peripheral administration, also suppresses food intake and mimics natural satiety.

This study was supported by USPHS grant AM 17240.

275.4 LATERAL HYPOTHALAMIC INJECTIONS OF BOMBESIN SUPPRESS FOOD INTAKE IN RATS. J.A. Stuckey* and J. Gibbs. (SPON: B. Kaplan). Cornell University Medical College and Bourne Laboratory, The New York Hospital, White Plains, NY 10605.

Bombesin (BBS) has been shown to reduce food intake after intraperitoneal or intracerebroventricular administration. We examined the possibility that BBS acts at a CNS tissue site to reduce food intake by injecting BBS into the hypothalamus, an area implicated in the control of food intake and which contains BBS-like immunoreactivity (Villarreal & Brown, 1978) and BBS receptors (Moody et al., 1978).

Eleven male Sprague-Dawley rats weighing approximately 350g were adapted to a 3-h food deprivation schedule; tap water was always available. Following implantation with bilateral stainless steel cannulae directed toward the lateral hypothalamus (from bregma posterior 2.5 mm, lateral 1.75 mm, and 8.2 mm ventral to the skull surface) and recovery, rats received bilateral injections of 0.5, 5.0, or 50.0 ng BBS in 0.5 µl 0.15 M NaCl alternating with equivolumetric 0.15 M NaCl control injections in a crossover design. Liquid food (40% Bioserve EC 116) was presented immediately following injections. Food intakes were measured and behavior was recorded using a reliable time-sampling observational method.

Results of BBS injections on the size of the first meal were as follows:

Dose of BBS (ng)	0.5	5.0	50.0
Percent change ± SEM	+1.5±7.4	-18.3±6.0*	-23.6±8.2*

Both the 50.0 ng and 5.0 ng doses produced statistically significant decreases in the size of the first meal (*p <.02). Analysis of the behavioral data revealed that rats displayed all of the behavioral characteristics of normal satiety in the appropriate sequence following the first meal. The latency to apparent sleep was increased following BBS administration. No abnormal behaviors occurred. Injections of 50 ng BBS had no significant effects on body temperature or water intake. To determine if the structural constraints for this feeding effect are the same as for previously documented biological effects of BBS, rats were injected with [D-Trp^8] BBS (courtesy of J. Rivier, Salk Institute, La Jolla) an analogue which shows only 1% of the biological activity of natural BBS (Rivier & Brown, 1978). Fifty ng of [D-Trp^8] BBS injected bilaterally failed to suppress food intake.

We conclude that BBS injected into the lateral hypothalamus of rats suppresses food intake. It remains to be determined if the satiety effect of peripherally-injected BBS is due to its action at this and/or other brain sites.

This study supported by USPHS grant AM17240.

275.5 BOMBESIN INDUCES GASTROINTESTINAL ABNORMALITIES WHICH REDUCES FOOD INTAKE. J.A. Deutsch* and W.G. Young. (SPON: L. Kromer). Department of Neurosciences, University of California, San Diego, La Jolla, CA 92093.

Bombesin (BBS) (2-16 mcg/kg) reduces food intake in rats (Gibbs et al, Nature. 282, 208-210, 1980). BBS (16 mcg/kg) also produces a conditioned taste aversion to flavored water (Deutsch and Parsons, Behav. Neur. Biol. 31, 110-113, 1981) suggesting that BBS produces malaise. Also, BBS (2-16 mcg/kg) produces gastrointestinal (GI) abnormalities in the form of elevated intragastric pressures and intragastric contraction amplitudes (Young, W.G. Soc. Neurosci. Abstr. 6, 165, 1980). Both the GI abnormalities and the food intake reduction are antagonized by the concurrent administration of diazepam (5 mg/kg) (Young, Deutsch, and Tom, Fed. Proc. 40, 941, 1981). The GI abnormalities suggest that aversion to ingested substances develops through an interaction of the ingested substance and the abnormal GI physiology prevailing after injections of BBS. Therefore, replacing the flavored water in the aversion studies with flavored nutrient would exacerbate the BBS-induced GI abnormalities and produce a quicker aversion with lower dosages. This did in fact occur.

8 Sprague-Dawley rats were trained to feed on undiluted condensed Carnation milk for one week and then divided into two groups. The first group was injected with saline (S) and the second group was injected with 8 mcg/kg of BBS. Both drank banana flavored milk. On the next day, the injections were reversed and the animals were fed on almond flavored milk. On the third day, the animals chose between the two flavored diets. The mean intake of the S-paired flavor was 12.5 ml + 8.7 ml SD and the mean of the BBS-paired flavor was 4.0 ml + 5.6 ml SD. This difference was not yet significant ($t = 1.79$, $p > 0.10$). The pairings were repeated in reverse order on days 4 and 5 and a choice was again presented on the day 6. The mean intake of the S-paired flavor was 16.8 + 7.6 ml and the mean of the BBS-paired flavor was 4.1 + 7.2 ml. This difference was significant ($t = 2.59$, $p < 0.05$).

The aversion to flavored nutrient was swift with this medium dose of 8 mcg/kg. A significant aversive effect was produced with the second pairing. When water was the unconditioned stimulus, three pairings with a higher dose of 16 mcg/kg was necessary. This data suggest that interaction with dietary substances after BBS injections produces aversion greater than with water or when no ingestive substance is used, perhaps through such clinically defined mechanisms as increased gastric emptying, producing a "dumping syndrome".

275.6 FEEDING AND DRINKING ARE AROUSED BY CHEMICAL INJECTION INTO THE INFANT BRAIN. S. Ellis* and A.N. Epstein. Inst. of Neurol. Sci. and Dept. of Biology, Univ. of Penn., Phila., PA 19104.

The responsiveness of the infant brain to neurochemical agents that arouse feeding and drinking in the adult has already been shown by Misantone, Ellis & Epstein (Brain Res., 186: 1980) who elicited water drinking with intracranial injection of angiotensin in new born rats. These experiments extend this line of research to include: 1) norepinephrine (NE), which increases food intake and 2) carbachol (Carb), a well-known intracranial dipsogen in the rat.

In examining their effects on the pups ingestive behavior, we employed the anterior oral cannula developed by Hall that allows the suckling rat to ingest independently of its mother. Weight gain was the measure of intake (fecal and urine losses being prevented by celloidinization of the perineum). Pups of various ages were removed from their mother, placed in a warm (32°C) and moist incubator. They were then satiated with 15s infusions of either milk (Bordon's Half & Half) or water every 2 min for 20 min after which they were reweighed (total infused = 5% body weight). This regime was continued until weight gained tapered off, typically after 1 hour. Animals were then injected intraventricularly with either 100ng AII, 2µgNE or 200ng Carb in a vehicle of 50% India ink and 50% isotonic saline or with 1µl of vehicle alone (cf. Misantone, et al for injection procedure). Pups were replaced in the incubator, the injection regime was continued for 30 min, and they were reweighed at 10 min intervals. After the final weighing they were decapitated and the site of injection was determined by examination of the surface of a coronal section through the head. For AII and Carb-injected pups, the lateral ventricle was designated as the site of a successful injection. For NE-injected animals the IIIrd ventricle was chosen, since the paraventricular nucleus is sensitive for adrenergic stimulation of feeding.

AII-injected rats as young as 2 days old drank significantly more water ($\bar{x}$=.09±.01g) than their vehicle-injected controls ($\bar{x}$=.02±.01g). Similarly, Carb was dipsogenic in 8 day old pups (Carb, $\bar{x}$=.25g; veh, $\bar{x}$=.03). Norepinephrine, when deposited into the IIIrd ventricle of 11-13 day old, milk-sated rats significantly increased milk intake ($\bar{x}$=.52±.08g) over both vehicle controls ($\bar{x}$=.2±.03g) and rats with NE deposited outside the IIIrd ventricle ($\bar{x}$=.2±.03g). Water intake was not affected by injections of NE. Further experiments, i.e, dose-response and blocking studies, are planned.

This work shows that the neurochemical systems for control of ingestive behavior are competent in the infant brain. Their role in suckling and in the development of adult feeding and drinking is under study. Supported by NINCDS 03469 and ITG 07517.

275.7 **BRAIN REGIONAL SEROTONIN IN HYPOTHALAMIC OBESITY: DIFFERENT DEPLETION PROFILES USING LESIONS VS. PARASAGITTAL KNIFE-CUTS.** **D.V. Coscina, J. Chambers*, J. Chang*, A. Chiu* and J.J. Warsh. Sects. Biopsychol. and Biochem. Psychiat., Clarke Inst. Psychiat., Univ. Toronto, Toronto, Canada.**

The possibility that impaired metabolism in brain serotonin (5HT) neurons produces chronic overeating has received considerable experimental attention. At last year's meeting, we presented data (Coscina et. al., Neurosci. Absts., 1980, 6, 783) showing that bilateral radiofrequency lesions of the medial hypothalamus (MH) sufficient to elicit protracted hyperphagia and obesity in female rats was associated with reliable (p<.05) 5HT depletion in hypothalamus (15%), hippocampus (-41%) and midbrain (-30%). Some of this depletion was apparently due to overeating per se as restricting food intake to normal in other MH-lesioned rats produced less depletion in hippocampus (-33%) and no depletion in midbrain. To test further the possibility that deficit brain 5HT levels are necessarily associated with overeating and obesity, independent of method used to induce them, we conducted similar experiments in adult female rats sustaining 3 mm bilateral parasagittal knife-cuts (KCs) between the MH and fornix (n=22 across two replications). As before, half of the KC rats were permitted to overeat ad lib while the remaining half were restricted to normal food intake (n=14 for sham-operated controls across two replications). While KCs were effective in eliciting hyperphagia and obesity similar to MH lesions, brain regional 5HT patterns were quite different. In hypothalamus, KCs were associated with elevations (+23-95%) rather than depletions of 5HT. In hippocampus, KCs produced either no 5HT change or much larger depletions (90%) than lesions. In midbrain, KCs produced no effects on 5HT. Furthermore, for all three brain regions studied, food restriction had no effects. We conclude that while MH lesions and KCs can produce similar effects on ingestive behavior and weight gain, the neuronal systems damaged by each technique appear quite different. This fact questions the ability of either or both technique to provide specific information about the neural substrates of overeating. Moreover, these data show that specific patterns of brain regional 5HT depletion are not necessarily the same in different experimental preparations who overeat and are obese.

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275.8 PANCREATIC GLUCAGON INHIBITS REAL FEEDING, BUT NOT SHAM FEEDING IN THE RAT. Nori Geary* and Gerard P. Smith. (SPON: H. Kissileff) Dept. Psychiatry, Cornell Univ. Med. Coll. White Plains, NY 10605

Pancreatic glucagon (PG) may be a hormonal satiety signal. Endogenous PG is released during meals (De Jong et al., 1977; Unger & Orci, 1976) and exogenous PG elicits a specific dose related inhibition of food intake (Martin & Novin, 1977) accompanied by hepatic glycogenolysis and hyperglycemia (Geary et al., 1979). These effects of exogenous PG were demonstrated within the context of all the endogenous satiety mechanisms normally activated by real feeding. To investigate the relative potency of PG to inhibit food intake and elicit satiety when endogenous satiety mechanisms are minimized, we measured the effect of PG on sham feeding. Rats (n=12) were equipped with chronic gastric cannulas and trained to sham feed (SF, cannula open) or really feed (RF, cannula closed) a palatable milk diet after 3hr food deprivation. In the RF condition rats fed for 8-12 min and then displayed the satiety sequence (baseline 30 min FI: 14.5±0.8, $\bar{x}$±SEM, ml). SF rats fed almost continuously and rarely showed satiety (baseline FI: 47.5±2.8 ml). Injection of PG inhibited RF, but not SF:

Change in 30 Min Food Intake (% Control, $\bar{x}$ ± SEM)

PG (µg/kg, i.p.)	0	100	500	2500
REAL FEEDING	-1 ± 9	-8 ± 5	-21 ± 5	-35 ± 8
SHAM FEEDING	6 ± 6	3 ± 5	-1 ± 9	0 ± 8

The possibility that PG did not inhibit SF because it did not elicit hyperglycemia during SF was tested by sacrificing SF rats 8 min after PG injection.

Change in Plasma Glucose (mg/dL, $\bar{x}$ ± SEM)

PG (µg/kg i.p.)	0	500
Portal vein	+3 ± 17	+89 ± 26
Hepatic vein	-16 ± 23	+111 ± 45

These results show that PG fails to inhibit SF despite producing marked hepatic hyperglycemia. The glycogenolytic effect of PG, which has been proposed as the mechanism of PG's satiety effect on RF (Martin & Novin, 1977; VanderWeele et al., 1979), is therefore not sufficient to inhibit SF under these conditions. Some other preabsorptive or postabsorptive mechanism(s) not activated during SF must be necessary for PG's satiety effect.

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275.9 EVIDENCE FOR IN VIVO AND IN VITRO MODULATION OF THE OPIATE RECEPTOR BY GLUCOSE. J.E. Morley, A.S.Levine*, S.A. Hess*, D.B. Brown* and B.S. Handwerger*. Neuroendocrine Research Lab, Minneapolis VAMC, Minneapolis, MN 55417.

Food intake in diabetic animals (genetic C57 BL/Ks-db+/db+ and streptozotocin-induced diabetic mice) is suppressed by a 100-fold lower dose of naloxone than in control animals. As the genetic animals are hyperinsulinemic and the streptozotocin animals hypoinsulinemic, this suggested that glucose per se modulates the opiate receptor. Further evidence of glucose involvement in the opiate receptor is suggested by the finding that feeding induced in the insulin hypoglycemic model is highly resistant to the suppressive effect of naloxone (no significant effect of 20 mg/kg compared to a significant effect at 1 mg/kg for starvation induced feeding). We next measured ^{3}H-naloxone binding over a range of 5 nM to 100 nM in whole brain minus cerebellum from obese diabetic animals (db+/db+) and their controls as well as in obese (ob/ob) animals.

	n†	K_D (nM)	B_{max} (cpm/mg protein)
Db/db	5	23.0 ± 2.3	23335 ± 1306
m/m	5	16.1 ± 1.2	21834 ± 2289
Db/db WR*	2	19.5	20972
Ob/ob	3	26.3 ± 3.3	11590 ± 1030
Ob/-	2	26.9	11433

†each number represents results from 3 pooled brains
*WR = weight restricted to control weight.

Db/db animals had a reduced affinity compared to m/m controls with weight restricted animals falling into an intermediate range. Obese animals and their controls had a decreased B_{max} compared to diabetic animals. Next we added glucose at varying concentrations to the opiate receptor assay in vitro. Glucose at 100 mg/dl and 300 mg/dl in the presence of sodium decreased affinity for the antagonist and increased the B_{max}. These data are compatible with glucose shifting the opiate receptor from the antagonist to the agonist form in the presence of sodium.

	n	K_D (nM)	B_{max} (fmol)
Tris + Na	4	16.7 ± 1.0	146 ± 15
Glu(100 mg%) + Na	2	28.2	181
Glu(300 mg%) + Na	3	33.8 ± 3.3	212 ± 23
Tris-Na	4	35.4 ± 8.8	146 ± 24

We conclude that glucose has potent effects on the opiate receptor in both the intact animal and in vitro.

275.10 MORPHINE-INDUCED HYPERPHAGIA: A BEHAVIORAL ETHOGRAM. M. Heft, G. Daniels*, A. Buller*, and A. Riley*. Psychopharmacology Laboratory, The American University, Washington, D.C. 20016.

Because of the efficacy of naloxone hydrochloride in suppressing feeding and drinking under a wide range of conditions (Siviy & Reid, East. Psychol. Ass., 1981), the endorphins have been implicated in regulatory eating and drinking. The fact that narcotic agonists often decrease consumption, however, questions this general role of endorphins in regulatory consummatory behavior (Holtzman, Life Sci., 16:1465-1470, 1975; Stapleton et al., Bull. Psychon. Soc., 13:237-239, 1979). In an account of these effects of agonists on feeding, Riley et al. (Neurosci., 6:783, 1980) suggested that while morphine produces hyperphagia, it also induces catatonia, a response which could mask any morphine-elicited changes in feeding.

To examine if the interaction of catatonia and feeding is responsible for the reported decreases in feeding produced by morphine, groups of animals were injected with either 40 mg/kg morphine sulfate or equivolume distilled water for 28 consecutive days. A range of behaviors including catatonia, feeding, drinking, grooming, sleeping, locomotion, and rearing was monitored for 6 hr post injection.

Following the first morphine injection, rats were catatonic with only minimal activity in the two hr post injection. As catatonia dissipated, the rats ate, peaking at 4 hr following morphine. Over repeated injections, the morphine-induced catatonia was less pronounced and dissipated more quickly. This decrease in catatonia was immediately paralleled by an increase in eating. Grooming and then sleeping always followed eating for the morphine-injected rats throughout the 28-day injection procedure. Throughout the study, control animals slept during the 6-hr observation period.

These data suggest that as the masking effect of catatonia is reduced with time following a single injection and over repeated injections, morphine-induced hyperphagia occurs. As such, these data offer a basis for the aforementioned effects of morphine on feeding and support the view that endorphins may be involved in regulatory consumption.

275.11 PSYCHOPHYSIOLOGICAL SUBSTRATES OF NORMAL AND ABNORMAL FEEDING AND DRINKING. S. Grossberg. Dept. of Math., Boston Univ., Boston, MA 02215.

Psychophysiological mechanisms will be derived which are capable of explaining some difficult motivated behaviors in a unified fashion. These phenomena include hyperphagia, notably the possibility of differential effects on finnickiness and obesity; cholinergic vs. angiotension drinking; schedule-induced polydipsia and related nonhomeostatic effects; self-stimulation to inject insulin at lateral hypothalamic sites; inverted U effects of amphetamine. Basic to our analysis is the notion of a gated dipole, which shows how drive, reinforcer, and arousal inputs are modulated by competitive feedback and transmitter gating effects in the regulation of motivational baseline and reset. Cholinergic-catecholaminergic interactions occur in the theory when conditional reinforcer inputs (cholinergic) are joined to drive and arousal inputs (catecholaminergic) to generate incentive motivational outputs. Hyperphagia emerges as an example of an underaroused depressive syndrome whose properties help to explain symptoms of Parkinson's disease and juvenile hyperactivity in other subsystems.

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276.1 EFFECTS OF BOTULINUM NEUROTOXIN DIRECTLY ON THE CNS: HYPOTHERMIA AND DEATH. J. L. Minnich, H. Sugiyama*, R. LoPachin* and T. Rudy. Food Research Institute and School of Pharmacy, Univ. of Wisconsin, Madison, WI 53706.

Botulinum neurotoxin's effects on the central nervous system (CNS) are not well known. We studied the effects of highly purified botulinum neurotoxin, type A (isolated by affinity chromatography) on adult rats. The toxin was injected intracerebroventricularly (i.cvt.), through an indwelling cannula, into the left lateral ventricle. Three experimental groups, of four adult rats each, were employed. In Group (1) the rats received anti-toxin (2.8 IU given i.p. at 1 hr before i.cvt. injection) plus i.cvt. toxin (390 nanograms/10 µl). The complete effectiveness of the anti-toxin dose against the toxin dose was confirmed by injections into mice. The rats in Group (2) received i.p. anti-toxin plus i.cvt. saline. Those in Group (3) received i.p. saline plus i.cvt. saline. All the animals in Group (1) died in hypothermia at 3-5 hrs after toxin injection. In the toxin-injected rats the core (colonic) temperature was 34.72° ± 0.58° at the time of death. This was significantly lower than their initial temperature of 37.75° ± 0.32°. None of the animals in Groups (2) or (3) died or showed temperature changes. The specific toxicity of the toxin used was 100 mouse i.p. LD50's per nanogram. The change in core temperature that we observed after this large dose of botulinum neurotoxin was relatively small, and it seems unlikely that the animals died from hypothermia per se. In at least some of the rats that died, it appeared that the cause of death was respiratory failure. Since both hypothermia and death occurred in animals in which peripheral effects were prevented by systemically injected anti-toxin, it appears that both these effects of botulinum neurotoxin were mediated by a direct action on the CNS.

276.2 SITES OF ACTION FOR THE EFFECTS OF INTRATHECAL NOREPINEPHRINE ON THERMOREGULATION. R. M. LoPachin* and T. A. Rudy. Univ. of Wisconsin School of Pharmacy, Madison, WI 53706.

We have examined the thermoregulatory effects of norepinephrine (NE) (0.01-0.30 µmoles) injected into spinal subarachnoid space of rats via chronic indwelling spinal catheters. Intrathecal (i.t.) NE produced dose-dependent falls in core temperature (T_c) associated with a correlated rise in tail skin temperature (T_{sk}). At the 0.30 µmole dose of i.t. NE, the hypothermia was preceded by a small, transient rise in T_c. Several lines of evidence indicate that the hypothermia and increase in T_{sk} were mediated by an influence of i.t. NE on spinal processes. NE (0.3 µmoles) injected i.t. reduced neural activity recorded from the lumbar sympathetic chain of anesthetized rats. These results suggest a NE-mediated decrease in efferent sympathetic activity. Studies with ^{3}H-NE injected i.t. demonstrated that this amine does not ascend higher than the rostral cervical cord. The changes in T_c and T_{sk} were therefore not mediated by noradrenergic stimulation of supraspinal areas. The studies with ^{3}H-NE have also showed that only after the 0.3 µmole i.t. dose are physiologically significant levels of NE found in the plasma. Thus, it seems reasonable to suggest that the hypothermia and increase in T_{sk} produced by the 0.01-0.1 µmole dose of i.t. NE were mediated by an action at spinal sites. However, the appearance of NE in the plasma is temporally related to the hyperthermia elicited by the 0.3 µmole dose of i.t. NE. This evidence, in conjunction with the finding that mecamylamine (10 mg/kg, i.p.) did not influence the hyperthermia, suggest that the hyperthermic effect of the 0.3 µmole dose was due to an action at peripheral sites. The hypothermia produced at this dose of NE also seems to involve a peripheral component since intraperitoneal injection of 0.3 µmoles NE caused a small fall in T_c, whereas the lower doses tested were ineffective. Thus, i.t. NE produces a dose-dependent change in thermoregulation which, for the 0.01-0.1 µmole doses, is mediated by an effect on spinal processes. In contrast, the effect of the 0.3 µmole dose on T_c and T_{sk} represents an action at both spinal and peripheral sites.

276.3 MORPHINE TOLERANCE ALTERS NOREPINEPHRINE INDUCED HYPOTHERMIA IN SENESCENT RATS. J.N. McDougal, P.R. Marques* and T.F. Burks. Dept of Pharmacology, University of Arizona Health Sciences Center, Tucson, Arizona 85724.

Tolerance and the associated physical dependence are a characteristic feature of opiate use and abuse. These factors are major limitations of the clinical use of opiates. We have previously shown that senescent rats acquire tolerance to the thermoregulatory effects of morphine less readily than postpubertal rats, but rats of all ages will acquire morphine tolerance with pellet implantation. How aging affects the phenomenon of tolerance to drugs is not known.

Several central nervous system transmitters, including serotonin, norepinephrine, dopamine and acetylcholine, have been shown to be involved in either the acute response to morphine or morphine tolerance. Among other receptor changes, β-adrenergic receptor density was shown to be decreased in senescent rats. Norepinephrine levels of the preoptic and hypothalamic regions of rat brain have been shown to decrease with aging. Norepinephrine injected centrally has been shown to cause hypothermia in rats and rabbits. The acute hypothermic responses to morphine are attenuated by intraventricular phentolamine, but morphine tolerance does not alter hypothermic responses to intraventricular norepinephrine is young rats. The purpose of this study was to determine if morphine tolerance alters norepinephrine-induced hypothermia in senescent rats.

Postpubertal (3 month), mature (10 month) and senescent (27 month) Fischer 344 rats from the colony sponsored by the National Institute on Aging were implanted with intraventricular cannulas and allowed to recover for at least 3 days. Norepinephrine hydrochloride (75 ug) was given intraventricularly in a volume of 5 ul. Rats were restrained in a specially designed wire-mesh restrainer which avoids the heat buildup of conventional plastic restrainers. Colonic temperature was monitored at an ambient temperature of 20-21°C. Results were expressed as change from baseline during a 4½ hour period. Norepinephrine caused a maximum hypothermia of 2-3°C in naive rats of all 3 age groups. Repeating the dose three days later showed no single dose tolerance to norepinephrine in any of the age groups. When the rats were made tolerant to morphine by subcutaneous pellet implantation, the hypothermic response to norepinephrine was reduced in the mature and senescent rats but unchanged in the postpubertal group. These results suggest that morphine tolerance alters noradrenergic sensitivity in mature and senescent rats but not in postpubertal rats. (supported by USPHS grants AG01289 & NS15420)

276.4 SET-POINT FOR BODY TEMPERATURE: THE EFFECTS OF SUPERFICIAL AND DEEP BODY RECEPTORS DURING EXERCISE. G.K. Savard*, K.E. Cooper and W.L. Veale. Department of Medical Physiology, Faculty of Medicine, University of Calgary, Calgary, Alberta, Canada T2N 1N4.

To examine the hypothesis that exercise in man does not cause a shift in the body temperature set-point, subjects were brought from a mildly hypothermic (tympanic temperature (T_{ty}) = 35.0 - 35.8°C) to an hyperthermic (T_{ty} = 37.6 - 38.2°C) state relative to the resting (set-point) temperature (close to 37°C). Subjects were seated in a quiet room for 30 min (T = 22 ± 1.0°C). Mean skin temperature (T_{sk}), T_{ty}, rectal temperature (T_{re}), heart rate (HR) and hand heat elimination (accurate measure of average hand blood flow, HBF) measurements were recorded. Subjects were then immersed in water at 17°C until their body temperature had fallen 0.6 - 1.0°C (30-45 min). They were then heated in one of 3 ways: (1) passive rewarming in a whirlpool bath (T_w = 40°C), (2) exercising on a bicycle ergometer at 65% max HR (at normal, resting body and ambient temperature) in a room maintained at 40°C (T_a), or (3) with subjects working in a suit with the water temperature at 40°C in an attempt to clamp T_{sk} between 37 and 40°C (T_a = 40°C) Resting HBF was sharply reduced when subjects entered the cold water, ie. there was almost complete skin vasoconstriction. Initial results show that the point at which HBF starts to increase from its very low level is independent of the T_{sk}, ie. it is similar whether T_{sk} is around 30°C (as in (2))or 40°C (as in (3)). On the other hand, this 'point of onset' of HBF would seem to depend on the core (T_{ty}) temperature. Preliminary results also indicate that this point is similar for both passive and active rewarming, and occurs at a slightly lower T_{ty} than resting T_{ty}.

Hence, peripheral receptor input (ie. removal of cold afferent stimulation and addition of warm receptor stimulation) would seem to have little effect on the return of HBF, whereas the deep body receptors (probably hypothalamic) may play a key role in the initiation of vasodilatation at these low body temperatures. Also because this central drive (T_{ty}) for the return of HBF was similar both during passive and active rewarming, it would seem that the 'controller', ie. the set-point temperature, remained unchanged, and was certainly not raised by exercise.

These results, using HBF as a peripheral indicator, support the hypothesis that there is no shift in the set-point temperature during exercise.

This work was supported by the Medical Research Council of Canada. G.K.S. is a predoctoral student of the MRC.

276.5 COLD EXPOSURE: ITS EFFECT ON CIRCULATING CATECHOLAMINES AND T_4 IN RABBITS RAISED AT 33°C. A.V. Ferguson, W.L. Veale and K.E. Cooper. Dept. of Medical Physiology, University of Calgary, Calgary, Alberta, Canada T2N 1N4.

We have recently demonstrated that the thermal environment in early life is significant to the development of temperature regulation in both the New Zealand White rabbit and the Sprague Dawley rat. Animals raised from birth at 33.0 ± 0.1°C show changes in their thermoregulatory ability which include a reduced resistance to cold exposure, a reduced and monophasic fever following intravenous injection of endotoxin and a reversal of the normal hypothermia following intrahypothalamic infusion of noradrenaline.

The present work was carried out in order to investigate the changes in circulating catecholamines and thyroxine in warm reared rabbits during cold exposure (4 hrs at 2.0 ± 1.0°C). Animals were implanted with intravenous cannulae a minimum of 1 week prior to experimentation. The cannulae were filled with heparinised saline and exteriorized through the ear. Colonic temperature was monitored continuously by use of Yellow Springs thermistor probes and blood samples were taken at 60 minute intervals throughout cold exposure. Samples were later assayed for adrenaline, noradrenaline and thyroxine.

Colonic temperature of warm reared rabbits fell 4.10 ± 1.20°C during the 4 hr cold exposure, while control animals maintained baseline temperatures throughout this time period (mean 0.07 ± 0.04°C). Baseline plasma levels of both adrenaline and noradrenaline were found to be significantly elevated in warm reared as compared with the levels of control animals. Adrenaline levels were 28 ± 11 pg/ml in control and 377 ± 87 pg/ml in warm reared rabbits, while noradrenaline levels were found to be 362 ± 34 pg/ml and 746 ± 184 pg/ml in control and warm reared animals respectively. Following 4 hrs cold exposure these levels were not significantly changed in control animals, while warm reared animals demonstrated significant increases in both monoamines, to 1980 ± 780 pg/ml for adrenaline and to 1776 ± 477 pg/ml for noradrenaline. T_4 levels in serum were found to be reduced in warm reared (mean 12 ng/ml) as compared to control (mean 26 ng/ml) rabbits, and no significant increases were observed in either group during cold exposure.

Further data on changes in TSH and T_3 during cold exposure are necessary to obtain a clearer picture regarding thyroid function in warm reared animals. However, the increase in plasma catecholamines during cold exposure suggests the sympathetic nervous system to be functional in warm reared rabbits.

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Thanks to L. Bauce for performing catecholamine assays.

276.7 PRECISE ANATOMICAL LOCALIZATION OF THERMAL BIOFEEDBACK SELF-REGULATION AROUND DIFFERENT FEEDBACK LOCI ON THE HAND IN HUMANS. Edward Taub*, Thomas Spalding and John Kunz. Inst. for Behavioral Research, 2429 Linden Lane, Silver Spring, MD 20910.

A biofeedback technique was developed in this laboratory for enabling most humans to establish rapid self-regulatory control of their skin temperature. It involves operant conditioning of small variations in skin temperature by means of changes in a visual information display. Pilot work had indicated that when feedback was given from a single location on the hand the learned temperature change is not diffuse, as might be expected of a response mediated by the autonomic nervous system, but rather develops considerable anatomical specificity around the feedback locus. To verify this observation subjects in this experiment were given feedback from one of several locations, and were trained for at least twenty sessions each. Temperature was recorded from five locations on each hand.

Nine subjects were given feedback electronically averaged from the first three fingers. For the last ten sessions, temperature change at two locations on the metacarpus was significantly less (43%) than on the fingers. Four subjects were given feedback from the web dorsum. For the three subjects who learned the response, the web dorsum exhibited a much greater (384%) effect than the fingers. In addition, temperature change at this location was 299% greater than at the hypothenar eminence, thus producing a clear fractionation within the metacarpus. Two subjects given feedback from the hypothenar eminence learned the response and exhibited a greater temperature response there than on the fingers (154%) or on the web dorsum (260%). Three subjects given feedback from the index finger exhibited the expected significant advantage of the fingers over the metacarpus, and one showed a greater effect on the index finger than on the other fingers. This subject also displayed an exquisite localization of the response to the ulnar surface of the index finger, from which feedback was originally given. When the feedback locus was switched without the subject's knowledge to the radial surface of the finger, the focus of the response gradually switched to that side over a period of days. Supplementary experiments indicate that these effects are not due to movement, isometric muscular contractions or respiratory manuevers.

The results indicate that with the present technique there is a marked specificity within the same hand of temperature self-regulation effect to the anatomical loci from which feedback is given. Thus, neural control over the peripheral vasculature, as reflected in skin temperature changes, can be far more precise than has been generally considered possible.

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276.6 MATERNAL INFLUENCES ON DEVELOPMENT OF FEBRILE RESPONSES IN KITTENS J.R. Villablanca and Ch.E. Olmstead. MRRC and Depts. of Psychiat. and Anatomy, UCLA School of Medicine, Los Angeles, CA 90024.

We have described the maturation of thermoregulation in the kitten, both in response to environmental challenge (Physiol. Behav. 23: 489, 1979) and to exogenous pyrogens. In both instances a feature of the developmental profile was the considerable amount of intra- and interlitter variability. Factors to be discussed led us to consider whether the immunological status of the mother, relative to certain feline diseases, might be a significant contributor to this variability. Feline Infectious Peritonitis (FIP) is a major factor in the survival of offspring within catteries and a radioimmunoassay is readily available for evaluating the serology of the mothers (Am. J. Vet. Res. 42: 368, 1981). Eighty three kittens from term litters received i.v. injections of typhoid vaccine diluted 1:100 (dose .5ml/kg at ~10^6 organisms per ml) in numbers and age groups shown in the table. Rectal temperature (T_B) was monitored at an ambient (T_a) of 20-23° for 1 hr prior to and for a minimum of 5 hrs post-injection. Fever was estimated using a thermal index (TI = sum of ΔTB/minutes x 100). Kittens were divided into high (>1:400) and low (<1:100) titer groups on the basis of maternal FIP antibodies. The results are summarized in this table.

		LOW TITER		HIGH TITER
AGE	N	TI(S.E.M.)	N	TI(S.E.M.)
0 - 10	8	3.40 (1.28)	10	2.49 (0.46)
11 - 20	11	1.87 (0.35)	11	1.89 (0.35)
*21 - 30	7	3.90 (0.36)	6	2.17 (0.28)
*31 - 40	9	5.57 (0.65)	7	2.95 (0.52)
*41 - 50	6	7.56 (0.70)	8	4.06 (0.52)

*P < .05, Mann-Whitney U for high versus low titers

In general, kittens from both groups showed higher T_B and fevers in the immediate postnatal period. The T_B of the high titer group was significantly higher than the low during this period. The febrile response developed across the first 5 weeks of life in both groups with the maturational process being significantly slower in kittens from the high titered mothers. No indications of behavioral thermoregulation were seen during the early days in either group. These data indicate that the immunological status of the mother has significant influence on the postnatal development of thermoregulation in the kitten.

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277.1 ENTRAINMENT OF CIRCADIAN LOCOMOTOR AND DRINKING RHYTHMS IN OPHTHALMECTOMIZED RODENTS. T.G. Hedberg and A.S. Feng. Dept. Physiology and Biophysics, University of Illinois, Urbana, IL 61801.

The entrainment of various cyclic behavioral functions such as locomotor, feeding and drinking activity to a 24-hour light:dark cycle has been frequently demonstrated in many species of mammals. Ocular photoreception is the primary if not sole mechanism of rhythm entrainment for most species. However, aside from Nelson and Zuckers' (1981) demonstration of the absence of circadian entrainment of wheel-running in blinded ground squirrels we know of no systematic investigations of entrainment mechanisms in diurnal mammals deprived of ocular photoreception.

In this study, the circadian rhythms of feeding, drinking and locomotion were monitored in ten intact Monogolian gerbils (Meriones unguiculatus) kept in a 10:14 LD cycle. Under a light source which approximated natural sunlight in spectral range and intensity, all three activities showed circadian rhythms which became entrained to the photic cycle. Drinking began uniformly within five minutes of photoperiod onset in the three animals monitored. Locomotion and feeding activities in all animals began within a 30 minute span before or after the start of the light phase. After the photoperiod was clock-shifted to begin six hours later, free-run ensued in all three parameters but was followed by entrainment after a delay of six days.

Four months after monitoring began all animals were enucleated bilaterally under sodium pentobarbital anesthesia and returned to the same testing regimen. Activity records compiled for three months after enucleation show persistance of the entrainment of drinking and locomotion but progressive aperiodicity in the feeding rhythm. A three hour clock-shift in photoperiod onset was done 40 days after enucleation. After 13 days of free-run, entrainment of the drinking rhythm in the same three animals became evident again.

We are unable to propose a mechanism for entrainment of these cycles at the present but investigations of the influence of potential zeitgebers such as temperature and sound are currently underway. In addition, the admittedly dim possibility of rhythm mediation by extraretinal photoreception cannot be discounted.

277.2 FEEDING AND LIGHT AS ENTRAINERS OF CIRCADIAN RHYTHMS. Friedrich K. Stephan, James A. Donaldson* and Ann Robbins*. Dept. of Psychology, Florida State Univ., Tallahassee, FL. 32306

Although rats with lesions of the suprachasmatic nuclei(SCN) show only weak or no circadian rhythms in activity in ad lib. feeding conditions, activity can be entrained to restricted feeding schedules with periods between 22 and 31 h (Boulos, Rosenwasser & Terman, Behav. Brain Res., 1:39-65, 1980; Stephan, J. comp. Physiol., in press, 1981). Activity of intact rats can also be entrained to restricted feeding; however, in constant light or darkness a free-running rhythm of activity usually is observed along with feeding entrained activity (Edmonds & Adler, Physiol. & Behav., 18:915-919, 1977; Gibbs, Am. J. Physiol., 236, R249-253, 1979).

The present experiment was designed to study the interaction between light- and feeding-entrainable activity rhythms. 16 rats were then placed on a 24 h feeding schedule with 2 h access to food/day, for 40 days, followed by 25 days on ad lib.

In only 4 cases was the free-running activity rhythm synchronized by the feeding schedule. The period of the free-running rhythm in these rats was very close (15 min) to that of the feeding schedule. In most of the remaining rats, a small change in tau (10-20 min) was systematically correlated with the introduction of the feeding schedule; however, the activity rhythm continued to free-run.

A 2 h light "pulse" delivered near the onset or offset of the free-running activity pattern resulted in the expected phase delays and advances, but had no effect on feeding entrained activity.

A 4 h phase advance of the feeding schedule resulted in advancing transients in feeding entrained activity for 5-7 days and affected the phase of the free-running activity in 2 out of 4 cases. Transients in response to a 4 h phase delay were less systematic and were partially obscured by the free-running activity rhythm.

Return to ad lib. conditions caused considerable instabilities in the free-running activity. These included large phase jumps and increased variability in the onset and in the acrophase of the activity pattern.

These results suggest that feeding entrainable oscillators can be "decoupled" from the free-running rhythm. The latter is light entrainable and presumably driven by the SCN. Entrainment of oscillators by feeding appears to exert some feedback on the light entrainable oscillator(s). The former can entrain the latter if their respective periods are sufficiently close.

277.3 SUPRACHIASMATIC NUCLEI: BILATERALLY DISTRIBUTED CIRCADIAN PACEMAKER SYSTEM? Alan M. Rosenwasser*, Ricardo Eng, and Norman T. Adler (SPON: D. HURVICH). Dep'ts. of Psychology and Anatomy, and Inst. Neurol. Sci., Univ. of Pennsylvania, Philadelphia PA, 19104.

The suprachiasmatic nuclei of the hypothalamus (SCN) have been identified as crucial for the normal expression of circadian rhythmicity in mammals. However, much evidence suggests that the circadian system consists of multiple "coupled" but potentially independent oscillators. Thus, the SCN may function as a master oscillator in a coupled multioscillator system. Furthermore, the SCN may themselves contain multiple oscillators since lesions which spare SCN unilaterally are consistent with considerable sparing of circadian function. In some non-mammalian species, bilaterally distributed pacemaker systems have been identified. Analyses of such systems reveal that the two pacemakers may oscillate independently and that overall circadian organization depends upon the interaction (coupling) between them. Recent reports that the mammalian SCN are connected neurally by fibers crossing the midline in the infundibular tract suggests that the SCN are a pair of neurally coupled bilaterally distributed circadian pacemakers. The present experiments were conducted to investigate this possibility by disrupting the coupling between the two SCN with midline knife cuts.

Female Long-Evans and Sprague-Dawley rats were given parasagittal midline knife cuts using a retracting wire knife. The cuts were aimed to interrupt neural connections between the SCN and to sever the optic chiasm throughout its anterior-posterior extent. These cuts should isolate the SCN from each other and restrict retinal input to that arising from the ipsilateral eye. The subjects were maintained with access to running wheels and exposed to light-dark cycles, light-dark phase shifts, and continuous illumination. While entrainment to the light-dark cycle appeared normal, an altered pattern of reentrainment was observed following phase shifts: the activity rhythm "split" into two components, one of which phase-advanced while the other phase-delayed into the new entrainment phase. These results are consistent with the idea that each nucleus is a potentially independent pacemaker, and that the two SCN are normally coupled by mutual innervation. We are currently further exploring this hypothesis by extending the range of lighting regimens employed and by placing unilateral SCN lesions.

Supported by NIH grants HD04522 and MH15092.

277.4 SPLITTING OF THE CIRCADIAN RHYTHM OF ACTIVITY IN THE GOLDEN HAMSTER IS ABOLISHED BY UNILATERAL LESIONS OF THE SUPRACHIASMATIC NUCLEI (SCN). G.E. Pickard and F.W. Turek. Columbia Univ., College of Physicians & Surgeons, Dept. Anatomy, New York, NY, 10032 & Northwestern Univ., Dept. Biol. Sci., Evanston, Ill., 60201.

The organization of the circadian system in multicellular organisms is based on the interactions of at least two circadian pacemakers. This is evident from the observation that exposure to constant light (LL) can lead, in many species, to a dissociation ("splitting") of the free running rhythm of activity into two distinct components. These two activity rhythms often free-run with different periods and stabilize approximately 180° out of phase. Little is known about the neural interactions underlying the organization of circadian rhythms or how multiple circadian pacemakers might be coupled to each other.

In searching for the neural location of the biological clock in mammals, attention has focused on the SCN since destruction of these nuclei leads to the loss of a number of different circadian rhythms. In view of the fact that the SCN are reciprocally innervated (Silverman and Pickard, Neuroscience Abst. 6:266 1980), we have begun our examination of the neural organization of the circadian system by examining the effect of unilateral SCN lesions on both intact and split circadian activity patterns of golden hamsters housed in LL.

Electrolytic lesions aimed at a single SCN were made in 19 hamsters demonstrating a split rhythm of locomotor activity (splitters) and in 11 animals displaying only a single circadian bout of activity (intacts). The extent of the lesions was determined by both Nissl stain and by the presence of labeled retinal terminals in the SCN following monocular HRP injection. Bilateral destruction of the SCN in splitters and intacts resulted in aperiodicty or ultradian activity patterns (N=6). Complete or partial unilateral lesions of the SCN in intact animals (N=10) did not alter the circadian rhythm of activity. Similarly, incomplete unilateral SCN lesions or lesions made in the neuropil adjacent to the SCN in splitters (N=6) did not alter the split activity rhythm. Importantly however, complete unilateral ablation of the SCN in splitters resulted in the termination of the split phenomenon and re-establishment of a single circadian activity component (N=8).

We have demonstrated that the splitting phenomenon is abolished following unilateral ablation of the SCN. While these findings could be interperted as suggesting that each SCN controls one component of the split activity rhythm, further studies are necessary to determine the complex interaction of both SCN in generating both split and intact circadian activity rhythms. Supported by a postdoctoral fellowship to GEP from the Pharmaceutical Manufacturers Assoc. and by NIH grants HD-09885 and HD-12622 to FWT.

277.5 CONTROL OF THE SUPRACHIASMATIC NUCLEUS TO THE VENTROMEDIAL HYPOTHALAMUS IN RAT BRAIN SLICES. Y. Oomura, S. Shibata, and H. Kita. Dept. Physiol. Facul. Med. Kyushu Univ., Fukuoka, 812, Japan.

It is well known that the suprachiasmatic nucleus (SCN) may act as a main source for many behavioral and hormonal circadian rhythmic changes in rats. Not only bilateral lesions of the SCN but also the ventromedial hypothalamic nucleus (VMH) abolished the circadian rhythms of food intake (Nagai, K., et al. Brain Res., 142: 384, 1978). From circadian rhythmic activity change in the SCN, the lateral hypothalamic area (LHA), and VMH (Koizumi, K. & Nishino, H., J. Physiol., 263: 331, 1976), as well as the anatomical evidence of direct projections from the SCN to the VMH and LHA (Swanson, L. W. & Cowan, W. M., J. Comp. Neurol., 160: 1, 1977), it appears that one of the control mechanisms of rhythmic feeding behavior may be a result of neural modulation of VMH and LHA activity by the SCN. Intracellular responses in the rat LHA neurons to SCN stimuli were demonstrated(Oomura, Y., et al. In: Biological Rhythms and their Central Mechanism. Eds. M. Suda et al., N. Y. Elsevier, p295, 1979).[1] Nevertheless, the effect of SCN stimulation on the VMH is still unclear. In the present study, using brain slice and intracellular recording, permitted clear demonstration of the effects of SCN stimulation of VMH activity.

Hypothalamic slices, included the SCN and VMH, were cut sagittally from rat cerebrum. For SCN stimulation, a bipolar tungsten wire electrode was used. Intracellular recording electodes were filled with 3M K-acetate. Out of 96 intracellurally recorded VMH neurons which had more than 40mV resting membrane potential, 18 elicited orthodromic responses and 6 produced antidromic spike (latency, 2.8±0.3: mean±S.D.). The main orthodromic response(78%) was EPSP's. Of 14 EPSPs, 10 were elicited with fixed latency (3.3±0.7ms), probably monosynaptic, for repetitive stimuli, and responses could follow to double pulse stimulation spaced shorter than 15ms. The reamining 4 exhibited polysynaptic EPSPs(latency, 4.9±0.4ms). Another kind of response which appeared to be an IPSP-like hyperpolarizing response followed by rebound discharges, were observed in 4 neurons(latency, 2.9±0.8ms). This hyperpolarizing response might be a reversed EPSP and not an IPSP since the amplituds of the rebound spike discharges were less than 30mV and the response could reversed by membrane hyperpolarization with a small negative injected current. This idea may be supported by a previous extracellular study, no inhibition of VMH activity by SCN stimuli was observed.[1] In our previous report[1] SCN mainly inhibited LHA neurons multisynaptically. From our present and previous data, it can be concluded that the SCN exerts excitatory influence on VMH activity and inhibitory one on the LHA. This SCN control may partly explain the nocturnal food intake of the rat. Antidromic spike response in some VMH neurons to SCN stimulation may indicate some functional influence on the SCN.

277.6 CIRCADIAN RHYTHMIC CHANGES OF SCN NEURONAL ACTIVITY IN THE RAT HYPOTHALAMIC SLICE. H. Kita,* S. Shibata, Y. Oomura (SPON: J. LaVail). Dept. Physiol., Faculty Med., Kyushu Univ., Fukuoka, 812, JAPAN.

Many studies have demonstrated that bilateral lesions of the suprachiasmatic nucleus (SCN) abolish both behaviolal and hormonal circadian rhythms such as feeding, locomotor activity, sleep-wakefulness, and plasma corticosterone levels in the rat, and suggesting that the SCN may participate in the regulation of these rhythms. Multipule neural unit activity recorded from the rat SCN showed circadian rhythmicity that still remained many days after the deafferentation of the retinal input to the SCN (Inouye,S.T. & Kawamura,H., Proc. Natn. Acad. Sci.,6,5962,1979). However, properties of single unit activity in the SCN were not demonstrated in that study.

The present study, utilizing rat brain slice preparations, demonstrated that SCN single neuronal activity showed changes corresponding with environmental light-dark (L-D) periods, even after the SCN was isolated from other brain areas. Adult wistar rats were adapted for at least one month to either a normal L-D (light, 0800-2000 hr) or a reversed schedule (light, 2000-0800). Food and water were available ad lib. A hypothalamic slice(about 300μm thick) preparation including the SCN was cut coronally from the brain at various clock times. Recordings of single unit activity were begun after a 30 min preincubation.

SCN spontaneous discharges showed very regular intervals with a range of 0.5 to 20 spikes/sec. The unit discharge rate of the SCN prepared from normal L-D rats was significantly different during the L and D-period: the highest (7.0 ± 2.7, mean ± S.D., n=24) from 1400 to 1500 and the lowest (3.8 ± 2.1, n=11) from 0200 to 0300. The unit discharge rate of the SCN prepared from the reversed L-D rats also showed a clear difference between the L and D-periods, however the activity change was shifted approximately 12 hs: the highest (7.9 ± 2.3, n=10) from 0200 to 0300 and the lowest (4.0 ± 2.1, n=13) from 1400 to 1800. Above results indicate that SCN activity itself can be entrained by the environmental L-D cycle with independent of afferent connections from other brain areas and humoral influences.

It has been reported that SCN stimulation produced mainly excitatory responses in the ventromedial hypothalamus and mainly inhibitory responses in the lateral hypothalamus in the rat (Oomura Y., et al., In: Biological Rhythms and their Central mechanism. Eds., M.Suga, H.Nakagawa & O.Hayaishi, N.Y., Elsevier, p 295, 1979). These present and previous evidence may provide a neuronal base for the circadian rhythmic feeding behavior observed in the rat.

278.1 HISTAMINE CHANGES INDUCED BY LOCAL INJECTION OF KAINIC ACID IN THE RAT STRIATUM. G.Sperk, H.Hörtnagl,M.Berger and O.Hornykiewicz. Institute of Biochemical Pharmacology, Univ.of Vienna, 1090 Vienna, Austria.

Intrastriatal injection of kainic acid in the rat results in selective destruction of intrinsic and efferent neurons of the striatum (Schwarcz,R.& Coyle, J.T.,Brain Res.127:235,1977). Recent studies showed that the lesion is followed by an activation of striatal dopamine and serotonin containing input neurons (Andersson,K. et al.,Nature 283:94,1980; Sperk,G. et al., submitted for publication). In the present study we investigated the function of the histamine system, another striatal input system (Schwartz,J.C. et al.,J. Neurochem.35:26,1980), following local injection of kainic acid.

Kainic acid (1 µg/µl) was infused stereotaxically into the left caudate of male Sprague-Dawley rats. The animals were killed after 2,10 and 70 days. Ten days after the injection, striatal glutamate decarboxylase and choline acetyltransferase activity respectively was 20 and 18 per cent of control.

There was a more than 100 per cent increase of striatal histamine levels persisting from two days after the lesion up to ten weeks. This accumulation of histamine seems to be associated with neuronal compartments because: (a) lesions of the median forebrain bundle abolished the histamine increase; and (b) a subcellular distribution study revealed an accumulation of the amine in synaptosomes but not in the P1-fraction. Concomitant with the increase in histamine levels there was a marked (50 per cent) decrease of histidine decarboxylase activity and a decrease in the disappearance rate of histamine after treatment with the histidine decarboxylase inhibitor α-fluoromethylhistidine (20 mg/kg, i.p., 2 hrs).

Our data demonstrate considerable changes in the neuronal histamine system taking place in the kainic acid injected striatum. The increase in histamine levels might be due to a decreased release of the amine, with a subsequent decrease in histamine synthesis rate.

We thank Dr.J.Kollonitsch for α-fluoromethylhistidine. Supported by the Austrian Science Research Fund, project S-25/02.

278.2 LOCALIZATION OF HISTAMINE-LIKE IMMUNOREACTIVITY IN RAT BRAIN. B.J. Wilcox and V.S. Seybold. Department of Anatomy, University of Minnesota Medical School, Minneapolis, MN 55455

In addition to its role in mast cells, biochemical and pharmacological evidence indicates that histamine may act as a neurotransmitter within the central nervous system. The distribution of histamine is reported to be uneven throughout the brain, with the highest concentration found in the hypothalamus, and pharmacological studies indicate that histamine may be involved in control of arousal states.

In order to obtain morphological information concerning histaminergic pathways in the brain, an antiserum against histamine has been generated in guinea pigs. This antiserum has allowed visualization of nerve fibers and cell bodies in rat brain by immunofluorescence.

The primary antiserum was generated in guinea pigs by immunization with histamine-methylated BSA complex. Ten micron frozen sections from normal and colchicine treated rats perfused with buffered 4% paraformaldehyde were processed for immunofluorescence. Adjacent sections were processed as absorption controls. Selected animals were injected with L-histidine (500 mg/kg, ip) thirty to sixty minutes prior to perfusion in order to enhance endogenous histamine levels.

Histamine-like immunoreactivity was observed in varicose fibers in the hypothalamus with the highest density found in the median eminence and in basal hypothalamic areas lateral to the median eminence. Scattered fibers appeared in the amygdala, hippocampus, and throughout the cortex. Histidine loading before sacrifice increased the quantity of visible varicose fibers. Absorption controls showed disappearance of fluorescent fibers when the primary antiserum was preincubated with the histaminemethylated BSA complex, while preincubation with the methylated BSA complex only did not alter labeling. Colchicine pretreatment revealed cell bodies within the lateral hypothalamus. These results are in agreement with existing evidence and support the hypothesis that a portion of the histamine in the brain is contained within a neuronal compartment.

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278.3 EFFECT OF RESERPINE ON MOUSE BRAIN HISTAMINE AND ITS SUBCELLULAR DISTRIBUTION. E.L. Orr* (SPON: I.M. Korr). Dept. of Anat., N.T.S.U./T.C.O.M., Ft. Worth, Tx. 76107.

Adult male mice were group-housed in plastic cages in air-conditioned animal quarters with a L:D cycle of 12 hr light:12 hr dark. In experiment 1, mice were injected i.p. with 0.08 ml saline or 5 mg/kg reserpine (Serpasil) and sacrificed 16 hrs. later. Brains were removed and homogenized in 2.5 or 12.5 volumes of ice-cold distilled water. The homogenates were heated, cooled and centrifuged at 40,000 xg for 10 min. Supernatants were assayed radioenzymatically for histamine (Hm) according to Orr & Eichelman (J. Neurochem. 30: 1539,1979). In experiment 2, mice were treated as in experiment 1, but whole brains were homogenized in 10 volumes of 0.32 M sucrose and crude subcellular fractions (P_1, P_2 and S) were prepared. The P_1 and P_2 pellets were weighed and homogenized in 12.5 vol. distilled water. After heating the P_1 and P_2 homogenates and the S fraction, supernatants were prepared and assayed for Hm.

		Saline	Reserpine	P (Sal. vs Res.)
Low Vol.	ng/g	67±2	89±3	<<0.002
High Vol.	ng/g	295±15*	353±19*	<0.05
P_1	ng/fraction	32±4	28±3	NS
P_2	ng/fraction	52±3	53±5	NS
S	ng/fraction	40±3	75±12	<0.02

*P<0.05 high vs. low volume

The above data show that whole mouse brain contains substantial quantities of Hm as previously demonstrated for rat brain (Orr & Eichleman, 1979). Further, reserpine increases the amount of Hm in the mouse brain, apparently by increasing the amount of cytoplasmic Hm. However, in saline-treated mice, the general distribution of Hm in the crude fractions is similar to the distributions reported by others in the mouse and rat brain, although in those reports, only a small fraction of the total brain Hm content was actually measured. In conclusion, mouse brain contains about 300 ng Hm/g wet wt. which is increased by reserpine. The increase is probably due to slow metabolism of intraneuronal Hm released by reserpine from synaptic vesicles into the synaptoplasm.

Supported by a faculty research grant from T.C.O.M.

278.4 HISTAMINE H2-RECEPTORS ON GUINEA PIG ILEUM MYENTERIC PLEXUS NEURONS MEDIATE RELEASE OF CONTRACTILE AGENTS. L.A. Barker and B. Jones Ebersole.* Dept. Pharmacology, Mount Sinai Sch. Med. of the City University of New York, New York, NY 10029.

The actions of the selective histamine H2-receptor agonist, dimaprit, were studied on the isolated ileum and the plexus containing ileal longitudinal muscle preparations of the guinea pig. Dimaprit caused a multiphasic contraction of both preparations. The initial phase was characterized by a twitch response which reached a maximum in 15 - 20 s and was followed by a partial relaxation. The secondary phase consisted of a slowly developing contracture which was sometimes accompanied by oscillatory changes in tension or consisted of a series of twitch responses. Dose-response curves were generated for the inital response; for intact ileal segments the EC50 was 5.1 ± 1.8 µM (mean ± s.d., N=7) and the Hill coefficient was 1.1 ± 0.2 and for longitudinal muscle strips the EC50 was 5.8 ± 1.2 µM and the Hill coefficient was 1.2 ± 0.1 (N=7). Both the initial and secondary components of the contractile responses to dimaprit were non-surmountably antagonized by 0.2 µM tetrodotoxin or 10 µM mefenamic acid and by the production of tachyphalaxis to either substance P or serotonin. Scopolamine, 0.001 - 0.1 µM, non-surmountably antagonized only the initial component of the response. Mepyramine (1.0 µM), hexamethonium (100 µM), and bromolysergic acid (0.25 µM) were without effect on the response to dimaprit. The histamine H2-receptor antagonists, tiotidine and cimetidine, produced parllel dextral shifts in the dose response curve for dimaprit with variable reductions in the maximal response which were reversed upon removal of the antagonist. The apparent pA_2 values for tiotidine and cimetidine antagonism of dimaprit are 7.7 and 6.1 respectively. We conclude that dimaprit acts on H2-receptors located on myenteric plexus neurons to cause the release of contractile substances. The mediators of the contractile response are tentatively identified as acetylcholine, products of the arachadonic acid cascade, and possibly substance P and serotonin.

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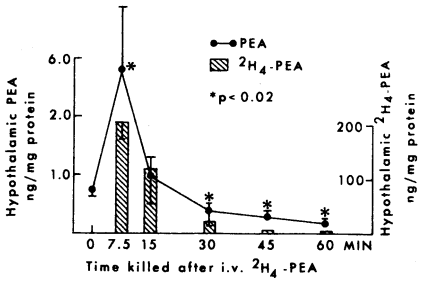
278.5 A SIMILARITY BETWEEN TYRAMINE-INDUCED NEUROTOXICITY AND THE ENCEPHALOPATHY OF REYE'S SYNDROME. B. A. Faraj, F. M. Ali* and E. Malveaux*. Dept. of Radiology (Div. Nucl. Med.), Emory University School of Medicine, Atlanta, GA 30322.

Reye's syndrome is a disease associated with acute encephalopathy with evidence of hepatic dysfunction in children. Clinically, cerebrospinal fluid pressure is frequently elevated, particularly in children who subsequently die from unresolved encephalopathy. Hypertyraminemia is a common metabolic abnormality in Reye's syndrome and correlates with stage and duration of coma (Pediatrics 64:76, 1979; Lancet 2:1097, 1979). Hypertyraminemia was caused primarily from decreased metabolism since lowered activity of monoamine oxidase (MAO) was noted in hepatic biopsy specimens of patients with Reye's syndrome (Pediatrics 65:647, 1980). The objective of the present investigation was to determine whether or not tyramine induces encephalopathy in experimental animals with impaired mitochondrial MAO function, and whether the encephalopathy in these animals was a function of increased CSF pressure. Ten mongrel dogs were treated (orally) daily with the MAO-inhibiting drug, phenelzine (3-9 mg/kg) over a period of one month. At the end of this period, the dogs were anesthetized with sodium pentobarbital (25 mg/kg) and catheters were inserted into the femoral vein and artery for tyramine administration and blood pressure recording. A 20 gauge intracath needle was inserted into the cisternal space for continuous monitoring of CSF pressure. Tyramine was infused (1 mg/kg) over a period of 10 min. The present studies indicated that tyramine infusion caused acute but transient hypertension followed by prolonged elevation in CSF pressure that exceeded 40 mm Hg (initial pressure was 7-10 mm Hg). This was followed by substantial accumulation of tyramine (25 ± 6.1 ng/ml vs 1.5 ± 0.3 ng/ml), dopamine (1533 ± 450 pg/ml vs 10 ± 3.2 pg/ml) and norepinephrine concentrations (6197 ± 1011 pg/ml vs 137 ± 37.5 pg/ml) in CSF of these treated animals at 60 min following dose administration. Intracranial hypertension in these animals correlated significantly with dogs behavior characteristic with the encephalopathy of Reye's syndrome including tremor, rigidity, convulsive seizure and coma. Contrastingly, the administration of tyramine to control dogs caused only the expected transient increase in blood pressure but with no significant effect on CSF pressure (≈7-12 mm Hg). These animals recovered fully from the experiment without any ill effect. The pathogenic role of tyramine in Reye's syndrome may be a consequence of (1) altered transport of tyramine at the blood-brain barrier; (2) increased sensitivity of the brain to tyramine; and/or, (3) increased release of catecholamines from central catecholaminergic neurons.

278.6 BIOCHEMICAL EVIDENCE OF POSITIVE FEEDBACK CONTROL OF PHENYLETHYLAMINE (PEA) RELEASE IN THE RAT BRAIN. F. Karoum, L.W. Chuang and R.J. Wyatt, Adult Psychiatry Branch, National Institute of Mental Health, Washington, D.C. 20032.

The observations that PEA shares many behavioral, electrophysiological and pharmacological properties with amphetamine and that amphetamine increases brain concentration of PEA, have prompted us to evaluate the effect of exogenously administered PEA on the production and release of endogenous brain PEA. For this, we administered a deuterated isomer of PEA ($^2H_4$-PEA), 2.5 mg/kg, i.v. to rats in groups of five and followed their hypothalamic and caudate nucleus concentrations of endogenous PEA and $^2H_4$-PEA over time. The results of our finding on the hypothalamus are summarized in the figure below. Similar results were obtained in the caudate nucleus.



Our results demonstrate that $^2H_4$-PEA very rapidly entered the brain and that it is equally rapidly destroyed. Furthermore, these results suggest a bi-phasic effect of elevated exogenous brain PEA (as demonstrated by $^2H_4$-PEA) on endogenous brain PEA. Increased exogenous brain PEA caused an elevation in endogenous PEA; an effect presumably related to increased PEA release. At a later period when practically all exogenous PEA is destroyed, endogenous brain PEA declined to concentrations significantly below normal. These two effects may respectively be related to a positive and negative feedback regulatory mechanisms of brain PEA release.

279.1 ETHANOL INHIBITS HARMALINE-INDUCED TREMOR AND CEREBELLAR INCREASES OF cGMP CONTENT. M. S. Rappaport*, R. T. Gentry,* D. R. Schneider*† and V. P. Dole* (SPON: N. E. Miller). The Rockefeller Univ., New York, NY 10021 and †Dept. of Pharmacol., Wayne State Univ. Sch. of Med., Detroit, MI 48202.

Neurophysiological and neurochemical evidence suggest that some pharmacological effects of ethanol may result from its facilitation of inhibitory neurotransmission mediated by γ-aminobutyric acid (GABA). We have selected harmaline-induced stimulation of Purkinje cell activity as a means to study the interaction between ethanol and GABA in cerebellum according to the rationale of Costa et al.,(Adv. Biochem. Psychopharm., 14: 113, 1975). Whole body tremor and an increase of cyclic guanosine monophosphate (cGMP) content of cerebellum are established endpoints of harmaline activation of Purkinje cells in mammals.

Tremor was assessed by observers blind to drug treatments; a numerical score of tremor was assigned to individual animals at one minute intervals throughout the study period (35 min). Male C57BL/6J mice (20-25g) were used in all studies. Harmaline HCl (10 mg/kg) was administered 20 min before vehicle (water) or ethanol (0.1, 0.3 and 1.0 g/kg, ip). Prior to measurement of cerebellar cGMP by radioimmunoassay, mice were killed by focused microwave radiation (6 kW, 480 msec) at 7 min following the second injection.

A dose-related suppression of harmaline-induced tremor resulted following ethanol. This effect was maximal within 7 min. Harmaline increased cerebellar cGMP content by 60% ($p<0.01$). This increase was completely attenuated by ethanol (0.3 and 1.0 g/kg).

Oxotremorine induces tremor by a mechanism known not to involve climbing fiber activation of Purkinje cells. Tremor induced by an equivalent tremorogenic dose of oxotremorine (0.25 mg/kg) was relatively unaffected by ethanol. Concentrations of cGMP were not significantly altered by oxotremorine.

These data demonstrate a pharmacological similarity between ethanol and the benzodiazepines which appear to act via GABAergic facilitation and are consistent with the hypothesis that certain pharmacological effects of ethanol are GABAergically mediated. (supported by grants from the John L. and Helen Kellogg Foundation and the Florina Lasker Foundation).

279.2 AN ANIMAL MODEL OF ALCOHOL DEPENDENCE. H. Rigter and J.C. Crabbe. CNS Pharmacology Dept., Organon, Oss, The Netherlands.

Animal models of alcohol dependence should meet a number of requirements. Importantly, the animals need to voluntarily ingest so much alcohol, that the intake exceeds the metabolic capacity of the organism, and that tolerance and physical dependence are induced or maintained. One reason for the relative failure to develop animal models of alcohol dependence may be the aversiveness of the taste of alcohol, or other aversive factors associated with the drinking of alcohol solutions. Accordingly, we wondered whether using another route of administration of alcohol would help the development of an adequate animal model. Forced inhalation of alcohol vapor can produce physical dependence in mice. We have found that mice learn to voluntarily inhale intoxicating amounts of alcohol vapor. The apparatus consisted of two plastic boxes (110 x 60 x 40 cm). Groups of 20 male Swiss mice were used. The mice could move from one box to the other by climbing an interconnecting bridge (slope: 45°). Alcohol (ethanol) vapor (7-10 mg/l air) was led into one box, according to a daily alternating schedule; air was led into the other box. The mice were injected with pyrazole once a day (1 mmole/kg, i.p.), to inhibit metabolism of alcohol. The animals were then placed in the 'air'box. The number of mice selecting the 'alcohol'box was scored hourly. During the first phase of the experiment (days 1-3), the 'alcohol'box was covered with an opaque plate. Mice prefer dark over illuminated spaces; therefore, all of the mice selected the (dark) 'alcohol'box. During the second phase of the experiment (days 4-7) the plate was removed. Pyrazole-treated mice continued selecting the 'alcohol'-box. On day 8, their blood levels ranged from 0.3-1.6 mg/ml blood. When removed from the 'alcohol'box, alcohol-selecting mice exhibited withdrawal convulsions, when picked up at the tail and spun. Withdrawal signs were apparent in some mice for more than 24 h. The mice also were tolerant to the hypothermic effect of alcohol, when tested 6 h after withdrawal. Mice receiving only pyrazole during days 1-3, either avoided alcohol, when alcohol vapor was introduced from day 4 onwards, or had a spatial preference for one of the boxes (resulting in a '50%' alcohol preference). Pyrazole treatment was necessary to produce consistent selection of alcohol within the short time period studied.

279.3 TETRAHYDROISOQUINOLINES ADMINISTERED TO THE NEONATE ALTER ALCOHOL PREFERENCE OF THE ADULT RAT. H.S. Swartzwelder and R.D. Myers. Center for Alcohol Studies and Departments of Psychiatry and Pharmacology, UNC School of Medicine, Chapel Hill, NC 27514.

Certain tetrahydroisoquinolines (TIQs) have been implicated in the abnormal drinking of alcohol. Tetrahydropapaveroline (THP) infused ICV can induce alcohol self-selection in the normally abstinent rat, and salsolinol has been found in the CSF of the alcoholic patient. However, the effect of such compounds upon the developing organism and the characteristics of its subsequent alcohol consumption is not known.

Within 24 hrs after birth, Sprague-Dawley male and female rat pups were given systemic injections of either THP (2.5 µg or 25 µg) or a control CSF solution. Within each of the six litters, the two doses and control vehicle were injected. In another set of five litters, three protoberberine (PBN) compounds and control vehicle were administered according to the same protocol in doses based on their LD-50 toxicity level. All rats were weaned at 21 days and placed in cages individually. Alcohol preference testing was begun at 70 days of age with a two-bottle-three-choice method. An ascending series of alcohol concentrations ranging from 3-30% (v/v) was offered over an 8-day interval with tap water as the alternative choice.

The neonatal treatment with THP in both dosages caused a reduced alcohol preference in the male rat, whereas alcohol drinking in the females was significantly enhanced by this TIQ. PBN compounds differed in their potency in altering alcohol consumption, depending upon their chemical structure; however, the general enhancing and inhibiting effect on alcohol self-selection in females and males, respectively, was similar to those of THP.

Overall, these results suggest that exposure to an amine-aldehyde condensation product during early post-natal life may produce marked and long-lasting functional changes in the brain which influence subsequent addictive behavior. Moreover, this liability may be sex-specific.

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279.4 ETHANOL DEPRESSES SYNAPTIC EXCITATION OF LOCUS COERULEUS NEURONS. G. Aston-Jones, S.L. Foote and F.E. Bloom, A.V. Davis Center For Behavioral Neurobiology, Salk Institute, San Diego, CA

The effects of acute ethanol (E) administration on target neurons of the norepinephrine-containing locus coeruleus (NE-LC) system has been previously reported by our lab. We are currently studying the changes induced by E in the impulse activity and sensory responsiveness of the NE-LC neurons themselves.

Single cell recordings were obtained from chloral hydrate-anesthetized rats implanted with bipolar stimulating electrodes in the dorsal noradrenergic bundle and in the frontal neocortex. Baseline data were collected for spontaneous discharge rate, the latency and number of antidromic (AD) spikes stimulated from one of the above placements, and the number of spikes driven orthodromically from sciatic nerve stimuli delivered as foot-shock (FS). E was then injected at a dose of .5g/kg, i.p., and these same measures were again obtained. Data on 6 animals for 2 to 6 sequential, cumulative injections each (1 cell per animal) are presented here. Rectal temperature was maintained between 36 and 37 °C throughout recordings. All data were collected from histologically verified NE-LC neurons.

Our results indicate that E has no significant effect on the electrical excitability of NE-LC neurons, as E administration did not alter the efficacy of invasion of AD spikes into the soma-dendritic membrane. However, E did cause a small (1 to 3 msec) increase in AD spike latencies in a dose-related fashion, indicating that E may effect spike conduction along NE-LC axons to some extent. More pronounced effects were seen on spontaneous discharge rate and on the number of spikes driven from FS stimulation. In a dose-related fashion, E at a dose of 1.0 to 3.0 g/kg decreased spontaneous discharge an average of 27%, and also decreased the number of FS-driven spikes an average of 53%. This indicates that E may substantially alter the ability of excitatory synaptic inputs to affect NE-LC discharge.

Further experiments are in progress to test the effects of anesthesia and sciatic nerve fatigue on these results. We also plan to examine the effects of E on inhibitory synaptic inputs to NE-LC neurons, and on NE-LC axon conductivity during trains of high-frequency AD stimulation. Our results to date indicate, however, that some of the sedative effects of E on behavior may be partially due to a depression of sensory-evoked excitation of NE-LC neurons. Supported by ARC Grant 03504, AA 07273 and NS 16209.

279.5 ANTAGONISM OF DIAZEPAM-INDUCED SEDATION AND ALCOHOL POTENTIATION BY CGS 8216 (2-Phenylpyrazolo[4,3-c] Quinolin-3(5H)-one) IN THE RAT. P. S. Bernard*, D. E. Wilson*, R. Sobiski* and R. D. Robson* (SPON: C. Boast). Res. Dept., Pharma. Div., CIBA-GEIGY Corp., Summit, NJ 07901.

In the rat forebrain, CGS 8216 potently inhibited the in vitro binding of ^{3}H-diazepam to benzodiazepine receptors (Czernik et al., Pharmacologist, Aug., 1981) but did not block metrazol-induced seizures or conflict behavior (Bernard et al., Pharmacologist, Aug., 1981). This latter group characterized the significance of these findings by demonstrating that CGS 8216 antagonized the anticonflict properties of diazepam, meprobamate and sodium phenobarbital; and in addition, blocked the effects of diazepam against metrazol-induced seizures and rotorod performance. Therefore, CGS 8216 appears to be an antagonist of diazepam. In the present experiments we evaluated the ability of CGS 8216 to antagonize diazepam's sedative and alcohol-potentiating effects.

A measure of the sedative properties of diazepam (30 & 100 mg/kg p.o.) was obtained in a variable interval-30 sec operant task (VI-30) where food-deprived rats were trained to press a lever for milk reinforcement. CGS 8216 (20 & 40 mg/kg i.p.) significantly antagonized the diazepam-induced reduction in response rate in this procedure. At the highest dose tested (40 mg/kg i.p.) CGS 8216 alone caused a partial disruption in this behavior which was less evident when it was given in combination with diazepam.

The interaction of ethyl alcohol (1.6 g/kg, 20% soln. p.o.) and diazepam (7.5 mg/kg p.o.) were tested in a rotorod task where the rat had to remain on a rotating bar (16 rpm) for 30 sec, in at least 1 of 3 trials. Diazepam potentiated the alcohol-induced impairment of rotorod performance. CGS 8216 (20 mg/kg i.p.) which completely antagonized diazepam alone, also markedly antagonized the combination of diazepam and alcohol. CGS 8216 failed, however, to antagonize the effects of alcohol alone. By itself, CGS 8216 did not produce any limiting effects.

These unique properties of CGS 8216 indicate that it may be a useful research tool in elucidating the sedative, anxiolytic, anticonvulsant and muscle relaxing properties of diazepam. In addition, they may have implications for the clinical usefulness of diazepam antagonists.

279.6 CHANGES IN CORTICAL SYNAPTOSOMAL PLASMA MEMBRANE FLUIDITY AND COMPOSITION IN ETHANOL-DEPENDENT RATS. E. Majchrowicz*, F.T. Crews and R. Meeks*. Lab. Preclin. Studies, NIAAA, Rockville, MD; Department of Pharmacol., Univ. of Florida Med. Sch., Gainsville, FL and South. Res. Inst., Birmingham, AL

Low concentrations of ethanol (Et) are known to expand and fluidize biological and artificial membranes. The purpose of this study was to determine if there are changes in the fluid properties and composition of synaptosomal plasma membranes (SPM) (prepared according to Jones & Matus) which might correspond to the onset and decay of neurological and behavioral signs and responses characteristic of the phases observed during the Et withdrawal (Wd) period in rats. Physical dependence upon Et was induced (and rated) in male rats (250-350 g) by po administration of Et (20%, w/v) as previously described (Psychopharmacologia, 43:245, 1975). Four groups of animals were used: (1) water treated controls; (2) acutely treated rats (4 hr after po Et, 5g/kg); (3) prodromal detoxication phase group (PrDP) (dependent while still intoxicated), and (4) ethanol withdrawal syndrome group (EtWds) (4-6 hr after the onset of the overt signs and responses of CNS withdrawal hyperexcitability). The apparent microviscosity at various temperatures of SPM from control rats, acutely treated, and rats undergoing EtWdS were similar throughout the entire temperature profile. The decrease in the flow activation energy (ΔE) implied an increase in the organization of SPM from Et dependent rats. The cholesterol/phospholipid (chol/phosphl) molar ratio was highest in SPM from rats in the PrDP, whereas it was intermediate in rats undergoing overt EtWdS as compared with controls or acutely treated animals. The amount of total phosphl per mg of protein was not significantly different in any of the groups tested. Although there was a slight decreasing trend in arachidonic acid (20:4) content during the PrDP there were no significant changes in the fatty acid composition of the SMP phosphl. These results indicate that there is an increase in SPM chol content in rats dependent upon Et. In vitro addition of chol to control membranes altered the apparent microviscosity similar to the changes found in SPM of Et dependent rats. When Et was added to SPM isolated from rats in the PrDP there was an increase in ΔE in the direction of or even beyond the control ΔE value. These findings indicate that the decreased ΔE in SPM of Et dependent rats, which is at least partially due to an increased chol/phosphl molar ratio, could be related to the development of physical dependence upon ethanol and the ethanol withdrawal syndrome.

280.1 TWO MECHANISMS FOR SPECTRAL SLOWING OF TRYPTOPHAN HYDROXYLASE FLUCTUATIONS. S. Knapp and A. J. Mandell. Dept. of Psychiatry, University of California at San Diego, La Jolla, CA 92093

Spectral and exponential analyses of the kinetic fluctuations in rat raphe tryptophan hydroxylase activity over time revealed multiple coherent spectral peaks with wavelengths of from 4 to 40 minutes. Both the frequency and the amplitude (variance) of product concentration fluctuations were sensitive to influence by ions (calcium and lithium) and psychotropic agents such as chlorimipramine. Lithium induced a low amplitude, low frequency fluctuation pattern, whereas the chlorimipramine-induced spectral slowing was associated with higher amplitude of variation.

Continuously versus discontinuously sampled time series of rate processes revealed evidence that the chlorimipramine-induced slowing was related to variable coupling of dissipative nonlinear enzyme protein oscillators, creating multiple configurations of state, through which relaxation (recurrence time) is slow. Lithium-induced slowing, however, appeared to be related to an initial uncoupling of molecules, randomization, followed by the emergence of a single, phase-coherent gaussian population of oscillators whose average frequency is slowed by the large coherent mass as it approaches a limit cycle.

Both lithium and chlorimipramine are known to slow circadian rhythms, and this work suggests statistical mechanical mechanisms for those effects. This research is supported by DA-00265-09.

280.2 IONIC DEPENDENCE AND CHARGE CARRIERS OF THE CURRENTS UNDERLYING BURSTING IN APLYSIA NEURON R15. William B. Adams and Irwin B. Levitan. Friedrich Miescher-Institut, P.O.Box 273, CH-4002 Basel, Switzerland.

Normal bursting activity in Aplysia neuron R15 is mediated by three currents: (i) a steady-state depolarizing inward current (I_B); (ii) a transient inward current (I_{in}) which maintains the burst once it has begun; and (iii) a transient outward current (I_{out}) which terminates the burst and controls the duration of the interburst. The three currents are voltage-dependent and interact during all phases of the burst cycle. Other workers have shown that I_B is carried by Na^+ and/or Ca^{++} (Barker, J.L. & Smith, T.G.,1978, in Abnormal Neuronal Discharges (Raven) 359-387;Tillotson, D., 1979, PNAS 76, 1497-1500). We have found that I_{in} also appears to be carried by Na^+, but is sensitive to Ca^{++} blockers such as Cd^{++} and Co^{++} and to decreases in external Ca^{++}. The results suggest that I_{in} may be a Ca^{++}-activated Na^+ current. I_{out} is also sensitive to Ca^{++} blockers and to decreases in external Ca^{++}. In these respects its properties resemble those of the Ca^{++}-activated K^+ channel. However, it does not reverse at the K^+ equilibrium potential (nor at any potential between -40 and -120 mV), nor is it affected by changes in external K^+ or by K^+ channel blockers such as Cs^+ or TEA. It is also unaffected by ouabain and by changes in external Na^+. Our results are consistent with the possibility that I_{out} is not a true outward current, but rather is due to a closing of channels which normally carry an inward Ca^{++} current. This decrease in Ca^{++} influx is seen as an outward hyperpolarizing current when measured against the baseline current.

280.3 SPINAL TREMOR INDUCED BY 4-AMINOPYRIDINE. S. Rossignol, R. Dubuc, Y. Lamarre and C. Julien. Centre de recherche en Sciences Neurologiques, Département de physiologie, Université de Montréal, Montréal, Québec, Canada H3C 3JT.

4-Aminopyridine (4-AP) in small doses (1 mg/kg) facilitates excitatory and inhibitory reflex pathways in the spinal cord of cats (Lemeignan, Neuropharmacology 11: 551, 1972 and 12: 641, 1973); Jankowska et al, Brain Res. 136: 387, 1977). This could apparently be due to a greater release of neurotransmitters resulting from an increase in calcium permeability. At higher doses, 4-AP produces convulsions and tremor. In 8 decerebrate and paralyzed cats, we have found that a single i.v. injection of 4-AP (20 mg/kg) induced, for several hours, regular rhythmic bursts of activity at frequencies ranging from 3.5 to 7.0 Hz recorded with cuff electrodes in cut muscle nerves of all limbs. This tremor persisted after spinalization at C1. The mean tremor frequency was similar in both forelimbs and in both hindlimbs, but it differed in forelimbs and hindlimbs. Marked beating was observed in the auto-correlograms calculated for several seconds indicating periodic oscillations around the mean tremor frequency of each limb. Beating was also found in cross-correlograms of forelimb-hindlimb activity suggesting that the periodic oscillations in the respective auto-correlograms were due to an interaction between the forelimbs and the hindlimbs oscillators. Indeed, after spinalization at Th13, the rhythmic activity in hindlimbs continued independently of that of forelimbs and the beats in the respective auto-correlograms disappeared. In these low spinal preparations, the activity in the extensor nerves (medial gastrocnemius) and flexor nerves (semitendinosus) on both sides was highly cross-correlated as well as that of extensor and flexor nerves on the same side. The tremor persisted after a bilateral dorsal rhizotomy from L3 to S2. Stimulation of L7 ventral root on one side could reset the tremor rhythm in both hindlimbs. Prolonged unilateral ventral root stimulation could nearly abolish the tremor ipsilaterally while the contralateral tremor continued. It is concluded that 4-aminopyridine can induce a generalized and sustained tremor in the isolated spinal cord of the cat. The spinal mechanisms for generating this tremor as well as the interactions between limb oscillators and supraspinal structures are being investiguated. (This work is supported by a group grant of the Canadian MRC).

280.4 SLOW OSCILLATIONS OF TRANSMEMBRANE POTENTIAL IN MYENTERIC NEURONS. J. D. Wood, C. J. Mayer* and P. Grafe*. Physiology Dept., School of Medicine, University of Nevada, Reno, NV 89557 and Physiologisches Institut der Universität München, Munich, West Germany.

Intracellular recordings of electrical activity of myenteric neurons in guinea-pig small intestine were obtained in vitro. The recording electrodes were glass micropipettes, filled with 3M KCl, through which electrical current was injected into the neuron by use of an electronic bridge circuit. Slow oscillations of transmembrane potential were observed in about 2 percent of the ganglion cells. The oscillations were sinusoidal in appearance, they had amplitudes of 10 to 15 mV measured from trough to crest and occurred at frequencies of 1.8 to 2 per min at 37°C. They spanned membrane potentials between -50 and -65 mV. The oscillations occurred in neurons that fired repetitively during depolarizing current pulses and that showed nicotinic-cholinergic, fast synaptic potentials and also received serotonergic slow synaptic input. Action potentials evoked by intracellular injection of depolarizing current were followed by short-duration hyperpolarizing after-potentials of 2 to 5 sec. The hyperpolarizing after-potentials summated when spikes were evoked at 1 to 2 Hz, and the oscillations began as the hyperpolarization decayed at the end of stimulation. Once started, the oscillatory behavior was continous. Hyperpolarization of the membranes to potentials 10 to 15 mV greater than the trough of the slow waves by injection of current terminated the oscillations. The input resistance of the cells as determined by injection of constant-current hyperpolarizing pulses, progressively increased during the hyperpolarizing phase of the oscillation and decreased during the depolarizing phase. The excitability of the cell, as indicated by action potential discharge at the offset of the hyperpolarizing current pulses, was maximal at the crest of the oscillation. The ionic mechanism for the slow oscillations involves progressive increase in ionic conductance of the membrane during the rising phase (depolarization) and a drop in conductance during the falling phase (hyperpolarization) of the electrical wave.

280.5 OSCILLATORY PROPERTIES OF INFERIOR OLIVE CELLS: A STUDY OF GUINEA PIG BRAIN STEM SLICES IN VITRO. Y. Yarom* and R. Llinás (SPON: J. Rosenbluth). Dept. Physiology & Biophysics, New York Univ. Med. Ctr., New York, NY 10016.

Inferior olive (I.O.) cells are known to fire at a rather low frequency. Furthermore, if stimuli are presented at different time intervals, these cells demonstrate an optimal following frequency of approximately 10 Hz. This oscillatory-like property becomes especially clear in vivo following the administration of alkaloids such as harmaline (Llinas & Volkind: Exp. Brain Res. 18, 69-87, 1973; de Montigny & Lamarre: Brain Res. 82, 369-373, 1974). A central question has been whether this oscillation is generated by the electrophysiological properties of single cells or by the connectivity properties of the I.O. circuit. Intracellular recordings were obtained from I.O. neurons of guinea pig brain stem slices (Llinás & Yarom: J. Physiol. 315, June 1981). I.O. action potentials have several components: (1) a fast somatic Na^+ spike followed by an after-depolarization (ADP) upon which axonic spikes may be seen. (2) This ADP is Ca^{++}-dependent, occurs at dendritic level, and is followed by a larger after-hyperpolarization (AHP) produced by a Ca^{++}-dependent K^+ conductance change which may reach the level of -85 mV and last for 100-200 msec. (3) The AHP is abruptly terminated by a rebound Ca^{++}-dependent somatic spike. This set of ionic conductances was suggested by us as necessary and sufficient to produce the oscillatory behavior of I.O. neurons. In order to test the hypothesis, harmaline at 10 ugm/ml concentration was applied to the Ringer's solution. Following administration of this alkaloid, I.O. cells showed a hyperpolarization (5-10 mV) and clear oscillatory behavior at approximately 5-8/sec when directly stimulated either antidromically or with a short intracellular pulse. This oscillation which could last for several seconds was produced by the enhanced somatic Ca^{++} spike which accompanied the harmaline-dependent hyperpolarization. Because in many cases I.O. neurons could be demonstrated to fire independently from nearby neurons (simultaneous extracellular recordings), it was concluded that the oscillatory behavior of I.O. cells is cellular and not a circuit property produced by the interplay between the rebound somatic Ca^{++} spike and the Ca^{++}-activated AHP which follows the dendritic Ca^{++} action potential. However, because I.O. cells are electrotonically coupled, these neurons may behave as loosely coupled oscillators in the context of the I.O. nucleus as a whole. (Supported by USPHS research grant NS-13742 from NINCDS)

280.6 TETRODOTOXIN REVERSIBLY BLOCKS SODIUM PACEMAKER CURRENT IN R15 OF APLYSIA. K. Futamachi* and T.G. Smith, Jr. Laboratory of Neurophysiology, National Institute of Neurological and Communicative Disorders and Stroke, National Institutes of Health, Bethesda, Maryland 20205

For several years there has been a controversy over the ionic charge carrier in the persistent, inward pacemaker conductance in molluscan pacemaker cells; namely, is it sodium (Na) (Nature 253:450) or calcium (Ca) (J. Physiol. 254: 129)? We report here that freshly solubilized, high (100 μM) doses of the sodium conductance blocker, tetrodotoxin (TTX), completely and reversibly abolishes the bursting pacemaker potential (BPP) activity, the persistent inward current and, hence, the negative slope region (NSR) of the N-shaped, voltage clamp current-voltage curve in cell R15 of Aplysia californica. This concentration is three times higher than that required to block the Na-spike in this cell. Refrigerated but unfrozen TTX in sea water does not completely block BPP activity or the NSR in R15. The Ca-spike in R15 is not blocked by 100 μM TTX.

Since BPP activity and the persistent inward current amplitudes (1) vary directly with Na_o but inversely with Ca_o, (2) are enhanced, not blocked, by D600, a known Ca-conductance blocker and, (3) as reported here, blocked by Na-specific conductance blocker, TTX, we conclude that the persistance inward current in the depolarizing pacemaker conductance is primarily a Na and not a Ca conductance.

281.1 COMPARATIVE RECEPTOR BINDING EFFECTS IN BRAIN AFTER I.V. LORAZEPAM AND DIAZEPAM ADMINISTRATION. N.M. Spirt*, G. Bautz*, M. Zanko*, W.D. Horst and R.A. O'Brien. Dept. Pharmacology II, Hoffmann-La Roche Inc., Nutley, NJ 07110.

When administered i.v. to man, lorazepam exhibits a more prolonged period of amnesia and sedation than a clinically equivalent dose of diazepam (Brit. J. Clin. Pharm. 4:45, 1977). This occurs even though the elimination half-life of lorazepam is much shorter than that of diazepam. There is also a slower onset of the clinical effects of i.v. lorazepam compared to diazepam. The quicker onset of diazepam is probably due to its having greater lipophilicity than lorazepam, thus allowing for faster penetration of the blood brain barrier. We sought to investigate whether the longer sedative action of lorazepam, at times when blood levels were drastically reduced, could be explained by the persistance of lorazepam binding to central benzodiazepine receptors. In vitro, lorazepam bound to rat brain receptors with a 5-fold higher affinity than did diazepam. IC_{50} values were: lorazepam, 1 nM, and diazepam, 5 nM. Tighter binding to the receptor might explain the longer lasting sedation, since more of an equieffective dose of lorazepam would remain bound to BZ receptors over the period following drug administration. To evaluate this hypothesis, groups of male rats (4) weighing 150 g were administered i.v. lorazepam (0.3 mg/kg) or diazepam (0.75 mg/kg) and killed at varying times after dosing. The brain cortex was used as the receptor source in the ^{3}H-diazepam binding assay of Mohler et al. The percent inhibition of binding was evidence for receptor occupancy by either lorazepam or diazepam. The peak diazepam-induced inhibition occurred at 30 sec, that of lorazepam followed at 10 min after dosing. Subsequently, the inhibition of binding in the diazepam group had returned to baseline after 60 min, the lorazepam-treated rats still displayed 45% inhibition of binding at this time. The data agree with studies in man in which lorazepam slowly passed from plasma to CSF (Acta Pharmaco. tox. 46, 156, 1980) after i.v. dosing. In contrast, diazepam administration to man i.v. was characterized by fast entry into CSF and by CSF levels which paralleled free plasma levels (Psychopharmacol. 70, 89, 1980). We propose that ex vivo binding studies such as those we have described are useful for explaining clinical pharmacokinetic and pharmacological profiles for CNS active drugs in man.

281.2 FACILITATION OF BENZODIAZEPINE RECEPTOR BINDING: A MECHANISM OF ACTION FOR METHAQUALONE? B. Kenneth Koe. Central Research, Pfizer Inc., Groton, CT 06340.

Methaqualone possesses hypnotic, anticonvulsant, muscle relaxant, and anxiolytic properties (Brown and Goenechea, Clin. Pharmacol. Ther. 14: 314, 1973), a pharmacological profile not unlike that of the benzodiazepines. Naik, Naik, and Sheth (Psychopharmacol. 57: 103, 1978) pointed out that the anticonvulsant action of methaqualone may involve GABAergic mechanisms. Recently, benzodiazepine (BD) receptors have been suggested as the site of action, because methaqualone inhibits the binding of ^{3}H-diazepam (IC_{50} 150 µM) and ^{3}H-flunitrazepam (^{3}H-FNP) (IC_{50} 220 µM) to rat brain membranes (Müller, Schläfer, and Wollert, N.-S. Arch. Pharmacol. 305: 23, 1978; Antoniadis, Müller, and Wollert, Neuropharmacol. 19: 121, 1980).

Although methaqualone inhibits ^{3}H-BD binding in vitro, our studies demonstrate that following the administration of methaqualone, BD receptor binding is actually enhanced. In vivo, we found that methaqualone, in a dose-dependent manner, stimulates the binding of ^{3}H-FNP (200 µCi/kg i.v.) to mouse brain in vivo (83% increase at 100 µmol/kg s.c.). Moreover, in ex vivo experiments whole brain membranes from mice treated with methaqualone (100 µmol/kg i.p.) bound more ^{3}H-diazepam than control membranes. Scatchard plots for the ex vivo membranes suggested that the enhanced binding is derived from an increase in the number of binding sites (B_{max}, 33% increase). These in vivo and ex vivo effects of methaqualone were also shown by cartazolate (SQ 65,396), the anxiolytic pyrazolopyridine (Beer, Klepner, Lippa, and Squires, Pharmacol. Biochem. Behav. 9: 849, 1978). Methaqualone competitively inhibited ^{3}H-diazepam binding to rat cortical membranes in vitro (K_D, 187% increase at 320 µM), whereas cartazolate (1 µM) stimulated ^{3}H-diazepam binding in both unwashed and frozen/washed membranes via a B_{max} change (30% and 16% increase, respectively).

Our findings suggest that the benzodiazepine-like actions of methaqualone may be ascribed to a novel mechanism, in which BD receptor binding is facilitated. Possibly, binding of a "natural" BD agonist is enhanced, or in view of the B_{max} increase, a "natural" BD antagonist may be displaced from BD receptors by methaqualone in vivo, freeing more binding sites for a BD agonist. Alternatively, methaqualone may be facilitating BD binding in vivo via GABAergic mechanisms.

281.3 BENZODIAZEPINES INHIBIT THE ACTIVITY OF SUBSTANTIA NIGRA PARS RETICULATA NEURONS. R.J. Ross*, B.L. Waszczak and J.R. Walters (SPON: F.K. Goodwin). NIMH and NINCDS, NIH, Bethesda, Md 20205.

It has been postulated that the central effects of the benzodiazepines are mediated by the ability of these drugs to potentiate the inhibitory effects of GABA on neuronal activity. The substantia nigra pars reticulata has been identified as a region containing neurons which are sensitive to the inhibitory effects of both i.v. muscimol,a potent GABA agonist, and iontophoretically applied GABA and muscimol. This region receives a substantial GABAergic input from the corpus striatum. Here we report the results of electrophysiological studies of the effects of i.v. administration of two benzodiazepines, diazepam and flurazepam, on firing rates of reticulata cells, recorded extracellularly in chloral hydrate anesthetized rats. Both drugs exerted an inhibitory effect on the activity of pars reticulata cells, but there was considerable variability in the sensitivity of individual neurons. When diazepam was administered in exponentially increasing increments to a cumulative dose of up to 8 mg/kg (n=17), 27% of the cells were inhibited by 80% or more; 13% of the cells were inhibited by less than 20%. The average effect produced in the 5-min interval following systemic administration of a single 0.5 mg/kg dose of diazepam was inhibition to 58% of baseline (n=9). The effect could be reversed by the subsequent administration of picrotoxin 4-8 mg/kg (n=5). The diazepam vehicle, administered alone, produced either no change or a modest excitation.

Flurazepam, administered in increasing increments to a cumulative dose of up to 8 mg/kg,also inhibited reticulata cell firing, but had a less potent inhibitory effect (n=16). Sixty-nine percent of cells were inhibited by at least 20%. No cells were inhibited by as much as 80%. The average degree of inhibition following a cumulative dose of 0.5 mg/kg flurazepam was 2% (n=16); following a cumulative dose of 8 mg/kg, it was 39% (n=11). Cells which were insensitive to flurazepam could still be effectively inhibited by i.v. muscimol 3.2-6.4 mg/kg (n=4).

The differences in potency between diazepam and flurazepam may reflect differences in lipid solubility and kinetics of distribution. They are consistent with observed differences in clinical efficacy. For each drug, the variability in the degree of inhibition may be related to differences in tonic GABAergic input among neurons, to different levels of an endogenous ligand for the benzodiazepine receptor, or to differences in numbers or affinities of benzodiazepine receptors. The inhibitory effects of these two benzodiazepines on reticulata cells are of the same order of magnitude as those on locus coeruleus neurons (Grant et al., Life Sci. 27: 2231, 1980). The benzodiazepines may have multiple clinically relevant sites of action.

281.4 BUSPIRONE: AN ANXIOSELECTIVE DRUG WITH SIMILARITIES TO APOMORPHINE. L. A. Riblet, Duncan P. Taylor, J.A. Becker*, D. K. Hyslop* and R .C. Wilderman*. Department of Biologic Research, Mead Johnson Pharmaceutical Division, Evansville, IN 47721.

Buspirone HCl is a novel nonbenzodiazepine antianxiety agent with efficacy comparable to that of diazepam (Am. J. Psychiatry 136: 1184, 1979). In contrast to the benzodiazepines, this anxioselective agent lacks anticonvulsant activity, interacts minimally with CNS depressants and does not cause muscle relaxation. In vitro binding experiments indicate that buspirone is without significant activity at the following binding sites: α_1, α_2, β, cholinergic, glutamate, glycine, H_1, H_2, opiate, 5-H_1, 5-HT_2 and adenosine. Furthermore, it neither stimulates nor inhibits ^{3}H-benzodiazepine binding, does not affect the influence of GABA or halide anion on ^{3}H-benzodiazepine binding and does not interfere with GABA binding or uptake. Buspirone appears to interact only with the dopaminergic system with reasonable potency. In fact, buspirone and apomorphine share many pharmacologic actions. Quantitative cortical EEG profiles from the cat and rat, determined by zero-cross analysis, indicate similarities between buspirone and apomorphine. This was also evident in subcortical recordings from the hippocampus of cat. This similarity between buspirone and apomorphine was also apparent from the drug-induced turning in rats that had received 6-OH-dopamine lesions of the substantia nigra. In addition, in vitro binding experiments in the presence and absence of guanosine triphosphate indicate that buspirone inhibits ^{3}H-spiperone binding in a manner similar to that of other dopamine agonists such as apomorphine, N-propylnorapomorphine and piribedil. The fact that apomorphine is of value clinically in relieving agitation and excitement, may be relevant to the anxioselective action of buspirone.

281.5 ANTIANXIETY EVALUATION OF A NOVEL IMIDAZOBENZODIAZEPINE-3-CARBOXAMIDE COMPOUND (Ro 21-8384) IN THE RAT AND IN THE SQUIRREL MONKEY. F.S. Grodsky*, J.W. Sullivan*, D.N. Mitchell*, and J. Sepinwall (Spons. L. Klevans). Dept. of Pharmacology, Hoffmann-La Roche Inc., Nutley, N.J. 07110

Ro 21-8384† was administered orally to: 1) untrained, water deprived rats in a modified water lick conflict procedure (shock for each 20th lick), 2) to food deprived rats trained to bar press on a single lever during alternating components of a VI 30" (food)/FR 10 (food + shock) multiple schedule, 3) to food deprived squirrel monkeys trained on a two lever concurrent VI 1.5' (food) + VR 24 (shock)/VI 6' (food) schedule of reinforcement.

In the water-lick conflict test, Ro 21-8384 produced marked increases in punished licking rates at doses of 0.08, 0.31, and 1.25 mg/kg.

In rats in the multiple schedule, Ro 21-8384, like diazepam, had an inverted U-shaped anticonflict dose-response curve (M.E.D. = .01 mg/kg). It was 125 times more potent than diazepam and produced selective antipunishment effects over a wider range of dose doublings up to 0.31 mg/kg, while producing no significant change in unpunished behavior. At 0.63 mg/kg and higher doses, significant decreases in unpunished responding were seen, making this compound only 32 times more potent than diazepam with respect to depressant effects. At 6.2 mg/kg punished responding was also significantly decreased.

In the squirrel monkey, Ro 21-8384 also had an inverted U-shaped anticonflict dose response curve; it produced antipunishment effects over a wider range of dose doublings than diazepam, from 0.02 mg/kg (M.E.D.) to 80 mg/kg, the highest dose tested, and was 16 times more potent. Unlike diazepam, Ro 21-8384 did not significantly decrease responding, in either component, within the range of doses tested.

Thus, Ro 21-8384 increased punished responding in procedures used to detect potential anxiolytic properties and would be predicted to be a more potent anxiolytic than diazepam. However, there appeared to be less sensitivity to the rate decreasing properties of Ro 21-8384 shown by the monkey than by the rat, thus, suggesting a difference in sensitivity to the sedative-depressant qualities of Ro 21-8384. Whether this difference is species dependent (e.g., due to differences in biotransformation), schedule dependent, or both, remains to be determined.

† Ro 21-8384: 8-Chloro-6-(2-chlorophenyl)-4H-imidazo[1,4] benzodiazepine-3-carboxamide (Walser et al., J. Heterocyclic Chem. 15:577, 1978)

281.6 CLOZAPINE: AGENTS WITH SIMILAR BEHAVIORAL AND BIOCHEMICAL PROPERTIES. Ronald G. Browne and B. Kenneth Koe. Dept. Pharmacol., Central Research, Pfizer Inc., Groton, CT 06340.

Clozapine, a dibenzodiazepine, with known antipsychotic efficacy, differs from classical neuroleptics in producing few if any extrapyramidal side effects. The use of clozapine has been greatly restricted because of unacceptable incidences of agranulocytosis. Efforts have therefore been made to discover other agents with a clozapine-like mechanism of action. The present investigation examined the ability of various drugs to mimic the behavioral effects of clozapine in a drug discrimination paradigm. In addition, comparisons were made on a spectrum of biochemical alterations produced by clozapine, including changes in dopaminergic, adrenergic, cholinergic, GABAergic and benzodiazepine systems.

Male Sprague-Dawley rats were trained to discriminate 3.2 mg/kg of clozapine from vehicle in a two-lever operant procedure. Discrimination accuracy was established following about 40 training sessions as evidenced by most animals emitting their first FR-10 responses on the appropriate lever in nine out of ten consecutive sessions. Generalization testing indicated that RMI-81,582 (2-chloro-11-3-dimethylaminopropylidene morphanthridine) and cyproheptadine produce discriminative stimuli identical to clozapine, in contrast to neuroleptics such as haloperidol which were discriminated as vehicle.

Biochemical findings demonstrated that clozapine, RMI-81,582, cyproheptadine and thioridazine, in addition to producing similar effects on ^{3}H-spiroperidol and ^{3}H-QNB binding to rat brain membranes in vitro, also facilitated ^{3}H-flunitrazepam binding to mouse brain in vivo. These results support further the hypothesis that atpyical antipsychotic agents such as clozapine may exert their effects in part through GABAergic mechanisms.

The results of these behavioral and biochemical studies indicate that despite structural heterogeneity, RMI-81,582 and cyproheptadine share a pharmacological profile similar to clozapine and that this activity is distinctly different from neuroleptics such as haloperidol.

281.7 NEUROPSYCHOPHARMACOLOGICAL EFFECTS OF A NOVEL POTENTIAL ANTIPSYCHOTIC AGENT: Ro 22-1319 A.B. Davidson*, E. Boff*, D.A. MacNeil, and L. Cook*, Dept. of Pharmacol., Hoffmann-La Roche Inc., Nutley, N.J. 07110

Ro 22-1319, a novel pyrroloisoquinoline compound (Olson, et al., submitted to J. Med. Chem.) with potential antipsychotic activity, was identified in a rat discrete avoidance procedure. This procedure is highly specific for chlorpromazine-like antipsychotic activity and potency of known antipsychotic compounds in this procedure is highly correlated with their clinical potency (r=0.953). The potency of Ro 22-1319 in this procedure (ABD50=0.7 mg/kg, p.o.) approached that of haloperidol (ABD50= 0.4 mg/kg, p.o.) and it exhibited similar high potency in other rat and monkey avoidance procedures, on rat motor activity, and in antagonism of apomorphine induced emesis in dogs. However, although effects in s. nigra lesioned rats indicate activity at dopaminergic sites in the nigro-striatal system, Ro 22-1319 exhibited relatively weaker cataleptogenic and acute antistereotypic activity than haloperidol and minimal effects in a chronic supersensitivity model of stereotypic behavior. These results suggest that Ro 22-1319 would exhibit clinically efficacious antipsychotic activity approaching the potency of haloperidol, but would have fewer or less intense acute extrapyramidal effects and minimal potential for tardive dyskinesia. Ro 22-1319 produced no appreciable cardiovascular or peripheral autonomic effects in dogs. The electrocorticographic activity of this compound was described earlier (Davidson, et al., Neurosci. Abstr.6:366,1980); its biochemical profile, including the interesting separation observed between potent in vivo and weak in vitro dopamine antagonist activity, is discussed elsewhere (Bautz, et al., This Meeting).

	Oral ED50's in mg/kg	
	Ro 22-1319	Haloperidol
Discrete lever avoidance (rat)	0.7	0.4
Pole-climb avoidance (rat)	1.2	0.8
Continuous avoidance (sq. monkey)	0.4	0.3
Motor activity - rearing (rat)	1.0	0.6
Apomorphine emesis antag. (dog)	0.05(sc)	0.02(sc)
Amphet. rotation antag. (SN-X rat)	0.7	0.4
Acute apomorph. stereotypy (rat)	2.4	0.4
Catalepsy - maximum effect (rat)	48sec.@ 12.5	> 60sec.@ 2.5
Chronic apo. supersensitivity(rat)	+33% @ 4.4	+69% @ 2
	+33% @ 8.8	+95% @ 4

281.8 COMPARISON OF IN VIVO AND IN VITRO ANTIDOPAMINERGIC POTENCIES OF A NEW ANTIPSYCHOTIC-LIKE COMPOUND (RO 22-1319). G. Bautz*, R.A. O'Brien, K. Meyers*, T. Mowles* and W.D. Horst. Depts. of Pharmacology II and Cell Biology, Hoffmann-La Roche Inc., Nutley, NJ 07110.

In several pharmacological tests, Ro 22-1319 (3-ethyl-2,6-dimethyl-4,4a,5,6,7,8,8a,9-octahydro-4a,8a-trans-1H-pyrrolo[2,3-g]isoquinolin-4-one, HCl) exhibits antipsychotic-like activity with potencies comparable to haloperidol (A.B. Davidson et al., these abstracts). However, the in vitro antidopaminergic potency of Ro 22-1319 is considerably weaker than for haloperidol or spiroperidol. The IC_{50}'s for the in vitro inhibition of ^{3}H-spiroperidol binding to rat striatal tissue by Ro 22-1319, haloperidol or spiroperidol are 45, 7.4 and 0.3 nM, respectively. Ro 22-1319 is also less potent than haloperidol in disinhibiting the in vitro release of prolactin from cultured rat pituitary cells (EC_{50}'s = 535 and 25 nM, respectively).

To determine whether or not these discrepancies in the in vivo and in vitro potencies of Ro 22-1319 may indicate a site of action for Ro 22-1319 which is different than for haloperidol or spiroperidol, Ro 22-1319 was tested in vivo for its influences on ^{3}H-spiroperidol binding and prolactin release. Ro 22-1319 or spiroperidol were administered (S.C.) to rats 30 min prior to the i.v. injection of ^{3}H-spiroperidol (1 µg/kg). The rats were sacrificed 2 hr after the ^{3}H-spiroperidol injection. Plasma prolactin levels were determined 1 hr after the administration (i.p.) of Ro 22-1319 or haloperidol. The ED_{50}'s for the in vivo inhibition of ^{3}H-spiroperidol binding to striatal tissue by Ro 22-1319 and spiroperidol are 0.5 and 0.24 mg/kg, respectively. The ED_{50}'s for prolactin release by Ro 22-1319 and haloperidol are 0.30 and 0.29 mg/kg, respectively. Thus, the in vivo antidopaminergic potencies of Ro 22-1319 are similar to haloperidol and spiroperidol.

These observations do not suggest that Ro 22-1319 has a site of action different from haloperidol and spiroperidol. The apparent discrepancy between the in vivo and in vitro potencies of Ro 22-1319 does not appear to result from biotransformation, but may be due to some unidentified factor, inherent in these in vitro procedures, which selectively influences the activity of Ro 22-1319.

281.9 N-Oxides of Phenothiazine Antipsychotics Contribute to Their Pharmacological Actions. M.H. Lewis, E. Widerlöv, C.D. Kilts, & R.B. Mailman. Biological Sciences Research Center, Depts. of Psychiatry and Pharmacology, Univ. of North Carolina School of Medicine, Chapel Hill, NC 27514

The clinical effect of antipsychotic drugs may partly depend on formation of active metabolites, a fact that often has been overlooked. This laboratory recently initiated studies about one major class, the N-oxides. Several N-oxide metabolites of clinically used phenothiazines were screened for anti-dopaminergic activity. In vivo, this was accomplished by using direct lateral ventricular injection of the metabolites or parent compounds. Both fluphenazine N-oxide and trifluoperazine N-oxide (which are piperazinyl-4-N-oxides) had significant anti-dopaminergic activity in vivo, as shown by their antagonism of either amphetamine-induced locomotor behavior or apomorphine-induced stereotyped behavior. In vitro, both of these N-oxides significantly inhibited ^{3}H-spiroperidol binding to brain membranes and also inhibited dopamine-stimulated adenylate cyclase activity in striatal homogenates. In all tests the compounds were equal to or slightly less potent than the parent compounds. Conversely, chlorpromazine N-oxide (side chain oxidation) did not have significant anti-dopaminergic activity in any of these in vivo or in vitro systems. This suggests that oxidation of the nitrogen atom (three carbons removed from the phenothiazine ring) which is believed to mimic the catecholamine nitrogen, results in loss of anti-dopaminergic activity, whereas other N-oxides may possess significant pharmacological effects.

It has been reported by others that CPZ-NO has significant anti-dopaminergic activity when given peripherally. The present data suggest that this may be due to conversion of the N-oxide back to the parent compound or to other active metabolites. This possibility was assessed by measuring the reduction in vivo of N-oxides of chlorpromazine, trifluoperazine and fluphenazine to their respective parents after peripheral or central administration. The compounds were extracted from blood or other tissue, and measured by HPLC on radially compressed silica with 254 nm UV detection. These data also underscore the necessity of understanding both the metabolism and neuropharmacology of antipsychotic agents if valid hypotheses about their actions are desired. (Supported in part by PHS grant HD-10570, and Center Grants HD-03110 and MH-33127.)

281.10 LEVELS OF HALOPERIDOL AND BIOGENIC AMINES IN THE BLOOD, URINE AND CNS OF RATS DURING CHRONIC HALOPERIDOL TREATMENT. P.A. Shea, S.R. Dunlop*, S.E. Wade*, and H.C. Hendrie*. Dept. of Psychiatry and Biochemistry, Institute of Psychiatric Research, Indiana Univ. Sch. of Med., Indianapolis, IN 46223.

The purpose of this study was to determine the relationship between CNS and peripheral measurements of neurotransmitters and their metabolites after chronic neuroleptic treatment. The distribution of haloperidol was determined in various brain regions, blood, and urine. Six male Wistar rats were injected I.P. for 19 days with 0.25 mg/kg haloperidol along with 6 saline injected animals. On various days, pre-injections and during treatment two 12-hour urine collections were taken. On day 19 all animals were killed, blood collected and brains dissected into 7 regions. In each brain part, levels of norepinephrine, 3-methoxy-4-hydroxy phenylglycol, dopamine, 3,4-dihydroxyphenylacetic acid, 5-hydroxyindoleacetic acid, serotonin, homovanillic acid, and haloperidol were determined by HPLC (biogenic amines) and receptor assay (haloperidol). After 19 days of drug treatment plasma levels of haloperidol were 5.82 $\pm$ 0.98 ng/ml. In brain the highest drug levels were in the hippocampus, 220.86 $\pm$ 70 ng/ml and hypothalamus, 208.9 $\pm$ 80 ng/ml. The midbrain contained the least, 63.9 $\pm$ 27 ng/ml. When compared to saline-treated animals the only statistically significant changes in biogenic amines and metabolite levels were noted in the cortex, caudate nucleus, and septum and this occurred in the dopamine system where HVA and DOPAC were elevated. Urinary levels of haloperidol were highest at day 2, and fell by day 3 to remain constant during the rest of drug injections. There was a definite diurinal variation in drug excretion. The determinations of the neurotransmitter metabolites in blood and urine will be presented along with their relationship to the CNS measurements. (Supported by the Indiana Dept. of Mental Health, Grant 47-869-11).

281.11 REDUCTION OF LOCOMOTOR HYPERACTIVITY BY AMPHETAMINE IN NEONATAL RATS GIVEN 6-HYDROXYDOPAMINE IS MEDIATED BY BRAIN SEROTONERGIC NEURONS. Thomas Heffner and Lewis Seiden. Dept. of Pharmacol. & Physiol. Sciences, University of Chicago, Chicago, IL 60637.

In neonatal rats, destruction of brain dopamine (DA) neurons by intraventricular injections of 6-hydroxydopamine (6-HDA) results in locomotor hyperactivity. Whereas Shaywitz et al.(Nature 261:153,1976) reported that this hyperactivity is reduced by d-amphetamine (d-A),Pappas et al. (Psychopharmacol.70:41,1980)failed to confirm this result. We examined the ability of d-A to reduce hyperactivity caused by 6-HDA as well as the mechanism responsible for this effect. Neonatal rats pretreated with desipramine were given ivt injections of 6-HDA (100 µg) or the vehicle solution at 3 and 6 days of age. This treatment produces large-scale (70-90%) selective depletion of brain DA. Locomotor activity was measured in stabilimeter cages for 1 hr daily from days 12 to 55 of life. Locomotion in 6-HDA-treated rats averaged 866% of the level in control rats during days 15 to 55 of life. At 21 days of age, a d-A dose of 1.0 mg/kg(ip) increased locomotion by 766% in control rats but reduced locomotion by 51% in 6-HDA-treated rats. The d-A-induced reduction of hyperactivity was dose-dependent between 0.25 and 2.0 mg/kg and was consistently seen in rats tested at 10 different ages ranging from 21 to 55 days. This effect was not accompanied by stereotypy or other abnormal behaviors. Methylphenidate (4 mg/kg,ip) also reduced hyperactivity in 6-HDA-treated rats by 57% while increasing locomotion by 477% in control rats. Although the DA antagonist spiroperidol (25-200 µg/kg,ip) provided dose-dependent antagonism of the stimulant effect of d-A in control rats, spiroperidol failed to attenuate the effect of d-A in 6-HDA-treated rats. However, the serotonin antagonist methysergide (0.5-2.0 mg/kg,ip) produced dose-dependent antagonism of the effect of d-A in 6-HDA-treated rats while not altering the stimulant effect of d-A in controls. Fenfluramine (3 mg/kg,ip), a drug that releases serotonin from central neurons,restored locomotion to normal levels in 6-HDA-treated rats while not altering locomotion in controls. The serotonin agonist quipazine (0.5-4 mg/kg,ip) also reduced hyperactivity in 6-HDA-treated rats in a dose-dependent fashion while not altering activity in control rats. Pretreatment with propranolol (5 mg/kg, ip), phentolamine (5 mg/kg, ip), atropine (0.5 mg/kg) or naloxone (10 mg/kg, ip) failed to alter the reduction of hyperactivity caused by d-A in 6-HDA-treated rats. These results indicate that d-A can reduce locomotor hyperactivity caused by DA-depleting 6-HDA injections in neonatal rats and suggest that this apparent therapeutic effect involves augmentation of central serotonergic neurotransmission. (Supported by USPHS NS-12324, RSA MH-10562).

281.12 POTENTIATION OF BARBITURATE-AND HALOTHANE-INDUCED HYPNOSIS AFTER PROBENECID OR SULFINPYRAZONE PRETREATMENT. W.H. Lyness, S. Ramanadham and P.K.T. Pang. Department of Pharmacology and Therapeutics, Texas Tech University Health Sciences Center, Lubbock, Texas 79430.

Recent studies have shown that probenecid, a uricosuric agent, potentiates the duration of i.p., i.v. or intracerebroventricularly administered barbiturate-induced hypnosis. This drug interaction has been attributed to the sedative effects of probenecid, inhibition of hepatic oxidation of the barbiturates, displacement of plasma albumin bound barbiturates (thiopental) and altered renal excretion. All previous studies have used large doses of probenecid (200 mg/kg i.p.). The present study sought to establish whether this is a dose related phenomenon and to eliminate the possible renal, hepatic and pharmacokinetic bases of the potentiation. The latter was accomplished using inhalation halothane. Probenecid pretreatment significantly decreased ($p < 0.05$) the latency to onset of hypnosis after halothane (0.5 to 3.0%) and did so in a dose related manner (10-200 mg/kg i.p., 30 min before halothane). The potentiation of pentobarbital hypnosis was significant only with probenecid doses greater than 50 mg/kg. Sulfinpyrazone, produced results identical to those of probenecid in both the inhalation halothane and pentobarbital studies.

Possible mechanisms of action in the halothane-barbiturate hypnotic potentiation might include a synergism with the sedative effects of both uricosuric agents or increases in cerebral blood flow. Both agents reduce systemic blood pressure in conscious and pentobarbital anesthetized rats when administered i.v. or i.p. Furthermore, both agents will relax isolated helical rat tail arterial strips in a dose related manner (0.1 - 1.0 mg/ml). Probenecid induces a dose related reduction in rectal temperatures of rodents and potentiates pentobarbital-induced hypothermia, suggesting peripheral vasodilatory actions. Sulfinpyrazone does not lower rectal temperatures indicating differences in the vascular beds influenced by these uricosuric agents. (This work was supported by the Tarbox Parkinson's Disease Institute at Texas Tech University Health Sciences Center and the Pharmaceutical Manufacturers Foundation Association).

281.13 INTENSIVE CARE FOR PERIODIC SCHIZOAFFECTIVE ILLNESS-PREVENTION OF "REVOLVING-DOOR" SITUATION.

Eugene Ziskind, George Maison* (University of Southern California, Los Angeles), Irving Chelnek*, Helen Zimnavoda* (Gateways Community Mental Health Center, Los Angeles).

Preventing the "revolving-door" phenomenon in chronic mental illness is the most urgent mental health problem. Towards that end, we have utilized a three stage unique treatment program, all directed by the same full-time p.r.n.-available psychiatrist: I - Intensive hospital milieu therapy with medication, mostly parenteral, devoted to getting the patient out to work as soon as possible; II - Monitoring the patient on and off the job with three weekly psychiatrist sessions of individual, family and peer confrontation group therapies; III - Maintenance medication and therapy sessions to prevent rehospitalization.

The first three patients to have completed the program had been previously ill over an eight to nine year period with repeated hospitalizations. The "revolving-door" situation in these patients has been prevented for five, four and three years. These results are unprecedented in the authors' experience nor have they found similar reports in the literature.

This program is unique in that there is no interruption from one phase to the next, no change of therapist or discontinuance of therapy; and to ensure uninterrupted therapy, a conservatorship is recommended. Initial formidable financing results in long run savings.

Reduplication of these results is now necessary on a much larger scale by some major foundation such as NIMH or the Ford Foundation.

282.1 EFFECT OF COLD-PRESSOR STRESS ON VISUAL SENSITIVITY, d', IN MAN. W.C. Clark, D. Schimmel*, and M. Janal*. N.Y. State Psychiatric Institute and Queens and City Colleges, CUNY.

Stress alters autonomic activity and induces the release of various hormones and neurotransmitters. Certain of these substances, e.g. excess cortisol, have been shown to elevate sensory thresholds in Cushing's disease. However, there have been no investigations of the effect of cold-water stress on the visual sensitivity, d', of healthy subjects.

Measures of visual sensitivity were obtained on 12 volunteers using the 4-alternative spatial forced-choice procedure. The subjects were fitted with artificial 2mm pupils. Visual reports were obtained for an average of 1 min during ice-water immersion of the hand, and for two recovery periods which followed withdrawal, at 0-30 sec and 90-120 sec. For each subject, data were obtained over 10 cycles. For the control session the water was at room temperature.

ANOVA revealed a significantly lower visual sensitivity for the stressed group averaged over the 3 test periods (1.81 vs 1.48), $F(1,10) = 33.1$, $p < .001$. Within each period sensitivity was significantly less under the stressed condition, t-critical (40) $\geq .17$, $p < .01$).

The pain felt during the ice-water immersion period could have interfered with the perceptual task. However, this does not explain the persistence of this lower sensitivity during the subsequent recovery periods when the subject experienced no distress. The duration of the effect suggests the presence of a stress-induced hormone or sympathetic activity which interfered with vision, either by some central action or by shifting accommodation in the lens.

282.2 PITUITARY CYCLIC AMP IN RATS IS INCREASED BY PSYCHOLOGICAL STRESS. B.N. Bunnell, G.J. Kant, R.H. Lenox, L.L. Pennington*, D.R. Collins*, E.H. Mougey* and J.L. Meyerhoff. Dept. Med. Neurosciences, Walter Reed Army Inst. of Research, Wash., D.C. 20012 and Dept. Psychiatry, Univ. of Vermont, Burlington, VT 05405.

Cyclic AMP may be involved in the release or synthesis of pituitary hormones mediating the organism's response to stress. Recently we have shown that a number of different physical stressors, including footshock, produce a significant increase in pituitary cyclic AMP in rats. The present study attempted to extend these findings to psychological stressors by measuring pituitary cyclic AMP and plasma hormone responses in rats exposed to environmental stimuli previously paired with inescapable footshock.

Male rats, adapted to handling for one week, were assigned to three groups: Experimental, Shock Control, or No-shock Control. For four consecutive days, all rats were placed in conditioning chambers for 20 min sessions. Experimental and Shock Control groups were given footshock during the sessions on a variable interval 30 sec schedule. Shock duration was 5 sec. No-shock Controls were placed in the chambers, but no shock was administered. On the fifth day, all rats were placed in the chambers for either 5 or 10 min; Experimental and No-shock Controls did not receive shock, while the Shock Controls were given shock as before. The animals were then sacrificed by microwave irradiation (2.5 KW, 2450 MHz for 5 sec). Pituitary cyclic AMP and plasma prolactin were measured by radioimmunoassay. Behavioral responses to shock were rated throughout the study.

The results are given in pmoles cyclic AMP/mg wet weight ± S.E.M. and ng prolactin/ml ± S.E.M.

	CYCLIC AMP		PROLACTIN	
	5 MIN	10 MIN	5 MIN	10 MIN
EXP	1.47±.20(N=6)	2.97±.90(N=6)	72.4± 7.1	104.3± 6.6
SC	2.07±.50(N=6)	2.11±.35(N=6)	93.7±13.5	140.0±19.8
N-SC	1.06±.08(N=6)	1.06±.09(N=6)	17.0± 6.3	30.6± 8.3

(EXP = Experimental, SC = Shock Control, N-SC= No-shock Control)

Only the Shock Control group received shock on the test day - the other 2 groups did not. The Experimental group's cyclic AMP and plasma prolactin levels were significantly elevated over those of the No-shock Controls, indicating the biochemical responses had been elicited by environmental stimuli previously associated with shock. The 10 min Experimental group actually had higher levels of pituitary cyclic AMP than the 10 min Shock Control group. Behavior ratings indicated that considerable adaptation to shock took place in the Shock Control groups during five consecutive days of shock.

282.3 STRESS ELEVATION OF PERIPHERAL TRYPTAMINE TISSUE CONTENT . S. T. Christian and R. E. Harrison. Neurosciences Program, University of Alabama in Birmingham, Birmingham, AL 35294.

Recent investigations into the role of tryptamine (TA) in the central nervous system reveal that the compound has an unusually rapid turnover and is found in low nanogram concentrations both centrally and peripherally. In addition, TA is actively transported into the brain, and there are indications that peripherally produced TA is involved in neuromodulation of behavior. Alterations in peripheral TA economy which result in an increase in extracerebral TA concentrations could conceivably have behavioral implications. Since various stress paradigms are known to induce alterations in both behavior and biochemistry of test probands, and in order to elucidate the role of stress in peripheral TA metabolism, we have examined TA tissue concentration in young (60 days old at sacrifice) rats that were either group-housed or stressed by individual housing. TA concentrations in lung, liver, spleen, heart and kidney were determined by a sensitive and specific gas chromatographic/mass fragmentographic isotope dilution assay. Animals were either group-housed (control) or individually housed for 8 or 28 days. Group-housed control animals, and animals that had been individually housed for 8 days had low tissue TA concentrations consistent with earlier reports using radioenzymatic assay, while animals that were individually housed for 28 days showed considerably elevated TA content in all tested organs except the spleen. The greatest TA concentration increases were observed in the lung (62 x control), liver (11 x control) and kidney (6 x control). These results demonstrate that individual housing stress is associated with the production of TA concentrations previously shown to possess pharmacologic activity.

282.4 NUTRITIONAL STATE: EFFECTS ON BASAL AND STRESS-INDUCED INCREASES IN SYMPATHETIC ACTIVITY OF RATS. B. G. Weick, S. Ritter and R. McCarty. Dept. of Veterinary and Comparative Anat. Pharmacol. and Physiol., College of Veterinary Medicine, Washington State Univ., Pullman, WA 99164 and Dept. of Psychol., Univ. of Virginia, Charlottesville, VA 22901.

Food deprivation for 48 hrs decreases norepinephrine (NE) turnover in the heart, pancreas and liver of rats compared to controls fed ad lib. In contrast, ad lib feeding supplemented with an 8% sucrose drinking solution for 48 hrs accelerates NE turnover in several peripheral tissues (Landsberg and Young, New Engl. J. Med. 298: 1295, 1978). In the present experiments, we were interested in examining the effects of fasting and sucrose-supplemented feeding on basal and stress-induced increases in plasma levels of epinephrine (EPI) and NE in rats. Plasma levels of NE and EPI provide an accurate assessment of the functional activity of the sympathetic nervous system and adrenal medulla, respectively.

Sprague-Dawley rats (approx. 300 g) were implanted with tail artery catheters to allow for repeated sampling of blood in undisturbed animals. For 48 hrs following surgery, each rat was assigned at random to 1 of 3 treatments: (i) food deprivation (FD), (ii) food available, ad lib (CF), or (iii) food ad lib with an 8% sucrose drinking solution (SF). Two days after surgery, blood samples (0.5 ml) were collected and direct measures of mean blood pressure (MBP) and heart rate (HR) were taken while rats were undisturbed in their home cages. Rats were then stressed acutely by exposure to 1 min of intermittent footshock (2.0 mA, 0.6 sec duration, every 6 sec). Additional blood samples were collected immediately and 5 min after the termination of footshock. Plasma levels of NE and EPI were measured by a sensitive radioenzymatic assay and plasma glucose was measured by the glucose oxidase method.

There were no significant differences in plasma levels of NE and EPI. Basal blood glucose levels were lower in FD rats (86±6 mg %) compared to CF (116±7 mg %) and SF rats (123±5 mg %). However, stress-induced increments in plasma glucose were similar across groups. Basal HR was significantly lower in FD rats (301±14 bpm) compared to CF (364±12 bpm) and SF rats (384± 15 bpm). Basal MBP's did not differ across groups. These results suggest that acute changes in nutritional state are not attended by significant alterations in basal or stress-induced increases in plasma levels of NE and EPI. The lower basal HR of FD rats suggests that the sympathetic inputs to some but not all peripheral tissues may be altered by acute changes in food availability.

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282.5 TIME COURSE OF BETA ADRENERGIC RECEPTOR ADAPTATION TO RESTRAINT STRESS IN RAT BRAIN. E. A. Stone* and J. E. Platt* (SPON: A. J. Friedhoff). Dept. of Psychiat., New York Univ. Med. Ctr., New York, NY 10016.

The present study examined the time course of neurochemical and physiological adaptation to chronic stress. Independent groups of male rats were subjected to 2.5 hours of restraint per day for either 1, 4, 7, 9 or 14 days. Restrained rats and non-stressed controls were killed immediately after the last stress session. The hypothalmus, cerebral cortex and brainstem were assayed for beta adrenergic receptors by the method of Bylund and Snyder (Mol. Pharmac., 12:568-580, 1976) with {^{3}H}dihydroalprenolol ({^{3}H}DHA) as the radioligand, using isoproterenol in place of propranolol to yield a more conservative measure of non-specific binding. In the hypothalmus, restraint produced a highly significant reduction of {^{3}H}DHA binding first evident on day 4 and persisting through day 14. A significant decrease was evident in the cortex on days 7, 9 and 14. In the brainstem, restraint produced a biphasic effect indicated by a significant treatment x day interaction: {^{3}H}DHA binding was increased on days 1 and 4 and decreased on days 7 and 14. Adaptation to restraint stress was measured by the development of resistance to gastric ulcer formation. The incidence of gastric ulcers decreased from 100% on day 1 to 0% on days 9 and 14 with resistance first appearing on day 4 (50%). This decrease in ulcer formation was positively correlated with the loss of beta receptors in the brain (hypothalmus: r = 0.82; cortex: r = 0.70; brain stem: r = 0.76). The above findings suggest that reduced adrenergic receptor function, which is known to occur after antidepressant therapy as well as adaptation to stress, may play an important role in the development of resistance to the adverse effects of emotional stress.

282.6 DISSOCIATION OF PITUITARY-ADRENAL ACTIVATION AND BEHAVIORAL STRESS RESPONSE FOLLOWING TASTE AVERSION CONDITIONING. G.R. Sessions and J.L. Meyerhoff. Div. Neuropsychiatry, Walter Reed Army Inst. of Research, Washington, D.C. 20012.

Stress produces pituitary-adrenal activation in rats resulting in increases in plasma levels of corticosterone and prolactin, and in cyclic AMP in the pituitary. Conditioned corticosterone release has been demonstrated in rats forced under extreme food and water deprivation to ingest a conditioned aversive flavor previously paired with an illness-inducing agent (lithium chloride, LiCl). Non-deprived rats do not show this conditioned pituitary-adrenal response. This dissociation has been interpreted as reflecting greater pituitary-adrenal activation when conflict exists between conditioned taste aversion and deprivation (Smotherman, et al. JCPP, 1980, 94, 25). The present experiment investigated pituitary-adrenal activation in non-deprived rats forcibly exposed to a conditioned flavor.

Two groups of rats received daily 1 ml oral lavage with water followed 5 min later by saline injections i.p. Beginning on the 10th day one group (N=16) received conditioning trials consisting of 1 ml lavage with 0.1% saccharin solution followed 5 min later by injections of 1.2 mEq/Kg LiCl i.p. The remaining animals (N=16) received saccharin solution followed by saline injections. Conditioning trials were given on 4 separate days at 2-4 day intervals. Taste aversion conditioning was assessed in 2-bottle preference tests prior to the 3rd and 4th conditioning trials. After conditioning, the saline and LiCl groups were subdivided into 2 groups each. Half of the animals in each group received 1 ml lavage with saccharin solution, while the other half received water. A 4 point rating scale was used to rate the animals' behavioral responses to the lavage. Fifteen min later the animals were sacrificed by high-powered microwave radiation focused on the head. Trunk blood was collected for hormone assays, and the pituitary gland was dissected from the brains. The plasma was assayed for corticosterone and prolactin, and the pituitary was analyzed for cyclic AMP levels by radioimmunoassay.

Although all LiCl-injected animals demonstrated severe aversions for the saccharin solution in the 2-bottle preference tests and reacted more strongly to the saccharin than controls, no group differences were observed in plasma corticosterone or prolactin, or in pituitary cyclic AMP following exposure to the flavor. Forced exposure to a conditioned aversive flavor thus does not result in a conditioned pituitary-adrenal response in non-deprived subjects. These results are consistent with the view that pituitary-adrenal activation following exposure to a conditioned aversive flavor occurs only in situations involving severe conflict.

282.7 ADRENAL DEMEDULLATION ALTERS THE EFFECT OF AMYGDALA STIMULATION ON RETENTION OF AVOIDANCE TASKS. C.B. Brewton*, K.C. Liang* and J.L. McGaugh (SPON: Beatriz J. Vasquez). Department of Psychobiology, University of California, Irvine, CA 92717.

Performance in an aversive task can be greatly impaired if low-level electrical current is passed through the amygdala shortly after rats are trained. Amygdala stimulation of this kind has been found to affect hormone release from the adrenal medulla, and recent data suggest a role for these hormones in memory modulation. The present study investigates the role of the adrenal medulla in mediating the memory effects of amygdala stimulation.

Male Sprague-Dawley rats were bilaterally adrenal demedullated (ADMX) or sham-operated (SHAM). These rats had previously been implanted bilaterally with bipolar electrodes aimed at the basomedial/basolateral region of the amygdalae. Two weeks after the adrenal surgery, the rats were trained in a one-trial inhibitory avoidance task (1 mA, 2 sec footshock). Two weeks later, they were trained in an 8-trial active avoidance task (640 μA, 30 sec footshock). For each task, approximately half the rats received amygdala stimulation (AS) immediately after training (50 μA/electrode, 30 sec) and the remainder served as implanted controls (IC). Results of the 24 hr retention test are shown in the table.

In both the inhibitory and the active avoidance tasks, amygdala stimulation produced a retention deficit compared with IC for both SHAM groups. In both tasks also, adrenal demedullation impaired retention scores of the IC groups. For inhibitory avoidance, the AS/ADMX group performed significantly better than the AS/SHAM group, and amygdala stimulation of these rats did not produce a deficit compared with the appropriate control group, IC/ADMX. In active avoidance, retention scores of the AS/ADMX rats were comparable to those of the IC/SHAM group, but significantly better than those of either AS/SHAM or IC/ADMX.

The present findings indicate that adrenal demedullation alters the effects of post-training amygdala stimulation on memory. This suggests that the effect of this type of stimulation may be mediated in part by altering the release of hormones from the adrenal medulla.

	INHIBITORY AVOIDANCE Median entrance latencies (sec)		ACTIVE AVOIDANCE Mean no. of avoids (Day 2-1)	
	IC	AS	IC	AS
SHAM	471.4	75.7[a,e]	2.81	1.50[c,g]
ADMX	161.5[b]	347.7	1.65[d,f]	2.79

a. p<.001, b. p<.005, c. p<.01, d. p<.02 different from IC/SHAM
e. p<.05, f. p<.02, g. p<.01 different from AS/ADMX

Supported by USPHS grants MH 12526 and AG 00538; BNS 76-17370; and a grant from the McKnight Foundation.

282.8 EXAGGERATED CONDITIONED VASCULAR RESPONSES IN SPONTANEOUSLY HYPERTENSIVE RATS CAN BE DISSOCIATED FROM EMOTIONAL BEHAVIOR BY A CENTRAL ADRENERGIC AGONIST. J.E. LeDoux, L.W. Tucker* and D.J. Reis. Lab. of Neurobiology. Cornell Univ. Med. Coll. NY, NY 10021

We subjected spontaneously hypertensive rats (SHRs) to classical fear conditioning to determine: (a) whether conditioned changes in arterial pressure (AP) and heart rate (HR) are enhanced in SHRs relative to Wistar Kyoto (WKY) controls; and if so, (b) whether treatment with a centrally acting a-adrenergic agonist, a-methyl DOPA (Aldomet), may selectively affect the expression of such conditioned changes without altering conditioned emotional behavior. Six animals were instrumented for chronic recording of AP and HR while awake and unrestrained. The next day, the animals were subjected to 30 conditioning trials during which a footshock (.5 sec, 1.5 ma) was paired with the termination of the conditional stimulus (CS) (10 sec, 1200 Hz tone). Two hr later, during 3 extinction trials (CS presented without shock) cardiovascular (CV) data were acquired and stored by a microcomputer for analysis. Basal levels of AP (mmHg) were higher in SHRs (181 ± 13) than in WKYs (133 ± 8) ($p < .01$). In the presence of the CS, the average response of AP was greater in SHRs (12 ± 4) than in WKYs (1 ± 1) ($p < .01$) and AP was more variable in SHRs (S.D. of AP in SHRs, 7 ± 1; in WKYs, 2 ± 1; $p < .01$). The magnitude and variability of conditioned HR responses did not differ. An additional group of SHRs was treated with Aldomet (25-38 mg/kg i.v; n=7) or saline (n=4) 1h prior to assessment of conditioned pressor responses and conditioned emotional behavior, which was measured by the duration of freezing in the presence of a 120 sec CS. The conditioned pressor response (mmHg) was attenuated in treated (3 ± 3) vs control (9 ± 4) SHRs ($p < .05$). While the resting AP was lowered in 4 of the treated animals and thus accounted for a lower basal AP in the treated (140 ± 12) vs control (169 ± 8) ($p < .01$) group, there was no relation between the lowering of AP and the attenuation of the conditioned pressor response. Moreover, Aldomet did not reduce the elevations of AP (up to 35 mmHg) occuring during a natural behavior, drinking, nor did it suppress conditioned emotional behavioral responses (secs of freezing) in treated (43 ± 23) vs control (30 ± 23) (n.s.) animals. We conclude: (a) SHRs have larger and more variable conditioned pressor responses than WKY controls; and (b) these responses are selectively reduced by a-methyl DOPA independent of an action of the drug on resting AP. The results suggest that central neurotransmitter systems underlying the expression of conditioned CV responses may be distinct from those underlying the resting and reflex control of AP and also from those mediating the expression of emotional behavior. Supported by HL 18974 and Merck, Sharp, and Dohme.

283.1 A BEHAVIORAL STUDY OF PATTERN & MOVEMENT DETECTION SYSTEMS IN THE CAT. Adrienne L. Graves* (SPON: D.G. Green). Psychology Dept. and Neuroscience Lab., Univ. of Mich., Ann Arbor, MI 48109.

Previous studies have proposed that X- and Y-cells in the visual system may be related to pattern and movement detection respectively (Tolhurst, 1973; Kulikowski & Tolhurst, 1973). This hypothesis is based on the comparison of electrophysiological properties of cat retinal ganglion cells with human psychophysical data. Possible species differences complicate such a comparison. The purpose of the present experiments was to determine if psychophysical data on pattern and movement detection in the cat are consistent with electrophysiological properties of cat X- and Y-cells.

In one set of experiments behavioral contrast sensitivity functions were measured for stationary and drifting (1,2,3,8, & 22 Hz) sinusoidal gratings. While functions for stationary and very slowly drifting (1 Hz) gratings were indistinguishable, contrast sensitivity functions measured at higher drift rates revealed different properties. There was a decreased sensitivity for high spatial frequencies, and for intermediate speeds there was an increased sensitivity for low spatial frequencies. Both the high frequency decrease in sensitivity and the low frequency increase were a factor of 2 or more.

A second set of experiments used adaptation to investigate differences in pattern and movement detection. Viewing a low spatial frequency grating for 2 - 3 minutes caused a decreased sensitivity for that stimulus. A low spatial frequency stationary grating, however, was an ineffective adapting stimulus for low spatial frequency moving gratings.

Both sets of experiments suggest that pattern and movement detection may be separate mechanisms in the cat. Findings are consistent with the following conclusions (Tolhurst, 1973): 1)pattern detection may be related to X-cells, which respond to stationary gratings and to higher spatial frequencies; and 2)movement detection may be related to Y-cells, which respond to lower spatial frequencies and require temporal modulation. (Supported by: NEI 5T32-EY07022 & EY00379)

283.2 WHITE-NOISE MODELING OF MOTION SPEED SENSITIVITY IN THE CAT'S LATERAL GENICULATE NUCLEUS. C. Veraart*, I. Sallets* and V. Pluvinage* (SPON: M. Meulders). Lab. Neurophysiol. Univ. of Louvain. B-1200 Brussels, Belgium.

This work's aim was to develop a mathematical model which would allow to describe information collected in the lateral geniculate body in response to the movement of a specific stimulus in an animal's visual field.

Cats, anaesthetized with a mixture of nitrous oxyde (75%) and oxygen (25%) supplemented by a long acting local anaesthetic (Bupivicaine) were prepared for visual investigations following conventional methods. Sinusoidal gratings were displayed on a Tektronix 606A monitor (P-31 Phosphor) with the help of a HP-1350 graphic translator, driven by a PDP-11/44. Grating parameters could be choosen at will, motion being linear, sinusoidal, or with speed related to a gaussian white noise. Neuronal activity was collected by means of glass microelectrodes filled with a mixture of NaCl (5%) and Pontamine (4%). Neuronal spike trains were sent to the computer. After X-Y determination, neurones were stimulated by presenting grating moving with a constant speed, or with a sinusoidal speed, or with a speed function of a gaussian white noise. After filtering and windowing, the fast FOURIER transforms were computed in order to obtain power spectrum estimate of both input and output of the retinal geniculate system. After smoothing first order and second order WIENER kernels were determined.

Results have shown that non linearities in visual information processing would also be involved in speed coding. Mathematical models were then developed and were used to simulate motion speed sensitivity. Evaluation of fitness of model responses is in progress and will be discussed.

283.3 INVARIANT MODELING AND RECOGNITION OF BINARY PATTERNS FOR VISUAL STIMULI. Faris Badi'i* and Behrouz Peikari* (SPON: Robert T. Matthews). Southern Methodist University, Dallas, TX 75275 and DeVry Institute of Technology, Irving, TX 75062

A brief discussion of the historical background of invariant recognition of binary patterns (digital computer recognition of binary shapes independent of variations in size, location and rotation) is presented. Their limitations and restrictions are discussed. A size, rotation and location invariant recognition model of binary shapes via a geometrical feature extraction approach is introduced. A family of functions relating geometrical properties of shapes are found, and mathematically justified to be invariant under variations in size, location and orientation. A number of test patterns are also presented as experimental support of the model and discriminatory results are given in corresponding tables. The model is mathematically extended to three-dimensional case and a second family of functions is developed for invariant recognition of three-dimensional shapes. A discussion of practical considerations for digitized data is also given.

283.4 SIMULATION OF DECISION-MAKING BY MULTIMODAL NEURONS. R. Martin*, A. Lukton* and S.N. Salthe* (SPON: I. Abramov). Dept. of Chem., Brooklyn College of the City Univ. of N.Y., Brooklyn, N.Y. 11210.

Neuroanatomists have been puzzled by observations that the vertebrate limbic system, striatum, diencephalon, midbrain and hindbrain each receive inputs, either directly or indirectly, from all or most of the neocortex, and that many neurons within these regions receive a large number of heterogeneous inputs from several different sensory modalities. Nauta and Feirtag[1] have written that "an electrical engineer who had this situation described to him would frown; he would say that one could never hope to get anything but noise from it" since information mixed together in this way cannot subsequently be differentiated. The function of multimodal neurons has been examined during computer simulations of simple conceptualization, learning by simile, risk-benefit assessment and game-playing in associative neural networks governed by the following rule: connections are strengthened between neurons which discharge simultaneously or soon after each other, and are weakened when postsynaptic cells do not discharge after presynaptic activity. This learning rule is a modification of Hebb's postulate[2] that neurons become more strongly interconnected when they discharge simultaneously. It means basically that connections which are frequently used are strengthened, while those which are neglected wither, like branches of a tree which receive no sunlight. Networks of this kind are described in detail elsewhere[3-5]. The simulations indicate that multimodal neurons may function as decision-makers which recognize specific combinations of diverse stimuli and then activate appropriate motor and neuroendocrine response pathways. Multimodal neurons within the mid- and hindbrain may spread their dendrites over several millimeters "hoping, it seems, to catch any kind of message"[1] because they need as much information as possible for the complex decisions they make. The simulations also suggest that multimodal neurons may facilitate the use of concepts which include different sensory and response modalities. This model of decision-making suggests that Hebb's hypothesis may be tested with experiments that focus on input pathways to multimodal neurons which trigger motor and neuroendocrine responses.

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283.5 MODEL FOR BILATERAL NEURAL PATHWAYS IN THE VESTIBULAR OCULAR REFLEX (VOR). H.L. Galiana*and J.S. Outerbridge* (SPON: W. Feindel), Biomedical Engineering Unit, McGill University, Montreal, Quebec, Canada H3G 1Y6.

A new bilateral model of brainstem pathways in the VOR has been developed, based on known anatomical elements and connections. Leaky ($T \sim .3$ sec.) bilateral brainstem integrators are assumed, each linking burst-tonic (BT) and ipsilateral tonic vestibular (TVP) cells in the vestibular nuceli (VN). In normal operation the output of these integrators faithfully predicts eye position, and the effective integration time constant of the VOR in the slow phase is much longer ($\sim$15 sec.), as observed. The commissural positive feedback loop emerges as an important component of the system, and fast phase generation is achieved through its modulation by reticular burst cells (Hikosaka et al., Exp. Brain Res. 39:301-311, 1980). During nystagmus, the system switches alternately from a compensatory position-tracking mode (slow-phase), to an anticompensatory velocity tracking mode (fast phase). Detailed non-linear analysis of the system shows that modulated commissural inhibition has the effect of keeping BT firing levels above threshold during compensatory phases; as a result, linear analysis is frequently possible and is used here to predict response trajectories during slow phase and fast phase segments.

The model accounts for a number of behavioural and electrophysiological findings, e.g., a) 2^{o} TVP and 3^{o} BT VN cells have sensitivities and decay time constants greater than those of primary fibres; b) tonic type I VN cells reverse their response polarity on section of superficial commissural fibres; c) effective VOR integration deteriorates after cerebellectomy; d) the envelope of eye position at the end of fast phases is a low pass filtered version of head velocity; e) peak fast phase eye velocity is modulated by the current vestibular input as well as the error from target.

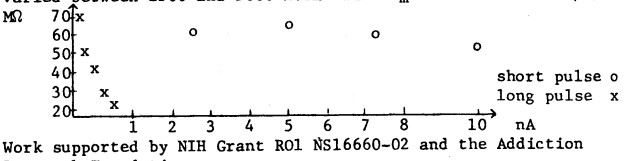
283.6 MEASUREMENT OF PASSIVE MEMBRANE PARAMETERS OF HIPPOCAMPAL GRANULE CELLS. D. Durand, P.L. Carlen, A. Ho, P. McMullen & H. Kunov*. Depts. of Biomedical Engineering, Physiology & Anatomy, University of Toronto, Addiction Research Foundation & Playfair Neuroscience Unit, Toronto Western Hospital, Toronto, Ontario.

Combined intracellular recordings and HRP injections have proven to be powerful techniques for analyzing electrotonic properties of nerve cells. Using the in vitro hippocampal slice preparation, HRP injections of adult rat granule cells showed that these cells have dendritic trees with few branching points that closely follow the 3/2 power law and dendrites of approximately identical lengths. These trees are therefore easily collapsable into a single equivalent cable.

Long (LP) and short (SP) hyperpolarizing current pulses (80 and 15 msec respectively) were injected into these cells at varying amplitudes (.1 to 20 nA). The decays of the voltage responses (a sum of exponentials) were analyzed by a computerized peeling technique and by a least-square fitting program (Provencher, Biophys. J., 16:27,1976). Cable equations for both open ended and characteristic impedence terminations then permitted the calculations of the electrotonic length L, and dendritic to somatic conductance ratio ρ.

We have found the short-pulse technique to be usually superior to the long-pulse technique for the following reasons: 1) The resolution of the several time constants is better since the coefficients associated with the faster ones are larger. 2) Provided that the pulse is short enough and the cell input resistance (R_N) linear, the short pulse voltage response is the derivative of the long pulse response eliminating the need for noisy differentiating techniques. 3) Although the hyperpolarizing long pulse sometimes generates a non-linearity (probably a voltage dependent conductance increase), the short pulse can give linear responses and therefore more accurate values of the cell resistance and time constants (see Figure below). (Note the large linear range of the short pulse response).

Preliminary analysis gives values of ρ ranging between 1.5 and 7 with L between .9 and 1.35. The corresponding values of R_m varied between 1500 and 5000 $\Omega.cm^2$ with C_m between 2 and 6 $\mu F/cm^2$.



Work supported by NIH Grant RO1 NS16660-02 and the Addiction Research Foundation.

284.1 ELECTROPHYSIOLOGICAL AND BEHAVIORAL CHARACTERIZATION OF THE NEURAL SUBSTRATE FOR BRAIN-STIMULATION REWARD. I. Kiss*, M. Lapointe* and P. Shizgal. Department of Psychology and Center for Research on Drug Dependence, Concordia University, Montreal, Quebec, Canada.

Electrodes suitable for both chronic stimulation and electrophysiological recording were aimed at various loci along the medial forebrain bundle under stereotaxic and electrophysiological control. Following a post-surgery recovery period of at least four days, the subjects, adult male Wistar rats, were trained to self-stimulate. After behavior had stabilized, estimates of the excitability cycles and conduction velocities of neurons subserving self-stimulation were obtained by psychophysical means.

Field potential recordings were obtained using the same electrodes, currents and pulse durations that were employed during the self-stimulation tests. During the recording sessions, which were interspersed with the behavioral tests, the subjects were anesthesized with sodium pentobarbital.

The field potential records demonstrate the plausibility of the inferences drawn from the behavioral results in that there is a considerable degree of overlap between the two sets of excitability cycle and conduction velocity estimates. In the recording data, recovery from refractoriness begins as early as 0.3 msec and ends as late as 2.0 msec. The behavioral recovery range is 0.4 msec to 2.0 msec. Both methods produce estimated conduction velocities ranging from rougly 2 to 10 m/sec.

Characteristics of directly activated reward-related neurons are inferred from the behavioral data; the electrophysiological findings indicate that neurons with congruent characteristics are, in fact, fired by electrodes and parameters that support self-stimulation. The possible sources of the correlation between data acquired by the aforementioned methods are discussed, as are 1) the sampling biases inherent in the two methodologies and 2) the potential application of this two-pronged approach as a tool for identifying the neural substrate for BSR.

284.2 A GEOMETRIC MODEL TO ACCOUNT FOR NON-LINEAR CURRENT-FREQUENCY TRADE-OFF DATA IN CIRCLING & SELF-STIMULATION. J. S. Yeomans and David Wen* (Dept. of Psychology, University of Toronto, Toronto, Ont., Canada).

Electrical stimulation applied just lateral to the median raphe in rats produces ipsilateral circling at currents as low as 18 uamps (100 Hz, 0.1 msec duration cathodal pulses). Paired pulse studies have suggested that the neurons mediating this behavior have very short (0.32-1.2 msec) absolute refractory periods (Miliaressis, 1981). In this experiment we examined the spatial and temporal summation properties of these neurons by means of current-frequency trade-off functions. Although similar in most respects to data from self-stimulating rats (Gallistel, Shizgal and Yeomans, 1981), the trade-off between current and 1/Frequency is not linear except under special conditions. The non-linearities found are different for different electrode placements, suggesting that the geometrical relationship between the electrode tip and the behaviorally relevant neurons is critical. A simple geometrical model is proposed which accounts for the patterns of non-linearities found. This geometric model also accounts for non-linearities that have been previously found in current-frequency trade-off data from self-stimulating rats (Shizgal, Howlett & Corbett, 1979). This model should be useful in localizing the substrate for behaviors elicited by brain stimulation.

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284.3 INTERMEAL INTERVAL, MINIMUM MEAL SIZE AND THEIR INTERACTION: A PARAMETRIC STUDY. T. W. CASTONGUAY*, M. B. P. LEUNG*, AND J. S. STERN* (SPON: V. MENDEL). NUTRITION DEPARTMENT AND FOOD INTAKE LABORATORY, UNIV. CA. DAVIS, DAVIS, CA 95616.

Before analyzing moment-to-moment feeding behavior, two parameters must be defined: intermeal interval (IMI) and minimum meal size (MMS). Typically, investigators choose one definition for determining the end of a meal (the IMI) and one definition for the minimum amount of food required to determine the onset of a meal (the MMS definition) While IMI definitions have been explored by several investigators, MMS and its interaction with IMI's have not been systematically studied. The present experiment details the interaction that is observed in daily meal frequency and average meal size by varying both definitions.

Five IMI definitions were chosen (1,2,5,10, and 15 minutes). Within each of these, four MMS definitions were explored, so that a total of 20 different analyses of the same feeding patterns were conducted. Four genetically obese (fa/fa) and four lean (Fa/-) adult female Zucker rats were used as experimental animals.

Results from the analyses revealed that under most definition combinations, obese rats eat significantly fewer meals than do their lean littermates. For example, when IMI was 5 minutes and MMS was .04 g obese rats ate 17.13 meals and lean rats ate 22.12 meals ($p < .05$). However, long IMI's in combination with small MMS definitions tend to obscure that finding. For a IMI of 15 minutes and a MMS of .01 g there were no significant differences in number of meals eaten by obese and lean rats (17.38 vs. 19.00 $p > .05$, respectively). Further, although the average meal size of the obese rats is significantly greater than that of lean rats, the size of the difference between these genotypes varies widely with the use of different definition combinations.

No one set of combination of definitions was ideally suited to assess the meal patterns of the two genotypes. Although no differences in meal frequency with 5,10 and 15 minutes IMI's were found in obese rats, these same definitions promoted changes in meal frequency when applied to lean rat meal patterns.

It is recommended that meal pattern analysis include more than the customary single set of definitions before drawing conclusions about differences between experimental and control groups.

(Supported in part by NIH grants AM 07355, AM 18899 and AM 13252.)

284.4 AN ON-LINE COMPUTERIZED FEEDING, DRINKING AND ACTIVITY PATTERNS ANALYSIS SYSTEM. P.M.B. LEUNG*, Q.R. ROGERS*, J.S. STERN* and V.E. MENDEL. (SPON: L. CHAPMAN). Depts. of Physiol. Sci., Nutrition, Animal Physiol. and the Food Intake Group, Univ. of California, Davis, CA 95616.

An on-line computerized feeding, drinking and activity monitoring system has been developed to determine detailed changes in each parameter using rats or other small animals under various dietary or choice regimens. The system consists of a plexiglass cage with inter-changeable food tunnels on both sides, Mettler electronic balance(s) fitted with spillage-proof food cup(s) positioned under the food tunnel(s), 3 electronic fluid volume detectors and an activity-wheel with electronic sensors. All devices, interfaced with an on-line computer are housed in a chamber with individual lighting and air circulating system is configured to run the RT-11 operating system (Digital Equip. Corp.). The computer is equipped with floppy and hard disk storage, a full compliment of analogue input and output, serial I/O ports and a custom designed interface to the Cage Communication Bus. Each cage is equipped with digital to analogue converters. The chamber is fitted with circuit boards, a status display panel and several internal Input/Output Registers which may be used as the experiments require. Feeding, drinking and running activities of animals can be monitored in short time lapses from 1/30 of a second to minutes. The detailed data output include: 1) the cage No. being monitored; 2) the type of activity being recorded; 3) the time for the initiation of activities; 4) the durations of such activities in seconds and fraction of a second; 5) the time for the initiation of the dark/light cycle and short-stop time of other events; and 6) the cumulative summations of all the activities during a specific (e.g. 12:12 hr) dark/light cycle. Software developments enable the determinations of meal size and frequency, drinking and running bouts, inter-activities intervals etc. in histogram form or group comparisons with statistics. The system has been used to determine feeding, drinking and running patterns of obese and lean Zucker rats fed diets varied in protein or fat contents, and of animals fed disproportionate amounts of dietary amino acids. These results will be discussed.

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284.5 STIMULATION OF SALT APPETITE IN HAMSTERS. D.A. Fitts*, E.S. Corp* & J.B. Simpson. Department of Psychology, University of Washington, Seattle, WA 98195. (Spon. I.L. Bernstein).

The existence of a salt appetite in hamsters has been questioned. Hamsters will normally avoid .15 M NaCl solutions in choice with water, even following DOCA or adrenalectomy. The present study examined free-choice selection of saline or water under two conditions which produce vigorous salt drinking in rats: hyperoncotic colloid dialysis (30% polyethylene glycol (PEG), 1.5 ml/100 g, sc) or a sodium deficient diet.

PEG treatment elevated saline intake, but not water intake, over 48 hr postinjection. This effect on saline intake was small compared to rats, increasing over baseline from 0.8±0.5 to 2.3±2.4 at 24 hr and 2.8±2.7 ml/day at 48 hr (n=10). Not all hamsters, however, responded with saline intake. Increases of sodium and water balances proceded slowly, with only one hamster approximating the proportional accumulations commonly seen in rats. Urinary sodium excretions during the first 24 hr of PEG averaged only 0.121±0.058 mEq, and the volume of urine declined by 50% (5.2±1.3 to 2.6±0.7 ml). Food was always present, with consumption declining from 8.4±0.6 to 4.8±1.8 g after 24-hr PEG. Water and food intakes were highly variable between subjects and were highly correlated ($r(14)=0.96$, $p<.001$) during the first 24-hr PEG.

The sodium deficient diet also increased mean saline intake in 8/10 hamsters over 15 days (from 0.4±0.3 to 0.8±0.6 ml/day). Consumption tended to be episodic rather than continuous, although 4/10 hamsters did develop a regular consumption pattern. The median excretion ratio for Na^+/K^+ during the low sodium diet was 0.02, or only slightly greater than the Na^+/K^+ ratio of the diet (0.01). After a return to the normal diet, saline intake returned to baseline within 6-12 days.

Together these studies indicate that hamsters do have a mechanism for salt appetite, although this species apparently relies heavily on renal mechanisms for compensation of hydromineral balances after PEG or low sodium diet challenges.

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284.6 TASK-DEPENDENT PROPERTY OF BRAIN STIMULATION ON DRL REWARD SCHEDULES. N.L. Freedman & J. Law*. Psychology Department, Queen's University, Kingston, Ontario, Canada, K7L 3N6.

Enhancement of lever-press responding for brain stimulation reward (BSR) by lengthening current duration to 300 msec. and gradual decline thereafter (Keesey, R.E., J. Comp. Physiol. Psychol., 58: 201, 1964) suggests that longer durations are aversive or produce incompatible responses. The effect is task-dependent (Lyons & Freedman, Unpublished). Identical stimulation delivered to the same rats for wheel-turning produces a monotonically increasing function of BSR duration over a variety of stimulus intensities. Thus, the incentive value of the stimulation depends on task rather than neural substrate underlying stimulation. With differential reinforcement of low response rates (DRL) successful reinforcement depends on withholding responses and is independent of interference by stimulation or response produced inertia in the wheel. The present experiment compares lever-press and wheel-turn over several DRL schedules (2, 4, 8, 16, 32 sec.) for BSR.

Four rats were trained to lever-press and wheel-turn (40 degrees) for 300 msec., 60 Hz, trains of medial forebrain bundle stimulation. Rats were tested one day on the wheel and one day on the lever for 15 min. at each successively longer DRL. Half the rats were trained on the wheel and half on the lever first.

Rats obtained a greater proportion of rewarded responses on the wheel than lever across all DRLs ($F\ 5,15 = 5.06$, $p = .0067$). Such results cannot be attributed to neurochemical substrates since at all DRLs stimulation of the same brains activated identical neurotransmitter systems for both tasks. Results support the previous conclusion that activation of relevant neurotransmitter systems provides task-dependent differential incentive for responding.

284.7 EFFECTS OF ANTERIOR AND POSTERIOR MEDIAL FOREBRAIN BUNDLE LESIONS ON LATERAL HYPOTHALAMIC SELF-STIMULATION REWARD. James R. Stellar and Scott Neeley. Dept. of Psych.& Soc. Rel., Harvard University, Cambridge, MA. 02138

A number of studies have focused upon the effects of electrolytic lesions in the medial forebrain bundle (MFB) on lateral hypothalamic (LH) self-stimulation reward (see Lorens, in Ed. Wauquier & Rolls, Brain and Reward, N.Y.: Elsevier, 1976, pp. 41-50). However, none of these studies have employed the recently developed quantitative techniques for characterizing the reward substrate (see Gallistel, Shizgal, & Yeomans, Psych. Review, in press). This report examines the effects of MFB lesions delivered anterior to, posterior to, and through the LH stimulating electrode on the reward summation function (RSF) described by Edmonds & Gallistel (JCPP, 1977, 91, 362-974).

A single point on the RSF is generated by finding the median speed of running a runway for one burst of LH stimulation. Other points on the RSF are generated by repeating the above step but using a burst with a different number of pulses. This RSF curve has a characteristic inflection point termed the locus of rise which is found by calculating the number of pulses required to sustain half asymptotic running speed. Changes in the locus of rise of the RSF have been shown to reflect changes in the effectiveness of pulses in generating reward; whereas, changes in asymptotic running have been shown to reflect changes in performance variables (e.g. task difficulty).

Large anterior lesions placed at the anterior border of the lateral hypothalamus and occupying about 75% of the crosssectional area of the MFB did not significantly affect the RSF from the LH electrode. Smaller posterior lesions placed just rostral to the MFB produced large shifts in the locus of rise of the RSF, indicative of decreased pulse effectiveness, as well as shifts in its asymptote. Small lesions through the electrode also produced similar shifts of the RSF. These findings suggest that the important reward relevant direction of conduction within the MFB is posterior. This agrees with previous work on LH self-stimulation in the hemiforebrain ablated rat (Stellar, Illes, & Mills, Neuroscience Abstract, 1979) and two electrode hyperpolarization blockade research (Shizgal, EPA symposium, 1981).

284.8 EFFECTS OF MODIFYING THE ENVIRONMENTAL CONDITIONS ON THE SELF-STIMULATION BEHAVIOR AT THE POSTERO-LATERAL AREA OF THE HYPOTHALAMUS. Ph. De Witte* and M. Meulders (SPON: B. Will). Lab. Neurophysiology, Univ. Cathol. Louvain, Faculté de Médecine, Bruxelles, Belgium.

In order to study the relationships existing between the self-stimulation behavior and the positive or negative properties of the environments, six situations were designed: home cage and novel cage into which was added or not a male or a female presenting or not a lordosis behavior. In each 10 min session of self-stimulation, the rewarding value induced by the electrical combinations (.1 msec pulse width delivered at the frequency of 100, 200 and 300 Hz, and .2 msec at 100, 200 and 300 Hz) was estimated by 4 parameters: (1) the total time each animal had effectively self-stimulated (T), (2) the number of bar presses (N), (3) the mean duration of bar pressing ($M=\frac{T}{N}$), and (4) the standard deviation of the duration of bar presses.

Results show that the total time the animal self-stimulates varies with the situation. In increasing order of self-stimulation time, the six situations are: 1° home cage, 2° home cage + female presenting a lordosis behavior, 3° home cage + male, 4° home cage + female not presenting a lordosis behavior, 5° novel cage, and finally 6° the highest total time of self-stimulation was found for novel cage + male.

Moreover, negative coefficient (-.72) of correlation relates the total time spent to self-stimulate to the mean duration of a bar presses as well as between N and S (-.64). Thus, the more the environment seems to be rewarding, the less animals self-stimulate, but the more the mean duration of bar presses increases, and the more often animal self-stimulated, the shorter became the mean duration of the bar presses. Furthermore, novel cage conditions seemed to have a clearly different influence from the other environment.

These experiments show that animals could either compensate the negative effects of the environment (novel cage) by increasing self-stimulation behavior or decrease the latter when environment is positively rewarding.

It is possible, therefore, that there exists a kind of homeostasis regulating the influx from the milieu externe relative to the affective data of the milieu interne, here excited by electrodes in the hypothalamus.

284.9 PREDICTION OF HYPOTHALAMIC "PREPOTENCY" WITH AMPHETAMINE-INDUCED STEREOTYPIES. Susan E. Bachus & Elliot S. Valenstein. Dept. Psychobiology, Univ. Michigan, Ann Arbor, Michigan, 48109.

Individual differences in behaviors elicited by electrical stimulation of lateral hypothalamus (ESLH), which range from locomotor agitation to consummatory behaviors, are not attributable to differential electrode placement; thus they may reflect individual variability in response predisposition or "prepotency" of the animal (Valenstein, Brain Behav. Evol. 2:295, 1969; Bachus & Valenstein, Physiol. Behav. 23:421, 1979). Rather than simulate behaviors exhibited during natural "drive states", responses seen during ESLH parallel in many respects the "stereotypic" behaviors elicited by stimulant drugs such as amphetamine (Valenstein, Nebraska Symposium on Motivation 1974, p. 251; Biology of Reinforcement, ed. A. Routtenberg, p. 39, 1980). However, amphetamine does not facilitate ESLH-induced consumption in rats(Wishart & Walls, J. Comp. Physiol. Psych. 87:741, 1974), presumably because of the anorexigenic effects of the drug.

When durations of discrete stereotyped behaviors (locomote, rear, "nose-hole-poke", groom) were recorded independently, 30 naive male Long Evans hooded rats, observed following 3 separate 1.5 mg/kg ip administrations of d-amphetamine sulphate, displayed consistent individual differences in predominant type of stereotypy elicited. Subsequently, these rats were implanted with bilateral stimulating electrodes in lateral hypothalamus and screened for elicitation of consummatory behaviors (eat, drink, gnaw) by ESLH. Across individuals, duration of stereotypy which entails orientation to external stimuli (nose-hole-poke) recorded during the first amphetamine experience was highly correlated with duration of ESLH-induced consumption ($r=.75$, $p<.01$).

To exclude the possibility that responses to ESLH were influenced by amphetamine history, 12 naive rats were first implanted with electrodes and screened for response to ESLH, and afterward received 3 injections with amphetamine. Again, the duration of ESLH-elicited consummatory behavior was significantly correlated with duration of nose-hole-poke evoked by amphetamine ($r=.62$, $p<.05$). Moreover, statistical analysis of histological determination of electrode placement (Mittleman & Valenstein, Physiol. Behav. 26:371, 1981), for these 42 rats, indicated that exact locus of stimulation did not interact with nose-hole-poke duration in predicting response to ESLH.

These results suggest that stable individual differences in behaviors evoked either by stimulant drugs or by ESLH may reflect more fundamental individual variation in tendency to respond to arousal either with locomotor excitation or with attention directed to stimuli in the external environment.

284.10 BEHAVIORAL SENSITIZATION EFFECTS FOLLOWING REPEATED ELECTRICAL STIMULATION OF THE MEDIAL PREFRONTAL CORTEX. Dale Corbett,* Andre Laferriere*and Peter M. Milner.* (SPON.: Alan Rosenquist) Dept. Psych., McGill Univ., Montreal, Quebec, CANADA H3A 1B1.

Intracranial self-stimulation (ICSS) from the medial prefrontal cortex (PFC) has different properties than ICSS from other brain regions. One such property is that PFC ICSS is acquired slowly, taking 5 or 6 days to establish. During initial training the animals appear little affected by priming stimulations. Over time, the priming stimulation begins to be effective as indicated by the animals beginning to orient towards the lever of the Skinner box.

This behavioral pattern suggested that the electrical stimulation was somehow "sensitizing" the neuronal circuitry involved in brain stimulation reward. Thus we sought to determine if we could hasten the acquisition of PFC ICSS by pretreating the animals with electrical stimulation prior to training. Forty PFC implanted rats were assigned to one of 3 groups: 1) SPACED - stimulation delivered at the rate of 1 train/4 sec for 20" per day for 6 days; 2) MASSED - stimulation delivered at the rate of 1 train/sec for 15" per day for 2 days; 3) CONTROL - treated like SPACED group but received no stimulations. Stimulation consisted of .5 sec trains of 60 Hz sine-wave stimulation at a fixed current intensity of 40 μa (r.m.s.). After the pretreatment phase the rats were placed each day in lever equipped boxes and given a single stimulation. The ICSS acquisition criterion was an overall 30" session response rate of 5/min. (150 total).

Nearly 50% of the SPACED group attained the acquisition criterion on Test Day 1. By the 2nd Test Day this proportion had increased to 80%. In contrast, it was 4 or 5 days before 50% of the other groups reached criterion.

The main finding of this study is that prior stimulation of the PFC results in a more rapid acquisition of ICSS than in animals without a prior stimulation history. Also, it appears that the stimulation is more effective when spaced since the MASSED group did not learn to self-stimulate any more rapidly than did controls. These data suggest that the initial effects of PFC stimulation are not, or are at best, minimally rewarding and that the rewarding effects become strengthened following the modification of as yet unidentified circuitry. However, it is worth noting that synaptic potentiation occurs in the perforant path-granule cell circuit of the hippocampus and that hippocampal ICSS exhibits similar characteristics to PFC ICSS (Campbell & Milgram, 1978).

284.11 MAPPING OF BRAIN-STIMULATION REWARD SYSTEMS WITH THE 2 DEOXY-GLUCOSE TECHNIQUE. E. Yadin and C. R. Gallistel. Department of Psychology, University of Pennsylvania, Philadelphia, Pa 19104.

The 2-deoxyglucose technique (Sokoloff et al., J. Neurochem., 28:897-916, 1977) was utilized to map the neural systems activated by self-stimulation in rats with unilateral electrodes in four different, well-established self-stimulation sites in the brain: the medial forebrain bundle (MFB) at the level of the anterior hypothalamus and at the level of the posterior hypothalamus, the medial prefrontal cortex, and the locus coeruleus.

Rats were trained to self-stimulate in a standard Skinner box. During the 2-DG session rats self-stimulated at a current that produced 3/4 of their maximum responding rate. After a short "warm-up" period the rats were injected intraperitoneally with ^{14}C-2DG and allowed to self-stimulate for 45 min at the end of which they were given an overdose of anesthetic, perfused for 30 sec with a 3.3% buffered formalin solution; the brain was promptly removed and frozen in liquid freon. The sectioning of the brain and the autoradiographic procedure were similar to those originally described by Sokoloff et al. (1977).

The determination of the neural systems exhibiting altered activity as a consequence of self-stimulation was performed using a computer-assisted image analysis. Since the electrodes were unilaterally implanted, comparisons could be made between the stimulated and nonstimulated sides of the same brain. According to such an analysis we found no overlap between the structures activated by the MFB electrodes and the medial prefrontal cortical electrode. The MFB electrodes produced darkening of the length of the MFB, from the anterior ventromedial tegmentum to the diagonal band of Broca and into the lateral nucleus of the septum. The medial frontal cortex electrode seemed to activate the piriform cortex, the anterior olfactory nuclei (both medial and posterior parts), the cortex adjacent to the rhinal sulcus, the enthorhinal cortex, the claustrum, and the dorsomedial nucleus of the thalamus. Indeed, observations of the animals' lever pressing behavior reveal the different nature of these two sites, the former being fast and vigorous, the latter slow and calm.

The neural pattern driven by the locus coeruleus electrode is virtually impossible to trace. In some animals, only the locus coeruleus itself was differentially darker on the stimulated side *and no other structures seemed to be labelled.* In other animals that were behaviorally excellent self-stimulators too, even the locus coeruleus did not appear to take up more labelled 2-DG on the stimulated side versus the nonstimulated side. It is possible that a system with many diffuse projections diverging from a small area cannot be easily detected with this technique.

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285.1 EEG AND BEHAVIORAL EFFECTS OF GAMMA-HYDROXYBUTYRATE IN THE RABBIT. R. Godbout* and R.T. Pivik. Lab. Neurophysiology, School of Psychology and Department of Psychiatry, Faculty of Health Sciences, University of Ottawa, Ottawa, Ontario K1N 5C8, Canada.

Gamma-Hydroxybutyrate (GHB), a metabolite of gamma-aminobutyric acid (GABA), has been reported to have hypnotic effects at low doses and excitatory effects at high doses. Although these effects have been reported in a variety of species, including man, they have not been examined in the rabbit. Moreover, in man this compound has recently been reported to facilitate sleep onset, reduce the latency to rapid eye movement (REM or PS) sleep, and increase the duration of PS episodes. The present study was conducted to provide data regarding the electrographic and behavioral effects of GHB in the rabbit, with particular reference to sleep-wakefulness variations.

New Zealand White rabbits (n=5) were chronically implanted for recording of EEG, EOG and nuchal EMG activity. Polygraphic recordings, which did not begin for at least two weeks post-operatively, were conducted between 9:00 and 15:00 hrs. Following IV administration of GHB or saline, two consecutive hours of recordings were taken in unrestrained animals housed in sound attenuated, climatically controlled cages. The following nine dosage levels were employed: 25, 50, 100, 150, 200, 300, 500, 750 and 1000 mg/kg, with at least 48 hour intervals between injections. Polygraphic recordings were analyzed in 30 second epochs for sleep-waking state changes. Results were analyzed using repeated measures analyses of variance with post-hoc comparisons based on Dunn's method.

GHB administration did not significantly facilitate sleep onset (drowsy or slow wave sleep: SWS) or latency to PS. At the highest dosages (750 and 1000 mg/kg) PS did not occur within the two hour recording period. This suppression of PS was accompanied by a significant ($p < 0.07$) increase in SWS and a decrease in wakefulness ($p < 0.10$). Other state variations following GHB administration were non-significant.

At dosages of 750 and 1000 mg/kg, high amplitude, slow-waves (250-500 uV; 2-4 Hz) appeared superimposed upon SWS patterns. Evidence of behavioral sedation was present in all animals at these dosages and although increased twitches were present in the nuchal EMG, EEG spike discharge or other epileptoid phenomena were not observed.

Whereas these results do not indicate the presence of hypnotic effects of low-dosages of GHB in the rabbit, the decrease in activated states (W and PS) and increase in SWS at high dosages are consonant with previous suggestions of a depression of the ascending reticular activating system by high doses of this compound.

285.2 THE INFLUENCE OF EXTRA-RETINAL AFFERENTS ON SLEEP STAGE RELATED NEURONAL ACTIVITY IN THE LATERAL GENICULATE NUCLEUS OF THE RAT. G.A.Marks, S.G.Speciale, Jr., J.Farber, H.P.Roffwarg, Department of Psychiatry, University of Texas Health Science Center, Dallas, Texas 75235

Levels of activity of single cells in widespread areas of the brain have been found to be influenced differentially by stage of sleep. This phenomenon appears to be a basic sleep behavior process, and one that might be related to the function of specific sleep stages. The brain mechanism controlling sleep stage-specific cellular activity is unknown, but it is likely to be controlled by just a few neural systems.

Our strategy is to use the dorsal lateral geniculate nucleus (dLGN) as a model for the study of sleep related influences. This area is well suited since dLGN cells exhibit sleep stage related activity (mean discharge rates in excess of 2:1 in REM sleep compared to slow wave sleep (SWS). Stage related activity is maintained after bilateral enucleation indicating that extra-retinal afferents are responsible for the regulation. By local and selective pharmacological manipulation of dLGN inputs it might be possible to identify the neurochemical mechanisms controlling sleep stage-specific cellular activity.

The present study utilized a microwire cannula guide tube assembly implanted bilaterally in the dLGN and standard electrodes used to define the sleep-wake stages. The multiple unit activity derived from the microwires yielded REM:SWS ratios of between 2:1 and 6:1. Specific neurotoxins or receptor blocking agents were pressure injected slowly through a cannula inserted into the guide tube. The contralateral side received a control solution. Mean frequencies of the multiple unit activity were determined within sleep-wake stages between ipsi- and contralateral dLGNs and during pre- and post-drug administration.

Initial data demonstrate that norepinephrine (NE)-depleting doses of 6-hydroxydopamine hydrobromide (4µg/0.5µl, in 0.1% ascorbate-normal saline) significantly increased the REM:SWS ratio by differentially slowing the rate of cellular activity in SWS. These data are consistent with the facilitatory effects of: 1)NE on P-cells; 2)Locus coeruleus (LC) innervation of dLGN and 3) the higher SWS than REM sleep discharge rate of LC cells.

Accordingly, these data not only demonstrate the feasibility of the employed technique, but further fail to support that it is the NE input to dLGN that can account for the differences in neuronal activity observed across sleep stages. Examination of the effects of other humoral systems on dLGN cells will also be presented.

285.3 EFFECTS OF PHENOXYBENZAMINE ON REM SLEEP AND BRAIN MHPG-SO_4 IN NORMAL AND REM SLEEP DEPRIVED RATS. R.C. Walovitch, R. Zak* and M. Radulovacki. Dept. Pharmacology, Univ. Ill. Med. Ctr., Chicago, IL 60612.

The aim of this study was to determine whether α-adenoreceptor blocking ability of phenoxybenzamine (Pbz), as indicated by an increase in MHPG-SO_4, could be correlated with the drug's ability to reduce REM sleep in normal, non-REM sleep deprived (NRD), as well as 48 h REM sleep deprived (RD) rats. To accomplish this, the amount of REM sleep and the amount of MHPG-SO_4, a final brain norepinephrine metabolite, was determined in the following groups of animals: 1. NRD group, 2. NRD rats which received Pbz (Pbz group), 3. rats deprived 48 h of REM sleep (RD group), 4. rats given Pbz prior to 48 h of REM sleep deprivation (Pbz RD group). Two hours before decapitation rats received probenecid, 50 mg/kg. MHPG-SO_4 was determined in the whole brains of rats by the fluorometric method of Meek and Neff (B.J. Pharm. 45: 435, 72). Continuous EEG recordings in the identical 4 groups of animals was done for 60 h. The results show that there was a reduction in REM sleep ($P < 0.002$) in the Pbz group during the first 10 h of EEG recording as compared to NRD groups. The effect of Pbz on REM sleep was over prior to 48-60 h of EEG recording period. This corresponds to the time when MHPG SO_4 level in the Pbz group was indistinguishable from the MHPG SO_4 level in the NRD group (Table 1). The increase in MHPG-SO_4 in the Pbz RD group (as compared to RD group) indicates that Pbz α-blocking activity may still be present 48 h after drug administration. Table 2 shows that REM sleep deprivation tends to potentiate Pbz ability to reduce REM sleep and increase MHPG-SO_4.

TABLE 1 - The Effect of Pbz on MHPG SO_4 in 48 h RD and NRD Rats

NRD(6)473*	Pbz(6)546	RD(7)655^{+}	PbzRD(8)813*$^{+}$

( )=Number of rats. The results are means of pm MHPG SO_4/g of brain tissue wt. $+p < .001$ when comparison is made to NRD, $*p < .002$ when comparison is made to RD using one way ANOVA with Scheffe test for multiple comparisons.

TABLE 2 -

	MHPG-SO_4 (pm/g)	REM Sleep (min)
%Pbz/NRD	+15 (68)	-10 (8 min)
%Pbz RD/RD	+24 (190)	-24 (33 min)

We conclude that, in non-REM sleep deprived rats, Pbz's termination of action on REM sleep corresponds to the termination of the drug's effect on central α-adrenoreceptors.

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285.4 SLEEP IN RATS WITH BASAL FOREBRAIN LESIONS. R. Szymusiak* and E. Satinoff. Dept. Psychology, Univ. Illinois, Champaign, IL 61820.

Damage to the medial preoptic area (MPOA) causes disturbances in body temperature (Tb) regulation and sleep/waking behavior. We have previously shown that active sleep (AS) in normal rats is extremely sensitive to ambient temperature (Ta). Therefore, a portion of the AS deficits which follow basal forebrain damage might reflect exaggerated sensitivity to thermal stress.

Male rats were chronically implanted for sleep recordings. Pre- and postlesion recordings were taken for 2 hours at each of 3 Ta's; 20, 25, 30°C. Data were collected at all 3 Ta's on the same day during the light portion of a 12:12 LD cycle.

Prior to lesioning, all rats showed similar sleep patterns. Total sleep time (TST) was longest at 30°C (5440 ± 134 (s.e.) seconds), less at 25°C (4721 ± 167 sec.), and least at 20°C (3954 ± 231 sec.). These differences were largely due to changes in AS: at 30°C, AS=1214 ± 50 sec. and AS/TST=.22 ± .01; at 25°C, AS=677 ± 43 sec. and AS/TST=.14 ± .01; at 20°C, AS=391 ± 57 sec. and AS/TST=.10 ± .01. One day following bilateral electrolytic MPOA lesions, 6 rats were hyperthermic in their home cages with Tb's ranging from 39.5-41°C. None could tolerate 30°C for more than an hour without approaching lethal hyperthermia, nor could they defend Tb when exposed to 5°C. TST was greatly reduced at all Ta's tested (e.g. TST=1296 sec. at 25°C). None of the rats exhibited AS and one was totally insomniac. At 4-5 days postlesion, TST was still depressed, but AS had become sensitive to Ta. For example, the rat that had been insomniac on day 1 was markedly hyposomniac at 20°C on day 5 (TST=690 sec. and AS/TST=0). However, on the same day at 25°C, TST was 2330 sec. and AS/TST was .20. Four of the 6 animals were hyperthermic, and exhibited the most AS at 25°C, whereas prior to lesioning all had shown the most AS at 30°C. The other animals, one of whom was hypothermic and one normothermic, had the most AS at 30°C. So, AS time is maximal at different Ta's in different lesioned rats. Testing animals at a single Ta might give the erroneous impression of an AS deficit.

In summary, we find a relatively Ta-independent reduction in TST immediately after MPOA lesions. However, as early as 4-5 days postlesion AS/TST may be markedly depressed at one Ta, but within control ranges at another. Thus, much of the AS deficit in rats with basal forebrain damage appears to be secondary to thermoregulatory disturbances. Later stages of recovery and results of 24 hour recordings will also be discussed.

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285.5 UNIT ACTIVITY OF NE AND non-NE PONTINE TEGMENTUM NEURONS IN THE RAT IN RELATION TO SLEEP-WAKE STATES AND REM SLEEP PHASIC ACTIVITY. P.Gatz*,H.P.Roffwarg and J.Farber (SPON: J.Herman). Dept. of Psychiatry,University of Texas Health Science Center at Dallas, Dallas, Texas 75235.

The role of the nucleus locus coeruleus (LC) and its neurotransmitter norepinephrine (NE) in the mediation and maintenance of REM sleep has not been incontrovertably resolved. Whereas lesion and pharmacological studies indicate significant contribution of the LC nucleus to REM sleep mediation (Jouvet, M., Ergebn.Physiol.64:166,1972),neurophysiological data indicate a paucity of LC unit activity in REM sleep (Hobson & McCarley, Science 201:269,1978;Foote et al,Proc.Natl.Acad.Sci.77:3033,1980). We have recently reported the presence of a PGO-type wave recordable during REM sleep in the pontine dorsal tegmentum,in the area of the LC,(Farber, J., et. al., Science 209: 615,1980). In order to establish whether the pontine PGO waves are events that are related in time to the increases in underlying neuronal activity and identify the characteristics of these neurons, we have studied the relationship of pontine neuronal unit activity to sleep-wake stages and pontine PGO waves.

Male albino rats (Sprague-Dawley, 400-500gms) were implanted with stainless steel recording wells, positioned above the area of the LC.Macroelectrodes were implanted for cortical EEG, neck EMG and pontine PGO activity.For 24 hours prior to the recording session, the rats were sleep deprived to increase the occurrence and to extend theduration of the various sleep stages.Single unit activity was recorded in these unrestrained rats in the LC, sub-LC, nuc. retic. pontis oralis (rpoo), and the dors. and vent. parabrachial nuc. (npd, npv), with glass-coated tungsten electrodes.Significant stage-dependent differences were found in the firing rates of the NE and non-NE cells.As recognized by their long duration action potentials (2msec.) and low firing rates (1. 5 to 3Hz), the NE cells of the LC showed a marked decrease in activity in REM sleep.No relationship was found between the unit activity and the PGO waves.

On the other hand, the activity of cells in the npd, npv and rpoo showed marked differences in firing rates in the four, mutually exclusive, sleep-wake stages: quiet awake(AQ),moving awake (AM), slow wave sleep (SWS) and REM sleep.In REM sleep and in AM, the firing rates of these units substantially increased compared to SWS and AQ.Bursts of activity were temporally associated with the occurrences of PGO waves in the perievent histograms. We conclude that pontine tegmentum PGO waves recorded in REM sleep reflect an increase in the cellular activity of the non-NE neurons in the area.

285.6 UNIT ACTIVITY OF DOPAMINERGIC AND NON-DOPAMINERGIC NEURONS IN THE REGION OF THE SUBSTANTIA NIGRA IN RELATION TO SLEEP-WAKE STATES AND REM SLEEP PHASIC ACTIVITY. J. Farber, J.D. Miller, P. Gatz*, H. Roffwarg and D.C. German. Depts. of Psychiatry and Physiology, U.T. Health Science Center, Dallas, Texas 75235.

It has been proposed that catecholamines may be important for the mediation of REM sleep (Jouvet, M., Ergebn. Physiol. 64:166, 1972). A major thrust in the study of sleep has been directed at the norepinephrine rather than the dopamine (DA) system. However, biochemical studies (e.g. Wojcik, W.J. and Radulovacki, M., Neurosci Abstr., 6:52, 1980) have indicated a role for DA in sleep. We now report on the spontaneous unit activity of DA cells and nearby non-DA cells during wakefulness and sleep.

Male albino rats (Sprague-Dawley, 300-400 gms) were implanted with stainless steel wells stereotaxically positioned above the mid-brain DA cell region. Also, electrodes were implanted for the recording of EEG, EMG and pontine PGO activity (Farber, J., et al, Science, 209:615, 1980). The rats were sleep deprived for 24-48 hrs before the recording session in order to increase the occurrence and extend the duration of the various sleep stages. Single unit activity was recorded in the substantia nigra and ventral tegmental area (VTA) regions, using glass coated tungsten electrodes in the unrestrained rat, while the sleep-wake stages were being polygraphically recorded. The DA cells were recognized by their long duration triphasic action potentials (> 2 msec) and slow firing rates (2-7 Hz). Also, microlesions were made at the DA unit recording sites. The firing rates of the DA cells were not different across four mutually exclusive stages of the sleep-wake cycle: quiet awake (AQ), moving awake (AM), slow wave sleep (SWS), and REM sleep. These cells exhibited a steady firing pattern across all stages. On the other hand, the faster firing (5-30 Hz), short duration (<·2 msec) non-DA cells of the VTA and zona reticulata regions showed marked and significant firing rate differences that were stage dependent: in AM and REM sleep their activity was faster (up to 100%) than in AQ and SWS. Spontaneous activity always increased as the rat entered a REM sleep episode. From perievent time histograms summated over at least 50 PGO wave events, we found a marked increase in the discharge frequency of each of the non-DA cells 20 msec preceding and following the peak of the PGO wave. These data suggest that the non-DA cells of the ventral mesencephalon behave, in relation to sleep-wake stages, in a manner similar to cells of the pons that have been implicated in the mediation of REM sleep. In addition, it seems that DA and nearby non-DA cells do not show reciprocal changes in firing rates across sleep stages. (Supported by NIMH grants MH-31402, MH-27574 and A.P. Sloan Foundation).

285.7 EFFECTS OF BRAIN STEM INFUSION OF PROTEIN SYNTHESIS INHIBITORS ON SLEEP-WAKING BEHAVIOR AND SINGLE-UNIT ACTIVITY IN THE MIDBRAIN AND PONTINE RETICULAR FORMATION OF THE CAT. S.S. Bowersox, R. Drucker-Colin, and D.J. McGinty. Neuropsychology and Neurophysiology Laboratory, VA Medical Center, Sepulveda, CA 91343

Chloramphenicol (CAP), a protein synthesis inhibitor with potent bacteriostatic properties, was shown in previous studies to selectively suppress REM sleep in cats when administered systemically at a dose of 100 mg/kg (Drucker-Colin, et al., 1979, Exp. Neurol., 63:458, Petitjean, 1979, Psycopharm., 66:147). Recent investigations in our laboratory disclosed that, in addition to suppressing REM sleep, chloramphenicol significantly attenuated unit activity in the brain stem reticular formation (RF). Administration of CAP congener, thiamphenicol (TAP), had no impact upon either REM sleep or RF unit discharge, indicating that these findings reflected a specific neuropharmacologic effect. The current study sought to determine whether local application of CAP into the medial reticular formation also depressed unit activity and REM sleep. Toward this end, a combination push-pull cannulae and microwire/microdrive system was developed to record single units within the diffusion field of substances administered through the cannulae.

Eight adult cats of either sex were surgically prepared for EEG, EOG, and EMG recording. The push-pull cannula microdrive system was chronically implanted into the gigantocellular tegmental field of the pontine RF. Experiments were initiated at least 10 days following surgery. All studies were carried out on unrestrained animals while they were exhibiting spontaneous sleep and waking behavior. As predicted, perfusion of RF foci with 10 mg/ml CAP resulted in a substantial attenuation of both midbrain and pontine RF unit activity. This accompanied an increased incidence of abortive REM periods, identified in polygraphic recordings by the presence of PGO spikes without muscle atonia or EEG desynchronization, and a significant reduction in total REM sleep time. In agreement with earlier findings, TAP perfusion had no effect upon either unit discharge or sleep-waking behavior. The increased incidence of transitional SWS→REM periods during CAP infusion indicated that REM deficits were not caused by interference with pre-REM conditions. It is hypothesized that REM was prevented by suppressing the capacity of reticular neurons to sustain critical firing rates necessary for maintenance of this state.

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285.8 NEURONAL ACTIVITY IN THE PREOPTIC AREA OF THE HYPOTHALAMUS DURING SLEEP AND WAKEFULNESS IN THE CAT. K. Kaitin* (SPON: E. S. Boyd). Dept. of Pharmacology, Univ. of Rochester School of Medicine, Rochester, NY 14642.

The preoptic area of the hypothalamus is considered to be a forebrain-synchronizing area with putative hypnogenic function. The present study was conducted to examine the relationship between the neuronal activity in the preoptic area and the behavioral state of the animal. Spontaneous activity of single neurons was recorded extracellularly from the preoptic area in the basal forebrain of chronically prepared cats. Neuronal firing was studied during alert wakefulness (W), slow-wave-sleep with cortical high voltage slow wave activity (SWS), and REM (paradoxical) sleep.

Of the 86 neurons recorded in three behavioral states, 44% and 40% showed their fastest discharge rate during REM and SWS, respectively, while 16% fired fastest during W. However, there were no significant differences in the mean discharge rates in the different behavioral states: SWS, $\bar{X}$ = 28.5 ± 20.5; REM, $\bar{X}$ = 26.4 ± 21.3; W, $\bar{X}$ = 25.6 ± 21.6.

Individual neurons often exhibited changes in temporal firing patterns associated with a particular behavioral state. In SWS, neurons tended to discharge less regularly than in W or REM. Occasionally, this irregular discharge was accompanied by sporadic bursts alternating with long periods of silence. Similar observations have been made in other brain regions. In the present study changes in the degree of variability of discharge were evaluated quantitatively by comparing the coefficient of variation for each behavioral state. The value obtained during SWS (1.11 ± 0.44) was significantly greater than values obtained during W (0.91 ± 0.24) and REM (0.99 ± 0.24).

Neurons were classed as either fast or slow on the basis of their discharge rate, in relation to the overall mean discharge rate, during SWS. Neurons classed as fast during SWS fired more slowly as the animal's behavioral state changed to either W or REM, whereas neurons classed as slow during SWS fired more rapidly during W or REM. This activity of preoptic area neurons is in contrast to the activity of medial brainstem and motor, primary sensory, and association cortex neurons.

285.9 SLEEP FACTOR FROM HUMAN URINE; LOCALIZATION OF ITS SITE OF ACTION. J.E. García-Arrarás* (SPON: J. Krueger). Dept. of Physiol. Biophys., Harvard Med. Sch., Boston, MA 02115.

Administration of sleep-promoting factor derived from human urine into the cerebral ventricular system is followed by prolonged excess slow-wave-sleep (SWS) in rats, rabbits, and cats (J.M. Krueger et al. Am. J. Physiol. 238: E116-E123, 1980, J.E. García-Arrarás, Fed. Proc. 40: 273, 1981). I now present the results of systematic investigations on the site of action of urinary sleep factor in the brainstem.

Microinjections of urinary sleep factor were made into rabbits with chronically implanted guide tubes. About 15 picomoles of urinary sleep factor dissolved in 1.5 µl artificial cerebrospinal fluid (art CSF) were injected unilateraly in each rabbit. After injection the EEG was recorded for 6 hours. Injections into specific areas of the ventral brainstem extending from the supraoptic area to the rostral mesencephalic reticular formation increased the amount ot time spent in SWS. Injections of art CSF into the same sites were ineffective. The average increase was from 45 ± 2% of 6 hours in SWS after injections of art CSF to 63 ± 3% of 6 hours after injections of urinary sleep factor. Numerous other sites (N=68) were found to be inactive.

The behavioral and electroencephalographic characteristics of the excess SWS following microinjections of sleep factor from human urine were similar to those following intraventricular infusions of Factor S. However, the latency to the effect is shorter after microinjections into the active area.

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285.10 EFFECT OF 3 OZS OF 80 PROOF ETHYL ALCOHOL ON THE SLEEP OF PATIENTS WITH OBSTRUCTIVE SLEEP APNEA. L. Scrima & M.A. Cohn*. Sleep Disorders Center, Mount Sinai Medical Center, Miami Beach, FL 33140.

Patients with obstructive sleep apnea (OSA) suffer from repeated upper airway obstruction during sleep, causing apneas that last more than 10sec. The apnea episodes usually end with an arousal. Research in our lab recently demonstrated that only 3ozs of alcohol greatly increased the incidence and severity of apneas in OSA patients (Scrima, L., Broudy, M. & Cohn, M.A., Sleep, 4(3), 1981). The sleep stages from the same study are herein analyzed to assess the impact of 3ozs of alcohol on OSA patients' sleep. Five diagnosed OSA patients, ages 28-67 (4 males, 1 female) were studied. EEG, EOG, chin-EMG, respiration and arterial oxygen saturation were recorded, 30 min after beginning to consume the alcohol. The sleep stages from this research night were compared to those of the previous diagnostic night sleep.

	WITH ALCOHOL					NO ALCOHOL				
	GWA	OSWA	AWA	LZWA	APWA	GNA	OSNA	ANA	LZNA	APNA
TIME IN BED	353	339	342	402	286	453	377	292	416	258
SLEEP PERIOD TIME	349	331	340	394	283	427	374	288	396	257
TOTAL SLEEP TIME	343	329	321	370	269	363	353	273	385	233
SLEEP EFFICIENCY INDEX	97%	98%	93%	92%	91%	86%	93%	93%	92%	89
SLEEP LATENCY (1)	4	2	2	10	3	26	3	4	28	2
SLEEP LATENCY (2)	11	12	6	20	27	33	11	6	25	17
NUMBER OF STAGES	24	66	56	39	58	56	35	53	34	42
NUMBER OF STAGE 1 SHIFTS	12	62	48	18	36	48	34	34	22	30
NUMBER OF AWAKENINGS	4	2	10	7	9	16	5	9	5	7
AVERAGE 1-REM DURATION	27	7	12	15	5	16	49	7	44	8
NUMBER OF 1 REM PERIODS	2	7	5	5	8	4	1	3	2	5
PERCENT STAGE 4	8%	0%	0%	9%	8%	8%	8%	0%	6%	12
PERCENT STAGE 3	8%	0%	0%	7%	12%	16%	6%	20%	5%	4
PERCENT STAGE 2	46%	12%	15%	64%	33%	23%	9%	35%	53%	46
PERCENT STAGE 1	5%	69%	59%	4%	27%	14%	71%	27%	15%	17
PERCENT STAGE REM	28%	18%	18%	20%	18%	18%	12%	12%	22%	15
PERCENT STAGE 0	2%	1%	0%	0%	0%	35%	0%	0%	7%	10

Overall, after ingestion of alcohol, OSA patients were awake 6.6% less, had more stages REM (4.2%), 1 (4%) & 2 (7%) sleep and 4.4% less stages 3 & 4, than during the no alcohol diagnostic sleep. It is important to note that stages 1, 2 & REM sleep are the very stages during which nearly all OSA episodes occur. Also, reduced stages 3 & 4 can reflect an increase in OSA severity, since more severe OSA during stage 2 causes arousals to stage 1 or 0, thereby preventing stages 3 & 4. These data appear to indicate that 3ozs of alcohol ingested by OSA patients before bed potentiates apneas by increasing the arousal threshold, rather than by depressing the CNS respiratory regulators. OSA patients with alcohol were less able to arouse themselves from apnea episodes, causing more prolonged apneas and less time in bed spent awake.

286.1 NALOXONE DIFFERENTIALLY AFFECTS THE ANALGETIC ACTIONS OF PHYSOSTIGMINE ON THREE BEHAVIORAL MEASURES OF NOCICEPTION. Romano, J., and King, J. US Army Biomedical Lab, APG, MD 21010.

Physostigmine, an anticholinesterase agent, produces an analgesia which is mediated, in part, by an opiate system. This is evidenced by the fact that (1) physostigmine (physo) and morphine interact (Fed. Proc., 1970, 29, 28), (2) cholinergic blocking agents, such as atropine or hemicholinium, attenuate both morphine analgesia (Ann. N.Y. Acad. Sci., 1976, 281, 262) and physo analgesia, and (3) analgesia produced by cold-water swims (CWS), presumably due to liberation of endogenous opiates, was shown to be blocked by benactyzine hcl (ben hcl), an anti-muscarinic drug (Soc. Neurosci. Abstr., 1980, 6, 149.17). These interactions are dependent upon dose and behavioral test since morphine potentiates physo on tail flick (TF) and flinch-jump (FJ), but not hot plate (HP) (Soc. Neurosci. Abstr., 1980, 6, 149.18). Ben hcl (1.8 mg/kg) blocks CWS analgesia on TF and FJ, but not HP.

Physo was tested in conjunction with naloxone (nal), a potent opiate antagonist. Dose response curves of the physo-nal relation were obtained on three behavioral tests of pain. Animals were tested on HP (latency, sec), FJ (threshold, mA), and TF (latency, sec). Analysis of variance showed physo X nal interactions on TF ($p<.03$) and FJ ($p<.03$), but not on HP. Results suggest that opiate and cholinergic systems, in combination, affect those responses since at 3.0 mg/kg nal there is an attenuation of physo analgesia (on HP), while at 3.0 mg/kg and 10.0 mg/kg nal the analgetic effect of physo is enhanced on TF and FJ. Nal alone has no significant effect on any of the tests. All drugs were administered i.p. SE_M's were 3.3 (TF), .06 (FJ), and 2.2 (HP), respectively.

		TAIL FLICK (SEC)				FLINCH JUMP (mA)		
Nal	(Dose)	Sal	3.0	10.0	Sal	.60	.71	.77
Physo	Sal	15.1	15.4	18.4	.32	.72	.67	.74
	.32	25.2	32.2	26.1	.65	.68	.81	.98
	.65	25.1	24.0	40.4				

Both opiate agonists and antagonists potentiate physo analgesia, at least on TF and FJ. These data suggest that (1) physo analgesia may involve both a direct cholinergic mechanism and an indirect (opiate) one, (2) that the relative contributions of the opiate and/or cholinergic components of physo analgesia are different in three behavioral measures of pain, and (3) that the contributions of opiate factors relative to direct cholinergic factors are more significant of HP.

HOT PLATE (SEC)	Nal	(Dose)	Sal	3.0	10.0
	Physo	Sal	12.8	9.4	13.5
		.32	18.5	13.8	17.5
		.65	17.9	14.9	20.1

286.2 DOSE-DEPENDENT REVERSAL OF PHYSOSTIGMINE AND COLD-WATER ANALGESIA ON THE HOT PLATE BY BENACTYZINE HYDROCHLORIDE, King, J. and Romano, J. US Army Biomedical Lab, APG, MD 21010.

Previous data from this lab and others have indicated that the analgetic mechanisms underlying the tail flick (TF) and flinch-jump (FJ) tests are cholinosensitive. An interaction between opiate and cholinergic mechanisms has also been shown. Physostigmine (physo), in addition to producing analgesia by itself, potentiates morphine analgesia, and physo, morphine, and cold water stress (CWS) analgesias are antagonized by anticholinergics such as atropine and benactyzine hcl (ben hcl). While results from TF and FJ studies present a relatively clear picture of cholinergic involvement, studies which have used the hot plate (HP) method to assess analgesia have yielded more complex results. Compared to TF and FJ methods, HP results do not indicate the same type or degree of interaction between morphine and physo, nor is HP-measured physo-induced analgesia effectively antagonized by ben hcl at a dose (1.8 mg/kg) effective in TF and FJ tests. Others have postulated that differences seen with HP analgesia may reflect the contribution of supra-spinal, endorphin-related mechanisms in addition to the descending, spinal-mediated mechanisms of TF and FJ analgesia (Brain Res., 1979, 160, 180). One question that arises in regard to potential cholinergic involvement in these analgetic mechanisms is whether physo or CWS analgesia are totally, or relatively, independent of antagonism by anticholinergic compounds such as ben hcl.

Following pretest, ben hcl was administered 4 min prior to analgetic treatment consisting of either (a) .65 mg/kg physo i.p., or (b) forced CWS. Animals were tested 30 min later. Five test doses were employed: saline, .57, 1.8, 5.7, or 18.0 mg/kg ben hcl (i.p.). Also five groups of animals were given ben hcl alone 34 min before HP test (latency, sec); ben hcl alone was without effect on this test. However, ben hcl pretreatment was capable of blocking either physo (5.7 mg/kg) or CWS (18.0 mg/kg) analgesia. The results obtained confirm and extend previous indications that cholinergic interactions with analgetic mechanisms are dependent upon both dose and test system employed. Results also support the postulated dual mechanism theory of HP analgesia in that differences in effective anticholinergic doses needed for antagonizing physo and CWS analgesia may reflect different degrees of cholinergic involvement in the neural mechanisms underlying the two treatments.

		HOT PLATE LATENCY (SEC., PERCENT OF BASELINE)				
DOSE	BEN HCL (MG/KG)	SAL	.57	1.8	5.7	18.0
CONTROL		95	99	101	87	104
PHYSO		203	153	145	112	122
CWS		161	150	163	140	106

286.3 MONOAMINERGIC MECHANISMS OF STRESS ANALGESIA. G. W. Terman*, J. W. Lewis and J. C. Liebeskind (SPON: L. Butcher). Dept. of Psychology and Brain Research Institute, University of California, Los Angeles, CA 90024.

A variety of stressors have been shown capable of producing analgesia. Stress analgesia, however, is not a unitary phenomenon: both opioid and nonopioid pain-inhibitory mechanisms exist that can be differentially activated by stress (Lewis et al., Science, 208:623, 1980). We have shown that exposure to brief continuous footshock reliably produces an analgesia that is neither antagonized by naloxone nor is cross-tolerant with morphine (Lewis et al., J. Neurosci., 1:358, 1981). The neurochemistry of this obviously nonopioid form of stress analgesia has, however, remained elusive. Studies of the neurochemistry of stimulation-produced analgesia suggest an important role for central monoamines in endogenous pain-inhibitory mechanisms (Akil and Liebeskind, Brain Res., 94:279, 1975). In this study, we examined the effects of reserpine, a depletor of all monoamines, on nonopioid stress analgesia.

Male Sprague-Dawley rats were randomly assigned to one of two groups (n=14) and administered either reserpine (2 mg/kg, i.p.) or saline on two consecutive days. One day after the second injection, each animal was tested for baseline pain responsiveness using the tail-flick test. Rats were then given footshock stress according to parameters previously shown to produce nonopioid analgesia (3 min, 2.5 mA, 60 Hz sine waves on continuously). Analgesia testing resumed immediately after stress and continued for 10 min.

Reserpine treated animals were significantly less analgesic to footshock than controls ($p < .01$). Their analgesia was reduced both in magnitude and duration. Baseline tail-flick latencies tended to be lowered by reserpine, although these differences were not statistically reliable.

These results demonstrate an important involvement of monoaminergic mechanisms in nonopioid pain-inhibitory systems activated by stress. The fact that adrenalectomy, which eliminates one major peripheral source of monoamines, has no effects on nonopioid stress analgesia (Lewis et al., Soc. Neurosci. Abstr., this volume), suggests that monoamines of central or sympathetic origin are important to this analgesic response. (Supported by NIH grant #NS07628 and MHTP grant #MH15345.)

286.4 EVIDENCE FOR THE INVOLVEMENT OF CENTRAL CHOLINERGIC MECHANISMS IN OPIOID STRESS ANALGESIA. V. E. Weinberg*, J. W. Lewis, J. T. Cannon and J. C. Liebeskind. Dept. Psychology and Brain Research Institute, University of California, Los Angeles, CA 90024.

Stress may be the natural activating stimulus for endogenous analgesia systems. A single stressor, inescapable footshock, can access neurochemically different analgesia substrates depending only on its temporal parameters (Lewis et al., J. Neurosci. 1:358-363, 1981). Thus, opioid peptides appear to mediate analgesia after prolonged intermittent footshock; nonopioid mechanisms subserve equivalent analgesia caused by brief, continuous footshock. We now report evidence suggesting a role for central, muscarinic cholinergic systems in opioid, but not nonopioid, stress analgesia.

Groups of male albino rats (n=8) were used. Prior to baseline pain testing (tail-flick method), either scopolamine (0.01-10.0 mg/kg, i.p.), methylscopolamine (1.0, 10.0 mg/kg, i.p.), naltrexone (3 mg/kg, s.c.), or saline was administered. Rats were then subjected to either the opioid (2.5mA, 1 sec pulse/5 sec of 60 Hz sine waves for 20 min), or nonopioid (same, except on continuously for 3 min) form of footshock stress, and pain sensitivity was reassessed. Each rat received only one combination of drug and stress treatment.

Both stresses caused potent analgesia lasting several min. The opioid form of stress analgesia was significantly antagonized by scopolamine ($p < .01$ at 0.1, 1.0, 10.0, but not 0.01 mg/kg), or naltrexone ($p < .01$), but was not affected by methylscopolamine (all comparisons made to saline controls). The nonopioid form of stress analgesia was unaffected by all drug treatments. None of the drugs altered baseline latencies or responsiveness of nonstressed control rats.

That scopolamine or naltrexone antagonized the analgesic response to prolonged, intermittent footshock suggests that a muscarinic cholinergic synapse exists in an endogenous opioid analgesia system. Since opioid stress analgesia was unaffected by methylscopolamine, a muscarinic antagonist with only peripheral activity, the cholinergic synapse appears to be central. The cholinergic-opioid interaction suggested by these data is supported by the presence of muscarinic receptors in brain areas known to be highly sensitive to morphine's analgesic action (Wamsley et al., J. Neurosci. 1:176-191, 1981). (Supported by NIH grant #NS07628; JWL was supported by MHTP grant MH15345.)

286.5 KINDLED SEIZURES APPEAR TO CAUSE ANALGESIA PREFERENTIALLY ON THE AFFECTIVE COMPONENT OF PAIN. S. Caldecott-Hazard, Y. Shavit and J. C. Liebeskind. Dept. of Psychology, UCLA, Los Angeles, CA 90024.

Analgesia following an amygdaloid kindled seizure in the rat is seen on hot-plate paw-lick latencies but not on the tail-flick test (D. W. Berman et al., this vol.). Similarly, ECS seizures elicit powerful analgesia on hot-plate escape behaviors but weak analgesia on the tail-flick test (Holaday and Belenky, 1980). Unlike the spinally mediated tail-flick response, the hot-plate test measures pain behaviors thought to be organized predominantly at supraspinal levels. The purpose of the present study was to further characterize the analgesia produced by amygdaloid kindled seizures by using the 3-level pain test of Carroll and Lim (1960). These researchers had shown that a tail withdrawal response to the lowest intensity of tail shock is mediated at a spinal level, a single squeak to intermediate shock intensities is mediated by the brain stem, and multiple squeaks, struggling, and teeth chattering to the highest intensity shock are mediated at diencephalic/limbic system levels.

Rats were implanted with chronic bipolar stimulating and recording electrodes in the amygdala. They were stimulated daily to produce kindled seizures until showing fully generalized (stage 5) seizures on 3 successive days. On test days, all rats were injected with either saline or naloxone (10 mg/kg). Half of each group was then given a kindled seizure and evaluted on the 3-level pain test. The remaining animals were evaluated in the same fashion without a prior seizure. In comparison with non-seizure trials, a kindled seizure significantly raised the threshold for the multiple-squeak behavior, but not for the behaviors elicited by lower shock intensities. This analgesia had a duration varying between 4 and 10 minutes, and it was partially blocked by naloxone. These results suggest that kindled seizures produce an analgesia by influencing principally diencephalic/limbic system structures, which are presumably involved in mediating the affective components of the pain experience. (Supported by NIH Grants #NS07628 and #NS06289.)

286.6 NALOXONE-REVERSIBLE HYPOALGESIA FOLLOWING INTRATHECAL 5,6 DIHYDROXYTRYPTAMINE. Mark S. Brodie* and Herbert K. Proudfit (SPON: L. Isaac). Dept. Pharmacol., Univ. Ill. Med. Ctr., Chicago, IL 60612.

It has been shown that neurotoxic lesioning of serotonin (5-HT) nerve terminals in the spinal cord leads to changes in the nociceptive threshold as measured by the tail-flick (TF) test. Depletion of spinal cord 5-HT by 5,6-dihydroxytryptamine (5,6-DHT) leads to hyperalgesia which subsides to pre-lesion threshold by fourteen days after the injection of neurotoxin. In light of this time-dependent alteration in pain sensitivity, this study was undertaken to observe any further changes in nociception.

Sprague-Dawley derived rats were implanted with intrathecal catheters (PE-10 tubing) extending to the lumbar cord, and were allowed to recover from the surgery for at least one week before receiving an intrathecal injection of vehicle (0.02% ascorbic acid in normal saline) or 5,6-DHT (20 µg in 15 µl vehicle). Animals were tested weekly on the tail-flick apparatus before and after a subcutaneous (sc) injection of morphine (5 mg/kg). There was a decrease of 39% in the mean TF latency compared to vehicle controls one week after 5,6-DHT injection. The magnitude of morphine induced analgesia in the control and 5,6-DHT treated rats was identical, despite the hyperalgesia of the latter group. This observation, reported previously, indicates that the descending 5-HT system is not involved in morphine analgesia. Two weeks after 5,6-DHT injection, both groups of animals had statistically identical TF latencies before and after morphine.

Eight weeks after 5,6-DHT treatment, the baseline TF latencies of the lesioned animals were elevated significantly (220%) above those of the control animals. Ten minutes after injection of naloxone (0.8 mg/kg, sc), mean TF latency was reduced to that of the controls.

While descending 5-HT neurons do not seem to play a role in morphine analgesia, lesioning of the 5-HT nerve terminals with 5,6-DHT causes a marked alteration in heat-induced nociception. Because the tonic hypoalgesia seen several weeks after the neurochemical lesion is reversible by naloxone, it is postulated that morphological changes subsequent to damage caused by 5,6-DHT lead to tonic involvement of enkephalinergic neurons in the pain transmission pathway in the rat.
(Supported by USPHS Grant NS 12649)

286.7 THE INDUCTION OF HYPOALGESIA BY CHRONIC RAPHE MAGNUS LESIONS AND ITS REVERSAL BY NALOXONE. Herbert K. Proudfit, Univ. of Illinois at the Medical Center, Chicago, IL 60612.

Bulbospinal neurons located in the nucleus raphe magnus (NRM) have been implicated in the modulation of nociceptive threshold. NRM neurons appear to be tonically active since destruction of these neurons produces decreased nociceptive threshold (hyperalgesia). However, the lesion-induced effects are highly dependent on the time at which measurements are made following the lesioning procedure. Thus, the nociceptive threshold is decreased seven days or less after lesions, but at longer intervals (28 to 35 days) the nociceptive threshold becomes elevated (hypoalgesia). Preliminary studies indicate that the lesion-induced hypoalgesia can be reversed by the systemic administration of the opiate antagonist naloxone. The present studies were done to replicate these preliminary observations.

Twenty female Sprague-Dawley derived rats were randomly divided into two groups: (A) nonlesioned control animals and (B) lesioned animals. Nociceptive thresholds were determined using the tail flick test. The animals in the lesion group received electrolytic lesions placed in the NRM. Seven days after surgery nociceptive thresholds were determined, morphine sulfate (5 mg/kg, sc) was given, and thresholds were again determined 30 min after morphine injection. The animals were similarly tested at 14,21,28, and 35 days after surgery. On days 42 and 49 each of the twenty animals was then given an injection of either saline or naloxone (0.8 mg/kg, sc) using a randomized cross-over design. Nociceptive thresholds were determined 5,15 and 55 min after the injection.

The mean pre-lesion tail flick latency (TFL) for Group A (3.5 $\pm$ 0.2 SEM) was not significantly different from that of Group B (3.4 $\pm$ 0.2). However, at 42 to 49 days after lesioning the mean TFL for Group B (8.1 $\pm$ 0.6) was significantly elevated over that for Group A (4.5 $\pm$ 0.2). The systemic injection of saline had no effect on the mean TFL for either group. Naloxone had no effect on the mean TFL for Group A, but produced a statistically significant reduction in that for Group B.

These results suggest that destruction of raphe-spinal neurons induces changes in either brain stem or spinal cord which augment the activity of enkephalinergic neurons. Similar results following the destruction of serotonergic terminals in the spinal cord (Brodie and Proudfit, Neuroscience Abstr., 1981) suggest that destruction of raphespinal serotonergic neurons is responsible for the hypoalgesia induced by NRM lesions.
(Supported by USPHS Grant NS 12649).

286.8 IDENTIFICATION OF NORADRENERGIC NEURONS PROJECTING TO MEDULLARY NUCLEI INVOLVED IN THE MODULATION OF PAIN. Jacqueline Sagen and Herbert K. Proudfit. Dept. of Pharmacology, Univ. of Ill. Medical Center, Chicago, IL 60612.

The nucleus raphe magnus (NRM) and magnocellular reticular formation (MC) in the caudal medulla receive a dense noradrenergic (NA) innervation as revealed by histochemical fluorescence studies. This NA input may enhance sensitivity to noxious stimuli since microinjection of α-adrenergic antagonists in these areas produces an elevation in the nociceptive threshold (Hammond et al, 1980). The present study sought to identify the origin of the NA projection to the NRM and MC using an adaptation of the method of Bjorklund and Skagerberg (1979). A retrograde fluorescent tracer, "True Blue" (TB), was used in conjunction with the glyoxylic acid fluorescence histochemical method. Under ether anesthesia, 0.2 µl of 5% TB was microinjected into the NRM or MC of rats. After 3-5 days survival, rats were rapidly decapitated, their brains removed and frozen on a cryostat chuck. Ten µ sections were cut and processed using the method of de la Torre and Surgeon (1976). Alternate sections were stained with cresyl violet to aid in the identification of the injection site. The sections were examined under a fluorescence microscope for perikarya containing both the retrograde fluorescent tracer and catecholamine (CA) fluorescence. Microspectrofluorometric recordings of individual neurons were performed to confirm the presence of both fluorophores.

Neurons containing both TB and CA were identified in the lateral tegmental groups (A1, A5, A7, and A8 of Dahlstrom and Fuxe, 1964) and in the ventral locus coeruleus (LC), with a preferential distribution depending on the injection site. With NRM injection sites, neurons containing both fluorophores were found primarily in A5 and occasionally in A1, A7, and A8. Few labeled neurons were found in the LC. Injections in the rostral raphe pallidus resulted in double-labeled neurons in the A5 and ventral LC, and occasionally in A1 and A7. When injections were made in the MC region, neurons containing both TB and CA were found primarily in A5 and the ventral LC.

These studies indicate that the CA neurons which project to the ventro-medial region of the caudal medulla are not situated in a single discrete locus, but instead are widely dispersed and are found in several brain areas. Each of these brain areas has been implicated in the modulation of nociceptive threshold. Thus, the present studies provide anatomical evidence to support the suggestion that CA neurons modulate nociceptive threshold by altering the excitability of bulbospinal neurons in the raphe magnus and magnocellular reticular nucleus.
(Supported by USPHS Grant NS 12649 and PMA Foundation Grant)

286.9 NALOXONE STIMULATION OF NUCLEUS TRACTUS SOLITARIUS BLOCKS ANALGESIC EFFECTS OF SYSTEMIC MORPHINE IN RATS+. N. Oley, C. Cordova,* M. Kelly* and J.D. Bronzino. Depts. of Psychology & Biomedical Engineering, Trinity College, Hartford, CT 06106.

Recent evidence of opiate receptors (Herkenham & Pert, Proc. Natl. Acad. Sci., 1980, 77, 5532-5536.) in the region of the nucleus tractus solitarius (NTS) suggests that this region of the dorsomedial medulla may participate in an opiate-sensitive pain-suppressive system. To test this hypothesis, an attempt was made to block the analgesic effects of systemically administered morphine with injections into the NTS of the opiate antagonist naloxone.

Sixteen naive male albino rats were each given 30 mg/kg of morphine (ip), morphine (ip) plus 10 µg of naloxone (in 0.25µl of Ringers (ic), morphine (ip) plus Ringers (ic), and morphine (ip) plus 30 µg of naloxone (ic). Injections were given at 1 wk intervals, and the order of naloxone doses was reversed in several animals to control for tolerance to morphine. Tail flick latencies were measured at 10 min intervals before and after morphine injections for up to 4 hrs.

Intracerebral injections of naloxone given 5 mins prior to systemic morphine injections delayed for up to 90 mins the onset of and reduced the magnitude of morphine-produced tail flick analgesia in dose-dependent fashion (in comparison with a Ringer's control injection). Catatonia was also blocked by naloxone. However, self-mutilation, which never occurred with systemic morphine injections alone, sometimes did occur following naloxone injections.

The results of this experiment indicate that the NTS plays a role in opiate analgesia. However, the failure of (ic) naloxone to completely block the analgesia produced by high doses of systemic morphine suggests that systemically-produced analgesia is only partly mediated by pathways leaving the NTS. This interpretation is entirely consistent with reports that systemic morphine can activate other supraspinal structures (such as the PAG) which mediate analgesia, and, in high doses such as were used in this experiment, can also act directly at the spinal level to reduce pain.

+Supported by NIGMS Grant #27226-01.

286.10 CHANGES IN BRAIN β-ENDORPHIN AND TOLERANCE TO MORPHINE ANALGESIA AFTER A SINGLE DEFEAT IN MICE. Michael L. Thompson*, Klaus A. Miczek and Louis Shuster. Depts. of Biochemistry and Pharmacology and Psychology, Tufts University, Boston and Medford, MA. 02111.

Analgesia following exposure to a variety of stressors has been well documented, but how endogenous opioid peptides are involved in this form of analgesia has been an issue of contention. We have previously reported that a large analgesic response is produced in mice by subjecting them to a social stressor,i.e.,repeated attack by another mouse, and that the analgesia produced by this form of stress does meet various criteria for involvement of endogenous opioid peptides,(i.e. naloxone reversibility, cross tolerance to and from morphine).

Recent experiments implicate brain, rather than peripheral β-endorphin, in this response. Behaviorally inexperienced B6AF$_1$ mice were introduced into the homecage of another mouse; the resident mice reliably attacked the intruder. After sustaining 70-100 attack bites, the intruder mice showed a 5-6 fold increase in tailflick latency. Mice pretreated with naltrexone (1 mg/kg) failed to show significant analgesia following defeat. In contrast, quaternary naltrexone (40 mg/kg), which does not enter the CNS but is active peripherally, was without effect. Dexamethasone pretreatment (400 µg/kg at 24 hrs. and 200 µg/kg at 4 hrs. prior to the test), has been reported to block stress induced release of ACTH and β-endorphin from pituitary but not from brain, and to block some forms of stress-induced analgesia. Such pretreatment did not reduce analgesia in mice subjected to defeat.

In a further experiment, mice were exposed for ca. 10 min. to residents and sustained 100 attack bites. After confirming their tailflick analgesia, their brains were removed, and their forebrain β-endorphin levels were determined by commercially available radioimmunoassay. Forebrain β-endorphin levels were reduced by an average of 40% following defeat.

In additional experiments significant tolerance to morphine was observed in mice tested 7 days after a single defeat experience resulting from 100 attack bites. We had previously reported such an effect after 14 days of daily defeat. Furthermore, these mice showed significantly enhanced naloxone-induced jumping after a small dose of morphine (5 mg/kg) when compared to mice not previously subjected to defeat.

Exposure to a biologically relevant stressor such as defeat appears to readily activate endorphin mechanisms in the brain which mediate a marked analgesis and produce long-lasting tolerance.

286.11 POTENTIATION OF NARCOTIC ANALGESIA BY H_1 and H_2 ANTAGONISTS. Renata Bluhm*, Bruce Diamond, Elemer Zsigmund, and Alon Winnie* Departments of Pharmacology and Anesthesiology, University of Illinois at the Medical Center, and Department of Anesthesiology, Mount Sinai Hospital, Chicago, IL 60612

In the present experiments we tried to identify antihistamines capable of naracotic potentiation and to correlate this with the anticholinergic action of the H_1 antihistamines and the H_1 and H_2 antagonist activity.

Analgesia was measured in mice using latency to jump from a hot plate at 55°C. Drugs were administered IP and animals were tested 45 minutes later.

Suboptimal doses of morphine sulfate (MS) (7.5 mg/kg) were potentiated by diphenhydramine (DH) (12.5 mg/kg), chlorpheniramine (CP) (20 mg/kg), hydroxyzine (HZ) (30 mg/kg) and cimetidine (CT) (200 mg/kg). At the doses used the increase in analgesic response when DH and MS were given together was 168%, with CP and MS it was 154%, for HZ with MS it was 460% and for CT and MS it was 168%. Benztropine did not potentiate morphine analgesia. These drugs had no analgesic effect alone. No measurable motor impairment occurred when measured by a grasp test using a hanging basket.

Apparently, H_1 and H_2 antagonists are effective potentiators of narcotic analgesia while anti-cholinergic activity does not produce this effect.

A possible explanation for the potentiating effect may be that the anti-histamines effect the disposition or metabolism of MS, change the amount of MS entering the brain or it may be due to a relationship of histamine and morphine receptors in the brain.

286.12 ANTINOCICEPTIVE INTERACTIONS AMONG NORADRENERGIC, DOPAMINERGIC, ENDOGENOUS OPIOID AND STRESSFUL MANIPULATIONS: PAIN INHIBITION OR HYPOACTIVITY. R. J. Bodnar, K. P. Merrigan*, N. Nicotera*, A. L. Kirchgessner* and G. Pasternak. Dept. of Psychology, Queens College, CUNY, and Dept. of Neurology, Memorial Sloan-Kettering Cancer Center, New York, NY.

Recent pharmacological and behavioral evidence indicates the existence of heterogeneous pain-inhibitory mechanisms. Acute exposure to stressors increase pain thresholds with some acting through endogenous opioid processes and others acting independently of these systems. Moreover, pharmacological manipulation of noradrenergic, dopaminergic and benzodiazepine systems alter nociceptive thresholds and interact with stress analgesia. In the first series of experiments, clonidine (0, 125, 500 and 1000 ug/kg, ip), a noradrenergic antinociceptive agent (Naunyn-Schmied. Arch. Pharmacol. 292: 119-126, 1976) which develops cross-tolerance with autoanalgesia (Soc. Neurosci. Abstr. 6: 247, 1980) was tested in the flinch-jump paradigm for interactions with morphine (5 and 2.5 mg/kg, sc) and cold-water swims (CWS: 2°C and 15°C baths for 3.5 min). Clonidine itself produced dose-dependent increases in flinch-jump thresholds with 60 min peak effects. The antinociception induced by the 2°C CWS and the 5 mg/kg dose of morphine were potentiated in a dose-dependent manner by clonidine. By contrast, clonidine failed to interact with the lower CWS and morphine analgesic stimuli. Activity levels were dose-dependently decreased by clonidine with the lowest dose exerting the greatest hypoactive effect. While morphine also produced hypoactivity, this was reversed by the higher doses of clonidine. Second, the neuroleptics haloperidol (HAL) and chlorpromazine (CPZ) which decrease restraint and heat analgesia (Life Sci. 27: 185-188, 1980) were tested in conjunction with 2°C CWS, morphine (5 mg/kg, sc), 2-deoxy-D-glucose (2-DG: 450 mg/kg, ip) and chlordiazepoxide (CDP: 15 mg/kg, ip) nociception on the flinch-jump test. HAL (10, 50, 100 ug/kg) and CPZ (1, 3, 5 mg/kg) each increased flinch-jump thresholds dose-dependently and each potentiated the antinociceptive effects induced by CWS, morphine, 2-DG and CDP. This potentiation was additive, not multiplicative. Third, the high-affinity opiate receptor antagonist, naloxazone (Science 208: 514-516, 1980) was evaluated for its central effects upon basal nociception and analgesic responses. Lateral ventricular injections of naloxazone (0, 1, 5, 20, 50 ug/5 ul: 1 ul/15 sec) decreased both basal flinch-jump thresholds and systemic morphine analgesia for up to 24 h after the injection. Preliminary data indicate naloxazone-induced increases in the non-opioid CWS antinociceptive response. (Supported by NIH Grants 14449, 5S05RR07064 and PSC/CUNY Grant 13493.)

286.13 ELIMINATION OF ANTINOCICEPTIVE RESPONSES FOLLOWING ACUTE EXPOSURE TO TAIL PINCH STRESS. D. A. Simone* and R. J. Bodnar (SPON: T.E. Frumkes). Dept. of Psychology, Queens College, CUNY, Flushing, NY 11367.

Acute exposure to a variety of environmental stimuli can alter a number of behavioral processes in the rat. While certain stressors, such as cold water swims (CWS) and cervical probing (CP) increase pain thresholds following stress exposure, other stressors like non-noxious tail pinch (TP) induces eating, licking and gnawing behaviors. The latter syndrome appears to be subserved by dopaminergic and enkephalinergic systems since selective depletion of each system abolishes TP hyperphagia. By contrast, stressors that induce analgesia may or may not be mediated by endogenous opioids, depending on the procedure and parameters employed. Until recently, no link between the hyperphagic effects of TP stress and the antinociceptive effects of other stressors has been investigated. Chiodo (Brain Res. 176 (1979) 385-390) demonstrated that simultaneous exposure to CP and TP stimuli eliminates the eating behavior induced by TP, an effect attributed to reciprocal alterations in dopamine. While CP decreases dopamine unit activity, TP increases it. The present study investigated the complementary relationship, that is, would the activating effects of TP alter basal nociception and change the analgesic responses to morphine and the non-opioid stressor CWS. In the first study, 14 female rats verified for their hyperphagic response to TP, displayed significant decreases in flinch-jump thresholds immediately following TP. This effect occurred whether or not food was available during TP. Second, the time course of TP-induced decreases in flinch-jump thresholds lasted up to 30 min following TP exposure. In a third study, 11 female rats displayed lower latencies following TP across three thermal levels on the hot-plate test. Only the high (55°C) stimulus elicited significant effects which persisted into recovery. Finally, the increases in flinch-jump thresholds following 10 mg/kg and 2.5 mg/kg doses of morphine were reduced and abolished respectively by either pre-injection exposure or pre-test exposure to TP. CWS in a 15°C bath was similarly affected. However, 2°C swim-induced flinch-jump increases were reduced significantly only when TP was administered during the swim-test interval. These data indicate important reciprocal interactions between activating and antinociceptive systems and question whether the observed increases in pain thresholds following antinociceptive procedures are the sole result of activation of pain-inhibitory processes. (Supported by PSC/CUNY Grant 13493 and NIH Grant 5S05RR07064.)

286.14 FUNCTIONAL MAPPING OF PAIN PATHWAYS IN THE CAT SPINAL CORD USING THE [^{14}C] DEOXYGLUCOSE TECHNIQUE. S.E. Abram*, D.R. Kostreva, and J.P. Kampine. Depts. of Anesthesiology and Physiology, Med. Col. of Wis. and VA Med. Ctr., Wood, WI 53193

The spinal cord projections of afferent pain fibers originating from nociceptors in the foot pad of the cat were studied in sodium pentobarbital (35 mg/kg i.v.) anesthetized cats (2-3 kg). Brachial artery pressure and lead II electrocardiogram were recorded using a Grass polygraph. Skin temperature was monitored using a 24 gauge needle thermister placed intradermally into the hindlimb foot pad. A radiant heat lamp was used to produce the noxious heat stimulus. The effectiveness of the noxious stimulus was determined by: recording afferent nerve activity from the cut distal end of a small slip of the medial portion of the tibial nerve; and by monitoring autonomic responses; i.e. heart rate, blood pressure, and breathing frequency, during the noxious stimulus. The threshold temperature at which the averaged afferent nerve activity began to increase was between 44 and 48°C. Elevations of blood pressure, heart rate and respiratory rate began to increase 1 to 5 seconds after the initial increase in afferent nerve activity. On cessation of the radiant heat stimulus, afferent nerve activity, heart rate, blood pressure, and respiratory rate promptly returned to pre-stimulation levels. Once reproduceable changes in heart rate, and blood pressure could be induced, a single bolus of [^{14}C] deoxyglucose (125 uCi/kg) was injected into a femoral vein. The foot pad was then repeatedly stimulated (45 sec on, 30 sec off) for a total of 45 minutes. The entire brain and spinal cord were then removed, frozen in -40°C isopentane, sectioned at 20 um increments and prepared for autoradiography. The sections were covered with Kodak MR-1 film and stored in X-ray cassettes for 12 days. The autoradiographs were then developed and analyzed for the areas having the greatest metabolic activity as indicated by the density of photographic emulsion. Sections from the lumbar and sacral regions of the spinal cord showed markedly increased metabolic activity in the region of the lateral spinothalamic tracts. Caudal sections in the sacral region of the spinal cord showed an increased metabolic activity in the area of the ipsilateral lateral spinothalamic tract. More rostral sections in the sacral and lower lumbar region showed that the activity was distributed in the lateral spinothalamic tracts bilaterally. More rostral sections in the lumbar region indicated that the activity was localized primarily in the contralateral lateral spinothalamic tract. This study demonstrates that the deoxyglucose technique is useful for studying pain pathways in the CNS. (Supported by NIH Young Cardiovascular Investigator Grant HL 21042 and VA Medical Research Service).

286.15 IN VIVO STUDIES ON THE EFFECT OF ZOMEPRIAC SODIUM (ZS = Sodium 5-(4-Cl Benzoyl)-1,4-dimethyl-1H-pyrrole-2-acetate dihydrate) ON CAT CSF LEVELS OF PROSTAGLANDIN E_2 (PGE_2). S. DIVINETZ-ROMERO*, T. L. YAKSH, L. D. MUSCHEK*, and P. J. O'NEILL* (SPON: P. L. SETLER). Depts. of Psych. and Neurosurg., Mayo Grad. Sch. Med., Rochester, MN 55905 and Depts. of Biol. Res. and Drug Metab., McNeil Pharmac., Spring House, PA 19477.

The analgesic ZS has been shown to inhibit prostaglandin (PG) synthesis in bovine seminal vesicles and in platelets. This prompted us to investigate if systemic administration of this drug would depress immunoreactive (IR)-PGE_2 levels in the CSF of unanesthetized cats. PGE_2 has been implicated in pain potentiation and thermoregulation. Adult cats of both sexes were implanted with chronic cannulas in the cisterna magna under Pentobarbital anesthesia. After a 24 hr recovery period, daily CSF samples (1-1.5 cc) were drawn, immediately frozen and extracted with 2 vol. of chloroform:methanol 2:1, evaporated to dryness under N_2, reconstituted with phosphate buffer and radioimmunoassayed within 2 weeks. Highly specific PGE_2 antibody (Pasteur Institute) was used. Values were corrected for extraction losses monitored with both tritiated and cold PGE_2.

Control IR-PGE levels were found to be stable, ranging from 1,042 ± 255 pg/ml CSF one day after surgery to 1,508 ± 369 on the seventh day (mean ± SE). ZS produced a marked effect. Twenty-four hours after a single 3 mg/kg i.v. dose IR-PGE_2 levels decreased from 2,176 ± 332 to less than 86 ± 27 pg/ml CSF ($p < .005$). In animals given a single 0.3 mg/kg i.v. dose, a 60% reduction was noticeable within one hour ($p < .0025$), with a further decrease occurring after 24 hrs (IR-PGE_2 levels falling from 1,850 ± 394 to 72 ± 15 pg/ml CSF, $p < .005$). In these animals IR-PGE_2 levels remained depressed for up to 5 days, returning to control values within one week.

The decrease in IR-PGE_2 levels in CSF was concomitant with detectable levels of ZS in serum and in CSF. Serum levels of the drug were 16 ± 1.5 µg/ml within 1 hr of a 3 mg/kg i.v. injection and 5.3 ± 2.3 µg/ml after 72 hrs. ZS appears rapidly in CSF (detectable at 10 min), reaching approximately 7% of plasma levels within 24 hrs. The prolonged depression of IR-PGE_2 levels in CSF may be related to the long-lasting circulating levels of ZS in this species.

These findings suggest that the analgesic-antipyretic effect of ZS could be mediated by PG synthesis inhibition.

286.16 DIET-INDUCED ALTERATIONS IN SEROTONINERGIC NEUROTRANSMISSION, PAIN SENSITIVITY, AND OPIATE ANALGESIC DRUG POTENCY. TIMOTHY G. BURNS*, PATTI KANNE*, AND LOY D. LYTLE. LABORATORY OF PSYCHOPHARMACOLOGY, DEPARTMENT OF PSYCHOLOGY, UNIVERSITY OF CALIFORNIA, SANTA BARBARA, CALIFORNIA 93106

BRAIN AND SPINAL CORD SEROTONINERGIC NEURONS ARE INVOLVED IN MEDIATING NOCICEPTIVE RESPONSES AS WELL AS SOME OF THE ANALGESIC EFFECTS OF OPIATE NARCOTICS [MESSING, R.B. AND LYTLE, L.D. *Pain 4:* 1 (1977)]. FURTHERMORE, RATS FED A TRYPTOPHAN DEFICIENT, CORN BASED DIET ARE HYPERALGESIC AND HAVE REDUCED BRAIN SEROTONIN LEVELS [LYTLE, L.D. *et al., Science 190:* 692 (1975)], AND SHOW NO ANALGESIA WHEN TREATED WITH MORPHINE [PHEBUS, L. AND LYTLE, L.D. *Proc. West. Pharmacol. Soc. 21:* 361 (1978)]. THE PURPOSE OF THE PRESENT STUDY WAS TO FURTHER EXPLORE THESE PHENOMENA, AND TO DETERMINE IF THE ABOLITION OF MORPHINE ANALGESIA IN CORN-FED RATS WAS DUE TO DIET-INDUCED ALTERATIONS IN CENTRAL NERVOUS SYSTEM SEROTONINERGIC FUNCTION OR TO OTHER FACTORS ASSOCIATED WITH THE MALNUTRITION.

DIFFERENT GROUPS OF MALE ALBINO RATS WERE OFFERED *ad libitum* ACCESS TO AN 18% CASEIN CONTROL DIET, TO A CORN-BASED DIET, OR TO A CORN-BASED DIET SUPPLEMENTED WITH AMOUNTS OF TRYPTOPHAN EQUIVALENT TO THOSE CONTAINED IN THE CASEIN DIET BEGINNING AT 22 DAYS OF AGE. ELECTROSHOCK JUMP RESPONSE THRESHOLDS WERE DETERMINED IN ALL ANIMALS AT WEEKLY INTERVALS FOR UP TO 5 WEEKS. SIX WEEKS AFTER IMPLEMENTATION OF THE DIETS DIFFERENT ANIMALS WERE INJECTED SUBCUTANEOUSLY WITH THE VEHICLE (0.9% SALINE) OR DIFFERENT DOSES OF MORPHINE SULFATE (5 OR 10 MG/KG; SALT WEIGHT) AND THEN WERE TESTED FOR PAIN RESPONSES TO ELECTRIC FOOTSHOCK. ALL ANIMALS WERE KILLED 1 WEEK LATER; PLASMA, MUSCLE, AND LIVERS WERE ASSAYED FOR TRYPTOPHAN AND PROTEIN CONCENTRATIONS, AND TRYPTOPHAN, SEROTONIN, AND 5-HYDROXYINDOLEACETIC ACID WERE ALSO DETERMINED IN BRAINS AND SPINAL CORDS USING FLUORIMETRIC PROCEDURES.

THOSE ANIMALS FED THE CORN DIET OR THE CORN DIET SUPPLEMENTED WITH TRYPTOPHAN WERE HYPERALGESIC TO FOOTSHOCK COMPARED TO THE CASEIN-FED ANIMALS AFTER 1 WEEK FOLLOWING IMPLEMENTATION OF THE DIETS. FURTHERMORE, RATS FED THE CORN DIET, REGARDLESS OF WHETHER OR NOT IT WAS SUPPLEMENTED WITH TRYPTOPHAN, ALSO SHOWED NO ANALGESIC RESPONSES FOLLOWING THE INJECTIONS OF MORPHINE. TISSUE CONCENTRATIONS OF TRYPTOPHAN WERE REDUCED IN ANIMALS WHO CONSUMED THE UNSUPPLEMENTED CORN DIET, AND WERE GENERALLY INTERMEDIATE IN VALUE IN ANIMALS FED THE TRYPTOPHAN-SUPPLEMENTED CORN DIET COMPARED TO CASEIN FED CONTROLS. BRAIN OR SPINAL CORD 5-HYDROXYINDOLE LEVELS WERE REDUCED IN THE UNSUPPLEMENTED CORN-FED ANIMALS BUT THOSE IN THE TRYPTOPHAN-SUPPLEMENTED CORN-FED RATS WERE APPROXIMATELY EQUAL TO THOSE OF THE CASEIN FED CONTROL GROUP. HENCE, MALNUTRITION ITSELF MAY BE A SUFFICIENT CAUSE OF ALTERED NOCICEPTIVE RESPONSES IN CORN FED ANIMALS.

287.1 TOUCH AND SOUND HAVE A COMMON BRAIN PROGRAM FOR THE PRODUCTION AND RECOGNITION OF EMOTION EXPRESSION. M. Clynes, J. Walker* and N. Nettheim*. Music Research Center, New South Wales State Conservatorium of Music, Sydney 2000, Australia.

Dynamic forms of expression for specific emotions appear to be governed by brain functions that are independent of the sensory mode, as evidenced in this report for touch and sound. Touch expressions (measured as pressure transients[1]) were transformed to sound expression so as to express the same quality. The required transform was found to conserve the dynamic form.

Transformation consisted of modulation of a sinusoidal tone in frequency and amplitude by the touch dynamic form, in specific ways.[2] For the frequency envelope instantaneous pressure transforms to instantaneous frequency, with a polarity and modulation index specific for each emotion. Thus the frequency envelope is an isochronous linear transform of the touch pressure dynamic form. The amplitude envelope is also derived from the pressure dynamic form but includes an adaptation time-constant.

Sounds transformed from touch for the emotions anger, hate, love, joy, grief, sex, reverence, were tested on 189 subjects drawn from the United States and Australian college population. Emotions were recognized with a $p < .00001$, except for a degree of confusion shown between love and reverence.

Forty non-english speaking Aborigines of the Walbiri Tribe in Central Australia (Yuendumu) recognized the sounds transformed from white urban touch as well as or better than Australian medical students, and students at MIT and Berkeley. Males and females did equally well.

A transform incorporating the visual sense is likewise being sought.

The results imply that there is a brain program for dynamic emotion expression and its recognition whose algorithm and function is similar regardless of the sensory mode.

1 M. Clynes, in Theories of Emotion, R. Plutchik and H. Kellerman, eds., pp 271-300, Academic Press, N.Y., 1980.

2 M. Clynes, ed., Music, Mind, and Brain, The Neuropsychology of Music, Plenum, N.Y., 1981.

287.2 COMPARISON OF FUNCTIONS BETWEEN PARKINSON PATIENTS AND AGED NORMALS. L. Z. Podbros. Dept. of Psychology, SUNY at Stony Brook, Stony Brook, NY 11794.

Parkinson's Disease has been likened to an accelerated aging process. To test this analogy, an individual intensive strategy was used to examine similarities and differences in behavior between 8 idiopathic male parkinson patients (age range 53-69 yrs.) and 2 aged male normals, ages 85 and 90 yrs. A control group of 8 normal males was employed in order to estimate normative performance levels. Subjects were administered a battery of psychomotor and cognitive tasks, yielding 100 dependent measures. Performance measures for the patients and aged were converted to standard z scores. Relationships among skills and abilities were then examined by combining z scores into 18 functional clusters. Clusters were derived on an a priori rational basis but were examined empirically by internal consistency measures. Relationships among the patients' age, parkinsonian medications, disease severity, and performance on tasks were examined by Pearson product moment correlations.

Examination of individual cluster profiles shows that strength for both aged normals was beyond the criterion cutoff level for impairment ($z = -1.96$); in contrast, the strength of all eight patients was within this level. However, the patients showed markedly greater difficulty for speed and for endurance compared to other fundamental skills than did the aged. The relationships among motor and visual-motor skills are similar for the patients and aged: fine motor, bimanual, and visual-motor skills presented the greatest difficulties while visual perception is within normal limits for all subjects. However, marked differences in magnitude of effects can clearly be seen between the patients' and ageds' clusters for motor and visual-motor skills. None of the ageds' clusters approach the levels of impairment observed for the patients. For cognitive skills, however, the opposite is observed: the aged overall show lower functioning compared to the patients, though the magnitude of effects for neither the patients or aged reach those seen for the patients in motor and visual-motor skills.

These differences between the patients and aged in fundamental and cognitive skills and in magnitude of effects for motor and visual-motor skills indicate that idiopathic Parkinson's Disease is functionally distinct from aging per se.

(Supported in part by a Grant-in-Aid of Research from Sigma Xi.)

287.3 EVALUATING THE PHOSPHODIESTERASE INHIBITOR, PAPAVERINE, TO FACILITATE PERFORMANCE IN SEVERE CLOSED HEAD INJURY PATIENTS. D. L. Chute. Division of Life Sciences, University of Toronto and Department of Psychiatry McMaster University, Scarborough Ontario, M1C 1A4, Canada.

A number of recent studies in animals have suggested that phosphodiesterase inhibitors like IBMX, Ro-20-1274, and papaverine may facilitate performance, persumably by increasing intracellular cAMP levels with a consequent effect on protein phosphorylation (Chute et al. Soc. for Neurosci. 1980). Papaverine has been investigated with mixed success in human clinical populations, especially the elderly with varying degrees of senile organic brain syndromes. Typically such studies have not directly linked the time of drug administration closely with the evaluation of performance as would seem to be required by the pharmacokinetics of papaverine. In our work using normal college age subjects as their own controls, 180 mg of papaverine or placebo was administered orally immediately after acquisition of a Sternberg type task. In a 24 hour retention test papaverine appeared to facilitate recognition. In a second test of short term memory 20 minutes after drug administration, there was no drug effect compared to placebo in reaction time or errors. Papaverine (200 mg/d) was evaluated in case studies of severe closed head injury patients from the comprehensive rehabilitation programme at Chedoke-McMaster Hospitals. Patients were evaluated on a number of clinical and psychological measures in a double blind, within subjects, placebo controlled repeated measures, clinical trial. Preliminary results suggest a modest performance increment may occur for newly acquired skills temporally contiguous with drug administration. A variety of possible explanations other than elevated cAMP levels may account for such observations.

287.4 THE HUMAN MEDIAL TEMPORAL LOBE CONTRIBUTES TO BOTH THE FORMATION AND RETRIEVAL OF RECENT MEMORIES. E. Halgren, C. L. Wilson, J. Engel, Jr., T. L. Babb and P. H. Crandall*. Calif Compre Epilepsy Ctr, VA Wadsworth Med Ctr, and Depts Psychiat, Neurol and Surg/Neurol, UCLA Med Sch, Los Angeles, CA 90024.

Bilateral damage to the human medial temporal lobe (MTL) is known to produce a profound, permanent, global and specific inability to remember events for more than a few minutes. Standard therapy for uncontrolled complex partial epilepsy is surgical removal of the epileptogenic anterior temporal lobe. Localization of seizure onset often requires bilaterally implanted MTL electrodes. In order to avoid global amnesia (produced if the contralateral MTL is nonfunctional)we evaluate unilateral MTL functional integrity by testing memory during brief biphasic charge-balanced pulses of weak electrical current. Each stimulus pulse induces synchronous neural activity lasting about 400 msec according to concurrent electrophysiological recordings. Multiple sites are stimulated simultaneously with single pulses separated by a minimum of 2 sec in order to avoid afterdischarges while still preoccupying a sufficient neural mass to produce a behavioral effect. 18 color slides of objects and/or people are presented every 2 sec for an exposure of 150 msec (Input). After a delay of about 20 sec with distraction, these 18 slides are presented again in random order with 18 novel slides (Output), while the patient is instructed to indicate whether or not each slide had been seen previously. Synchronization of cognitive processing with the phasic preoccupation is accomplished by presenting the stimulus pulse simultaneous with slide onset. In order to interpret the effects of unilateral stimulation (to be reported elsewhere), it is necessary to determine the effects of bilateral stimulation. Bilateral stimulation during both Input and Output produced a severe ($p < .001$) impairment in recognition. If an identical stimulus pulse was applied but the delay between Input and Output presentations was shortened to 2 sec, then no deficit was observed. There exists considerable controversy regarding whether the inability to remember after MTL lesions reflects insufficient encoding or consolidation, or retrieval. Therefore, we felt it important to determine if stimulation confined to the slides at Input or at Output would be as effective as stimulation at both presentations. It was not: Stimulation confined to either period was partially effective. Thus, the MTL contributes to mnestic processes both at the initial encounter with information, and again when it is presented for recognition.(Supported by NINCDS grant NS02808 and the Ralph Smith Foundation.)

287.5 LECITHIN EFFECT ON NORMAL HUMAN MEMORY. C. M. Harris, M. W. Dysken, P. J. Fovall* and J. M. Davis, Dept. Pharmacol., Univ. Illinois Col. Med., and Illinois State Psychiat. Inst., Chicago, IL 60612.

The level of function in the central cholinergic system has been postulated to be directly related to memory performance. Cholinergic agonists enhance some aspects of memory function, while the antagonist scopolamine has an amnestic effect. Experimental attempts to enhance human memory with the acetylcholine precursors choline and lecithin have produced mixed results, with, at best, small improvements, in young college graduates and medical students (Sitaram et al., Science 201:274, 1978).

We postulated that more striking improvements might be produced in an older, non-student, population. To test this idea, we conducted a double-blind, placebo-controlled, clinical trial of lecithin (Phospholipon-100, American Lecithin Co.) in in nine healthy paid volunteers with a mean age of 39.9 years ± std. dev. = 10.3 years (3 men, 6 women). Cognitive performance was guaged by a battery of psychological tests, including: a categorized serial learning task, a paired associates learning task with both high- and low-imagery forms, a word-recognition task, a test of retrieval, by category, of words from long-term memory, and a digit/symbol substitution test of psychomotor speed. Plasma choline was determined by the radioenzymatic method of Wang and Haubrich (Ann. Biochem. 63:195, 1975). After one practice-session, subjects received drug and placebo, in random order, and were tested on two occasions, separated by at least 48 hours. The drug and placebo were taken orally in single 80 ml doses. Active drug was 20 grams of lecithin in a sugar- and peppermint-flavored aqueous suspension. The taste- and appearance-matched placebo contained flour, water, gelatin, sugar, oil of peppermint and food color. Tests were conducted during the sixth hour after dosing, at the time of an expected peak in plasma choline. Results of t-tests for paired comparisons,performed on cognitive test scores, revealed no change in memory function, although plasma choline levels rose to nearly double the control levels (16.5 ± 2.4 nmol/ml after placebo versus 31.2 ± 7.2 nmol/ml after lecithin). Clinical failure of lecithin for memory enhancement cannot be attributed to slowing of psychomotor speed, or to other adverse drug effects, as no change occured in performance on the digit/symbol task, vital signs were not altered, and all subjects reported feeling well at the time of testing.

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287.6 PERSISTENT MEMORY DISORDER FOLLOWING CLOSED HEAD INJURY IN CHILDREN: LIMITATIONS ON CEREBRAL PLASTICITY: H.S. Levin, H.M. Eisenberg and M.E. Miner. Division of Neurosurgery, The University of Texas Medical Branch, Galveston, TX 77550.

Twenty children who were 12 yrs. of age or younger when they sustained a closed head injury and had no previous developmental disorder were studied for at least six months postinjury (median = 13.5 mos) to assess recovery of memory. There were 12 children with diffuse injury and 8 cases with a focal intracranial or contusion visualized by computed tomography. According to ratings on the Glasgow Coma Scale (GCS), the initial severity of head injury was severe (GCS ≤ 8) in 12 children and mild or moderate (GCS > 8) in eight cases. Despite the finding of low average intellectual level in both the severe (Verbal IQ = 91, Performance IQ = 88) and mild injury (Verbal IQ = 92, Performance Scale IQ = 91) groups on follow-up examination, impairment of long term memory storage and retrieval ($p<.05$) was relatively more frequent in the severely injured children as was continuous recognition memory ($p<.01$). These findings confirm and extend our previous report of frequent memory deficit in children during the early stages of recovery from closed head injury (Levin, H.S. and Eisenberg, H.M., J. Pediatr. Psychol., 4:389-402, 1979) and suggest that residual memory deficit may persist even in children who evidence normal intellectual ability. In contrast to the impressive evidence for greater recovery of language after cerebral insult in children as compared to adults, a similar sparing of memory may not occur after injury to the young brain.

287.7 MEMORY FOR STABLE VERSUS FLEXIBLE TARGETS IN HUMAN AMNESICS. John A. Walker, Michael I. Posner, Robert D. Rafal*, and Kevin P. O'Brien*. Neuropsychology Laboratory, Neurological Sciences Center, Good Samaritan Hospital & Medical Center, Portland, OR 97210

Recent research with human amnesics has suggested that the class of preserved memory capacities is broader than previously believed (Cohen and Squire, 1980). Current work with animals who have memory deficits suggests close similarities to the human work (Walker and Olton, 1981). Both sets of results suggest that simple, stable, procedural information seems spared in the amnesic, while specific, flexible, episode-related information is lost. However, the different nature of the tasks that have been used with humans and with animals has generally precluded any direct comparison of results. The present study was designed to test human amnesics in a direct analog of a task that has proven to be sensitive to memory deficits in experimental animals.

Chronic amnesics of several etiologies (CVA, temporal/hippocampal surgery, closed head injury, Alzheimer's, Korsakoff's) were tested in a task that required memory for a target word. Each target was chosen from a short list of words. Two types of target lists were developed: in one, the target word was always the same, single word chosen from the list (stable targets), while in the other type, each of the words could appear as a target (flexible targets). Several lists of each type were used repeatedly within a block of trials. On each trial, the target was presented and then, after a variable delay, the word list was presented. The words were presented on a CRT screen, and the subject was required to indicate the spatial position of the target word within the list by pressing the appropriate key on a keyboard. Choice accuracy and reaction time were recorded. At the end of the test session, the subject was required to indicate which words had been presented in the day's session from a list containing stable targets, flexible targets, presented nontargets and nonpresented words.

Analysis of the pattern of choice accuracy and reaction times suggested differences in the subject's abilities to remember target items based in part on whether the targets were stable or flexible. In addition, there were differences in the performance among the patients of different etiologies that suggest differences in the information processing deficit. Although a number of important differences may remain between the human and animal amnesias, there appears to be a common defict when flexible information processing is required.

Supported in part by NIMH Grant 1-R03-MH35802-01 to JAW, and the Neurological Sciences Center Foundation.

287.8 DEMENTIA IN PARKINSON'S DISEASE: A NEUROPSYCHOLOGICAL ANALYSIS. F.J. Pirozzolo, J.A. Mortimer, E.C. Hansch, D.D. Webster* & M.A. Kuskowski*, Geriatric Research, Educ. & Clinical Center, V.A. Med. Ctr., Minneapolis, MN 55417, and Department of Neurology, University of Minnesota Med. Sch., Minneapolis, MN 55455.

An increased association of intellectual deficits and cognitive impairment with Parkinson's disease (PD) has been repeatedly observed in clinical and psychometric investigations. While the use in these studies of global ratings of dementia or standardized tests of mental reasoning have documented the occurrence of cognitive impairment, little agreement has been reached on the issue of a specific vs. more generalized deficit in cognition in the disease. In the present study, an extensive neuropsychological battery was administered to patients with PD in order to elucidate the nature of the cognitive deficit.

Sixty patients with idiopathic PD and an equal number of normal age- and education-matched controls were given a battery of tests measuring such abilities as verbal and nonverbal memory, language, visual-spatial perception, concept set shifting, speeded categorical decision making, and fund of information. Patients performed significantly poorer ($p<.001$) on all measures than controls with the exception of tests for vocabulary and information. The strongest group differences, as revealed by the size of the t-ratios, occurred for tests involving visual-spatial performance and paired associate memory. In order to assess clinical classification efficacy, stepwise discriminant analysis was applied to the data for patients and controls. A derived variable subset of block arrangement, a difficult paired associate memory test, and a letter cancellation test correctly assigned 73% of patients and 87% of controls. This three variable discriminant function when derived from a randomly-selected half of the total sample continued to classify the remaining patients and controls at essentially similar (79% and 77%) levels. To take into account the match between patients and controls, difference scores on the discriminant function were computed for each patient-control pair. These scores indicated that 89% of patients were more impaired than their matched control counterparts.

These results support the contention that cognitive impairment in PD occurs across a wide domain of neuropsychological abilities. On the other hand, the observed strong decrements in several tests of visual-spatial function argue that this aspect of cognition is especially impaired in the disease. Lack of impairment on the vocabulary and information tests suggests a selective sparing of "crystallized" as compared to more "fluid" intellectual abilities. Finally, results of the discriminant analysis suggest that the neuropsychological profile may serve as an independent and relatively accurate predictor of the presence of the disease.

288.1 MORPHOMETRIC CORRELATES OF CORTICAL CELL PATTERNS OF LIMBIC LOBE IN DOLPHIN BRAIN. M.S. Jacobs, P.J. Morgane and W.L. McFarland. Dept. Pathobiol., NYU Coll. Dent., New York, NY 10010; Worcester Found. Exp. Biol., Shrewsbury, MA 01545; NIH, Bethesda, MD 20014.

Studies of the limbic lobe in cresyl violet stained serial sections of perfused brains of the bottlenose dolphin have included quantification of neuronal size, neuronal and neuroglial cell numbers and laminar thicknesses. The quantitative data have been valuable for interpreting organizational patterns of the cetacean limbic cortical formations which incorporate several special features of the dolphin's voluminous neocortex. These latter include (1) relative uniformity of cortical thickness, (2) regional cytoarchitectonic differences that occur gradually and are often less prominent than in terrestrial mammals, and (3) an apparent absence of a small celled internal granular layer IV.

The major cytoarchitectonic patterns of the limbic cortical formations at pregenual (Prgn), supracallosal and retrosplenial (RtSpl) levels reflect increasing laminar differentiation as the cortex is followed radially from its internal, peritectal origin to its external margin; increased cellularity in the posterior supracallosal (PScl) and RtSpl regions as compared to Prgn and anterior supracallosal (AScl) regions; and an unusual phalangeal cell pattern at mid levels of the cortical plate in the RtSpl peritectal zone. In addition, the cortical plate of the subrostral (parolfactory) limbic region exhibits gradual increasing cellularity, widening and laminar differentiation as it is followed radially from its origin. The temporal periarchicortical limbic formations reveal a complexly organized, anteriorized entorhinal region exhibiting numerous subfields, and a posteriorized, rather uniform presubiculum.

Respective listings of quantitative data of neuronal densities (cells/mm^3) and glia/neuron cell ratios for various sectors of limbic cortex proper are: Prgn sector, 5090 and 2.40; AScl sector, 3626 and 4.13; PScl sector, 6762 and 1.96; anterior RtSpl sector, 5638 and 1.89; and posterior RtSpl sector, 5522 and 2.08. The maximum deviation from the mean cortical thickness (1.45 $\pm$ .04 mm) is 0.11 mm, reflecting uniformity of cortical width. Differences in laminar widths are regionally slight, and mean values are: layer I, 0.35 $\pm$ .02 mm; layer II, 0.09 $\pm$ .01 mm; layer III, 0.31 $\pm$ .03 mm; layer V, 0.23 $\pm$ .02 mm; and layer VI, 0.47 $\pm$ .04 mm. In view of the lack of layer IV, it is of special interest that the dolphin counterpart of the granular type of posterior limbic cortex present in land mammals is denso- and parvicellular cortex. Here in the PScl sector, supragranular layers II and III contain cells that are more numerous and have major and minor axial dimensions that are significantly less than corresponding neurons in the AScl sector. (Supported by NSF Grant BNS 79-08660).

288.2 BEHAVIORAL FUNCTION OF SYMPATHETIC SPROUTING AND ITS RELATION TO LIGHTING CONDITIONS AND LIGHTING CYCLES. P. Moes*, D. Graves*, P. Kesslak*, F. H. Gage. Chem. of Behavior Program, Texas Christian Univ., Fort Worth, TX 76129.

Sprouting of superior sympathetic ganglion (SCG) fibers following lesions to the septal nucleus is well documented, and several investigators have studied mechanisms for growth of these fibers into the hippocampus. However, a functional significance of this ingrowth has not been determined. The loss of Ach fibers to the hippocampus appears to be critical for the sprouting of SCG fibers. For this reason, lesions to the medial septal (MS) area (which project Ach fibers to the dentate gyrus) were performed to induce the sprouting, and to produce a behavioral deficit which recovers over time. Since the time course of the ingrowth following the lesion corresponds roughly with the behavioral recovery, it was felt that this new innervation may be mediating the changes seen in the recovery. Because the SCG has also been implicated in the mediation of circadian rythms and response to levels of light, the following conditions were examined: housing in 24 hr. light vs. 12/12 light-dark vivarium, testing in morning vs. evening, and testing in a light room vs. a dark room.

Animals were given MS, or sham, lesions, allowed to recover for 3 weeks, and then received a ganglionectomy or sham ganglionectomy. Daily water consumption was measured and behavioral testing was done each week throughout the study, which included response to footshock and hotplate, and activity in a novel environment (open field). Results show an increase in reactivity following MS lesions that recovers over 1-2 weeks. Following ganglionectomy, there is an accentuation of differences in behavioral responses between light vs. dark testing conditions (compared to animals with sham lesions and ganglionectomies). These results demonstrate a functional significance for septal sprouting and suggest that lighting conditions alter the functioning of this new innervation.

288.3

WITHDRAWN

288.4 NON-THALAMIC EFFERENT PROJECTIONS OF THE POSTERIOR CINGULATE GYRUS IN THE RABBIT. J.L. Bassett* and T.W. Berger (SPON: A. Mallinger). Psychobiology Program, Department of Psychology, University of Pittsburgh, Pittsburgh, PA 15260.

Efferent projections of the posterior cingulate gyrus in the rabbit were examined using both horseradish peroxidase and autoradiographic axonal transport techniques. The cytoarchitecture of the posterior cingulate gyrus was defined according to Rose and Woolsey (J. comp. Neurol., 89:279, 1949) for the rabbit. The present analysis deals specifically with its granular cingulate and retrosplenial components.

Large injections of ^{3}H-proline (50 uCi/ul) that involved both retrosplenial and cingulate cortices, and smaller injections that involved only cingulate or retrosplenial subdivisions were made at various locations along their anteroposterior extent. Following a 24-48 hour survival period, the brains were processed in accordance with established procedures (Cowan, W.M. et al., Brain Res., 37:21, 1972). HRP (Sigma, type VI, concentration 30%) in volumes of 0.02-0.05 ul was injected into the various efferents of the cingulate gyrus after which tissue was prepared according to Mesulam (J. Histochem., Cytochem. 24:1273, 1976; ibid., 26:106, 1978).

After tritiated amino acid injections in the posterior cingulate gyrus, silver grain deposits were seen in the dorsal claustrum, medial and lateral pontine nuclei, caudate nucleus, anterior limbic cortex and pretectum.

Injections of HRP into these target regions identified cells in (1) layers V and VI of cingulate cortex as projecting to the claustrum (2) layer V of retrosplenial cortex as projecting to the pons (3) layers V and VI of retrosplenial as projecting to the caudate and (4) layers II and III of cingulate and layers V and VI of retrosplenial as projecting to anterior limbic cortex.

In total, these results identify the cells of origin and site of termination of the major non-thalamic efferent projections of the cingulate gyrus in the rabbit.

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288.5 SUBICULAR PROJECTIONS TO RETROSPLENIAL CORTEX IN THE RABBIT. S.L. Semple-Rowland*, J.L. Bassett* and T.W. Berger (SPON: J. Jennings). Department of Psychology and Department of Psychiatry, University of Pittsburgh, Pittsburgh, PA 15260

Subicular efferents to the posterior cingulate gyrus in the New Zealand white rabbit were examined using anterograde and retrograde axonal transport methods. Injections of tritiated proline were made throughout the septo-temporal extent of the subicular cortex. Autoradiographic analysis revealed a concentration of silver grains in layers I and IV of the retrosplenial subdivision of the posterior cingulate gyrus. The highest density of silver grains was clearly located in the granular, layer IV. Higher than background grain counts were also evident in layers V and VI, though labeling in these layers was light compared to that seen in I and IV, and may have represented axons from the cingulum en route to superficial layers. Subicular-retrosplenial connections are topographically organized such that septal (rostral) subicular regions project to medial portions of rostrally located retrosplenial cortex; more posterior (though dorsal) subiculum projects to medial portions of corresponding posterior retrosplenial cortex; and temporal (ventral) subiculum projects to lateral aspects of the posterior retrosplenial cortex. In addition, results showed that efferents from rostral and (dorsal) posterior regions of subiculum terminate over a much wider region of retrosplenial cortex than efferents from temporal subiculum.

Retrograde analysis of this connectional system using horseradish peroxidase (HRP) revealed the same topographical organization described by autoradiographic methods. In addition, HRP results showed that the cells of origin for retrosplenial afferents were restricted to the subiculum proper, and did not include the presubiculum or other subicular subdivisions.

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288.6 NEOCORTICAL INPUT TO ENTORHINAL AND CINGULATE CORTICES IS RELAYED BY THE CLAUSTRUM. T.W. Berger, J.L. Bassett* and S.L. Semple-Rowland*. Psychobiology Program, Department of Psychology and Department of Psychiatry, University of Pittsburgh, Pittsburgh, PA 15260.

The nature of claustral afferents to the limbic system was studied in the New Zealand white rabbit using anterograde and retrograde tracing methods. Horseradish peroxidase (HRP) injections that involved both medial and lateral entorhinal cortex resulted in dense retrograde labeling of a large number of neurons within the claustrum. Labeled neurons were seen throughout the entire structure, though the majority were located in the dorsolateral and ventral regions. There was also evidence of reciprocal connections between entorhinal cortex and the claustrum, i.e., anterogradely transported HRP was often visible in the claustrum after entorhinal injections. Injections of tritiated proline in the claustrum resulted in silver grain densities that were greatest within the deeper cortical layers (V-VI) of both medial and lateral entorhinal areas.

Injections of HRP in the posterior (granular) cingulate gyrus also resulted in dense labeling of a large number of claustral neurons, though these were restricted in location to dorsomedial regions. Anterogradely transported HRP was also evident in the claustrum after cingulate injections, indicating that both entorhinal and cingulate cortices are reciprocally connected with the claustrum. Autoradiographic results showed that claustral afferents terminate in layers VI, V and possibly I of cingulate (though not retrosplenial) cortex.

These experiments demonstrate the existence of a prominent claustral afferent system to two allocortical brain regions -- the entorhinal and the cingulate cortices. The entorhinal cortex, in particular, is known to be a major afferent to the hippocampal formation (Hjorth-Simonsen and Jeune, J. comp. Neurol., 144:215, 1972), and the posterior cingulate gyrus has been shown to project to the presubiculum (Domesick, Brain Res., 12:296, 1969). Previous studies have demonstrated that the claustrum receives input from virtually all of neocortex (Carmen, Cowan and Powell, J. Neurol. Neurosurg. Psychiat., 27:46, 1964). As a result, the claustrum could function as a major "conduit" for neocortical information to reach the limbic system.

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288.7 AUTORADIOGRAPHIC DEMONSTRATION OF THE SPROUTING OF SEPTAL AFFERENTS TO THE DENTATE GYRUS AFTER LESIONS OF THE ENTORHINAL CORTEX IN ADULT RATS. B.B. Stanfield and W.M. Cowan. Salk Institute, La Jolla, CA 92138.

Following lesions of the entorhinal cortex in adult rats there is a striking intensification of the histochemically demonstrated acetylcholinesterase (AChE) activity in the molecular layer of the dentate gyrus. Although it has been shown that the AChE intensification is dependent on the integrity of the septum, the evidence that it is due to collateral sprouting of septo-dentate fibers remains indirect.

To examine this we have employed the autoradiographic method following injections of ^{3}H-proline into the septum of control rats and of animals in which lesions of the entorhinal cortex had been placed one month earlier. By using fairly large injections (20-40 μCi in 0.2-0.4 μl) and relatively long survival times (2 to 5 days) we have been able to demonstrate more clearly the rather sparse septo-dentate projection. In addition, by processing one series of sections for autoradiography and an adjacent series for AChE histochemistry, we have been able to directly compare in each experiment the distribution of transported label in the septo-dentate fibers with the AChE staining pattern.

The septal projection to the dentate gyrus in normal rats is rather diffuse; following a septal injection of ^{3}H-proline the greatest density of transported label is found in the hilus immediately beneath the granule cell layer, but there is also above-background labeling over the molecular layer. Here the density of labeling is more-or-less uniform and we have been unable to identify a correlation between the pattern of labeling in the autoradiograms and the distribution of AChE staining in the adjacent sections.

Septal injections in animals that have had prior lesions of the entorhinal cortex give rise to a quite different pattern. In these cases there is about a ten-fold increase in grain density over the denervated portion of the molecular layer. The region of increased grain density corresponds closely to the zone of gliosis and AChE intensification. The width of these zones varies from case to case depending on the extent of the initial lesion and the distribution of the interrupted entorhinal afferents. Thus, after lesions of the medial entorhinal cortex the zone of AChE intensification and elevated grain density is confined to the middle portion of the molecular layer, whereas after lesions of the lateral entorhinal cortex, the corresponding zone is limited to the outer third of the molecular layer.

These results provide the first direct evidence that there is a proliferation of septo-dentate fibers following entorhinal lesions, and they are consistent with the view that the concomitant AChE intensification is due to this proliferation.

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288.8 CYTOARCHITECTONIC MATURATION OF RAT ENTORHINAL CORTEX (AREA 28). Russell E. Ruth and Sara K. Goldsmith*. Il. Inst. Dev. Disabil., Chicago, Il 60608.

Neurons destined to reside within rat cortical area 28 proliferate at the beginning of the third gestational week (Bayer, 1980). It was of interest to ascertain the temporal sequence of morphogenesis in this cortex after proliferation had ended. Brains were removed from anesthetized animals in embryonic or postnatal life, and were processed using routine histologic methods.

In adults area 28 has 3 clearly recognizable cytoarchitectonic divisions: medial (m), intermediate (i) and lateral (l). In fetuses removed by Caesarian section one day prior to parturition only a gross lateral/medial distinction can be made. Lamination is rudimentary, and although layer II of area 28l has split into a superficial and deep lamina cell clustering is not yet observed. Compared to adults (> 90 days old) cell density is high, due to numerous small, densely stained neurons.

By postnatal day 8 cell density has significantly declined, and the individual cells are larger. All three divisions are discernable, with distinct clustering of cells in layer II of area 28l. This division is more adult-like than either area 28m or 28i, paralleling the order of earlier cell proliferation. Finally there is a dramatic increase in the total cortical volume during early postnatal life. It is not clear whether the lowered cell density seen in adults is due to increased volume, to cell death or to both factors. These and other questions are currently being examined in greater temporal detail using reconstructive and quantitative morphometric methods.

288.9 TETANIC POTENTIATION IN THE CINGULATE CORTEX. R. W. Sikes, K.H. Taber, and J. F. DeFrance. Dept. of Neurobiology and Anatomy, Univ. Texas Medical School, Houston, Texas 77025

The projection from the mediodorsal thalamic nuclei(MD) to the cingulate cortex has been well established by anatomical studies. The lateral part of the nucleus sends fibers which terminate primarily in the deep part of layer III of the anterior limbic cortex. Very little, however, is known of the electrophysiology of this pathway. This report will describe the effect of electrical stimulation of MD on the anterior limbic cortex measured by extracellular field and unitary recording techniques.

Stimulation of the lateral part of MD at frequencies below 1 Hz elicit surprisingly little response in the cortex. By increasing the frequency of stimulation to 4-10 Hz, however, large extracellular field responses could be produced. The magnitude of this response increases to a maximum at 7 Hz and then decreases with higher stimulus frequencies. The response is a large negative potential with a 15 msec latency which is followed by a smaller positive potential at 30 msec. The magnitude of the response is greatest in the deep part of layer III and in the superficial part of layer V. This negative-positive response appears reversed at the surface of the cortex.

The magnitude of the response increases gradually during a train of stimuli; often requiring three or four stimuli to reach its maximum. If the frequency is them abruptly lowered to below 1 Hz, the response disappears: i.e., there is no significant posttetanic potentiation.

Single cell recordings show that many cells in the anterior limbic cortex are not affected by MD stimulation. Nevertheless, another group of cells do show frequency dependent excitatory responses. When the MD nucleus is stimulated at low frequencies, these cells discharge with no apparent relation to the stimuli. But when the stimulus frequency is increased to 4-10 Hz, the cells discharge in phase with the stimulus train with a 15-18 msec latency. These discharges occur in relation to the peak of the negative field. The cells do not generally discharge until the field is near maximal amplitude.

These data indicate that while the MD projection is excitatory to the cingulate cortex neurons, the effect is dependent on the frequency of stimulation and possibly to the number of stimuli in the train.

288.10 THE EFFECT OF HIPPOCAMPAL LESIONS ON RAT MATERNAL BEHAVIOR AND SUBSEQUENT ENDOCRINOLOGICAL AND BIOCHEMICAL CONDITIONS PRESENT IN THEIR DEVELOPING OFFSPRING. H. M. Murphy and C. H. Wideman, John Carroll Univ., Cleveland, OH 44118, D. W. Long*, Consolidated Biomedical Laboratories, Columbus, OH 43216, and T. S. Brown*, DePaul University, Chicago, ILL 60614.

Behavioral studies have indicated that maternal behavior is altered in animals with hippocampal lesions. Endocrinological and biochemical studies have also demonstrated a number of changes in such animals. For example, these animals have an altered glucocorticoid response to stress. They have significantly lower levels of serum TSH, total T_4, and free T_4. Animals with hippocampal lesions have significantly higher levels of blood urea nitrogen and significantly lower levels of serum triglycerides and cholesterol. The present study was undertaken in order to gain further insight into the maternal behavior of animals with hippocampal lesions. In addition, the levels of certain chemicals in the serum as well as body weight were ascertained from offspring of female animals with hippocampal lesions.

In the behavioral aspect of the study it was shown that pup survival to weaning (21 days) was significantly lower in animals with hippocampal lesions than in control animals. Mean body weights were significantly lower in pups born of mothers with hippocampal lesions than in controls 5 days after birth. These weight differences disappeared by day 14. Nursing behavior and nest building were significantly poorer in animals with hippocampal lesions. Some differences were also noted in retrieval behavior. Although there were no significant differences in retrieval behavior when pups received no treatment, poorer retrieving was noted in rats with hippocampal lesions when the pups were covered with vaseline or food powder. Poorer retrieving was also noted in these animals when the pups were contaminated with the scent of another rat.

In the endocrinological and biochemical aspect of the study the following chemicals were measured in the serum of the pups: TSH (RIA), total T_4 (RIA), free T_4 (RIA), sodium, potassium, chloride, phosphorus, iron, glucose, uric acid, blood urea nitrogen, creatinine, cholesterol, triglycerides, total protein, albumin, and bilirubin. The tests were conducted 21 days after birth. There were no significant differences between pups born of animals with hippocampal lesions and control animals in any of the above chemical substances. It thus appears that if pups born to mothers with hippocampal damage can survive until weaning, they will not show endocrinological and biochemical abnormalities typically found in the brain damaged mother.

288.11 THE OLFACTORY BULB AND ENTORHINAL AREA HAVE DIRECT PROJECTIONS TO THE DORSAL HIPPOCAMPAL RUDIMENT IN THE MOUSE. M.T. Shipley, G.D. Adamek and Marc S. Sanders*. Dept. of Cell Biology and Anatomy, Northwestern Univ. Med. School, Chicago, IL 60611 and Grad. Prog. in Neurosci., Northwestern Univ., Evanston, IL 60201

The dorsal hippocampal rudiment (DHR) is an enigmatic cortical field classically felt to be a part of the hippocampus (HC). In the mouse, the DHR lies just dorsal to the corpus callosum at the base of the anterior half of cingulate cortex. In coronal sections the field is small but constitutes a fairly long rostro-caudal strip. The connections of the DHR are unknown.

The Timm's staining pattern of the DHR is reminiscent of a mini-dentate gyrus (DG) comprising a layer of granule cells with 2-3 bands of staining in the molecular layer. In the DG these bands correspond to inputs from the lateral and medial entorhinal area (LEA and MEA) and the ipsi-and contralateral association systems. Using anterograde transport of HRP we have found that the LEA and MEA terminate in the molecular layer of the DHR in a manner similar to their projection to the DG. This suggests that the DHR is a displaced portion of the DG.

The olfactory system is known to have a strong indirect influence on the HC via primary and secondary bulbar projections to the LEA. Wheat germ agglutinin-HRP injections confined to the main olfactory bulb (MOB) show a direct projection from the MOB to the DHR.

Both the olfactory bulb itself and retrobulbar structures such as the piriform cortex (PC) convey olfactory information to the LEA; the LEA supplies a major input to the DG. Our results suggest that there is a much more direct pathway whereby olfactory information may influence a cortical region whose histochemistry and direct afferents from the entorhinal cortex suggest that it is part of or closely related to the DG. Thus, the DHR may represent a phylogenetically old olfacto-recipient outpost of the hippocampus.

It has become apparent that there are parallel functional olfactory systems originating in the MOB and accessory olfactory bulb. Taken with the other connections of the mouse MOB (Adamek and Shipley, this volume), the present findings suggest that there may be parallel functional subsystems associated with the MOB itself.

Slotnick and Berman (Br. Res. Bull. 1980) have shown that rats with total transections of the lateral olfactory tract (LOT) can relearn odor discriminations. If the MOB to DHR projection we describe exists in the rat, it may have escaped the LOT lesions and provide a substrate for the animals' ability to relearn.

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288.12 EVIDENCE FOR POSTSYNAPTIC ALTERATIONS IN PAIRED PULSE POTENTATION OF CA1 PYRAMIDAL CELLS IN HIPPOCAMPAL SLICES. David Whitehorn, Walter C. Low and Spencer L. BeMent, Dept. of Physiology and Biophysics, Univ. of Vermont, Burlington, Vermont and the Bioelectrical Sciences Laboratory, Univ. of Michigan, Ann Arbor, MI.

The evoked activity of CA1 pyramidal cells in the hippocampal formation has been shown to potentiate in response to paired electrical stimuli of afferent pathways; and evidence suggests that the potentiation of this neuronal population is a result of an enhancement of synaptic transmission. We have previously shown (Brain Res., 1980, 198:472) that an augmentation of the afferent volley is involved in paired pulse potentiation produced by activation of Schaffer collaterals. We now report evidence demonstrating that postsynaptic alterations also contribute to the overall potentiation of CA1 pyramidal cell responses. Our data show that the potentiation of population discharge is greater than would be predicted from the enhancement of synaptic transmission alone.

In rat hippocampal slice preparations, Schaffer collateral fibers were electrically stimulated over a wide range of intensities. Input-output functions (I-O functions) relating the amplitude of the summated discharge of the pyramidal cells (population spike) to the amplitude of the summated dendritic depolarizations (population EPSP) were determined using linear regression equations. I-O functions were determined for both control responses and for potentiated responses obtained with the second of a pair of stimuli delivered with a 30 msec interstimulus interval.

If the paired pulse potentiation of the population spike is a result of potentiation of the population EPSP alone, then the control and potentiated I-O functions should be coincident. On the other hand, if potentiation involves postsynaptic mechanisms, control and potentiated I-O functions should be non-coincident with potentiated functions displaying a greater slope, and/or intercept.

I-O functions were obtained in 28 slices. Sixteen slices exhibited non-coincident control and potentiated functions. In all of these 16 slices slopes and/or intercepts were greater in the potentiated condition. Data from slices exhibiting non-coindent control and potentiated function were further analyzed to determine the relative contributions of postsynaptic and synaptic factors to the overall potentiation. We found that 57 ± 6% of the population spike potentiation could be explained by the enhancement of the population EPSP while 43 ± 6% arose from apparent postsynaptic alterations. These results suggest that alterations in postsynaptic excitability can be a major contributing factor to the enhancement of CA1 cell activity under conditions of paired pulse potentiation. (This work supported by NIGMS grant GM 01289 and USPHS grant NS 08478).

288.13 DOPAMINERGIC STIMULATION OF N. ACCUMBENS CAN REDUCE THE BEHAVIORAL CONSEQUENCES OF HIPPOCAMPAL DAMAGE. J. H. Hannigan, Jr., J. E. Springer* and R. L. Isaacson . Dept. of Psychology and Center for Neurobehavioral Sciences, SUNY-Binghamton, Binghamton, NY 13901.

The intracerebral administration of a specific dopamine agonist, 3,4-dihydroxyphenylamino-2-imidazoline (DPI; see Cools & Van Rossum, 1980) into n. accumbens can reduce several of the usual consequences of bilateral hippocampal damage (Reinstein, Hannigan, & Isaacson, 1980). This suggests that at least some of the behavioral changes following such damage may be due to lesion-induced secondary changes in n. accumbens and possibly other basal ganglia sites.

Secondary changes of behavioral importance may also be occurring in the caudate since alteration in membrane phosphorylation can be found there after hippocampal destruction (Bär, Gispen, & Isaacson, 1981), despite the lack of direct hippocampal formation afferents to the area. Therefore, we undertook to investigate the behavioral effects of intra-accumbens and intra-caudate administration of dopaminergic agents to determine the degree to which the usual behavioral changes found after hippocampal damage would be alleviated.

Rats with sham, cortical, or hippocampal lesions were implanted with a cannula into either the caudate nucleus or the nucleus accumbens. On 4 consecutive days starting 6 days after surgery, rats were injected with increasing doses of DPI into nucleus accumbens or similarly incrementing doses of haloperidol into caudate. The animals' behavior (locomotion, rearing, grooming, and hole poking) was measured in an open field/hole board afterwards. One month later, the injections were reversed (i.e., DPI into caudate, and haloperidol into accumbens) and behavior observed again.

Hippocampally-lesioned animals exhibited characteristic behavioral deficits in locomotion, rearing, and grooming which were selectively attenuated by injection of DPI into accumbens. Haloperidol into either area or DPI into caudate were generally ineffective. Further, control lesion groups were not selectively affected by either drug in either brain area.

These results indicate that the hippocampal lesions induce an enhanced sensitivity to low doses of DPI into n. accumbens and that the presumed activation of the DAi receptors by DPI can result in the normalization of several behaviors influenced by bilateral hippocampal damage. This suggests that the behavioral sequelae of hippocampal lesions may be in part due to alternation in the dopamine system in n. accumbens which develop secondarily to the lesion.

288.14 CELLULAR AND SYNAPTIC CHARACTERISTICS OF FETAL RABBIT HIPPOCAMPUS. P.A. Schwartzkroin and D.D. Kunkel*. Dept. of Neurological Surgery, University of Washington, Seattle, WA 98195.

The development of hippocampus has been well studied by morphologists (Bayer, J Comp Neurol 190:87, 1980; Stensass, J Comp Neurol 129:59, 1967). They have shown that many of the most dramatic changes occur prenatally, during a period when it is technically difficult for physiologists to investigate the development of cellular and synaptic activities. Although hippocampal neurons, initially taken from fetal animals, have been studied using dispersed cell culture (Peacock, Brain Res 169:247, 1979) and tissue culture (Zipser et al., Brain Res 60: 489, 1973) techniques, neither of these situations provides an accurate picture of normally organized hippocampus at known stages of development.

Using the *in vitro* hippocampal slice technique, we have studied the hippocampal pyramidal cell region of fetal rabbits, correlating electrophysiological findings with cell and synaptic profiles shown by electronmicroscopy. Intracellular impalements of cells in the pyramidal cell region of hippocampus were obtained from fetuses 21 through 29 days' gestation (term - 30-31 days). In all experiments, cells were capable of generating action potentials, but spikes were of unusually long duration (sometimes greater than 10 msec). In good penetrations of cells from fetuses 21-25 days' gestation, cell input resistance could be as high as 200 megohms (time constants of 15-50 msec); in cells from older fetuses (25-29 days' gestation), input resistances of 80-90 megohms were not unusual. Intracellular current injection elicited trains of spikes only in the best penetrations. Repetitive discharge was followed by long duration afterhyperpolarizations in some cells.

No synaptic activity could be evoked in fetal hippocampus in the earliest stages we studied. However, by 24 days' gestation, stimulation in the stratum radiatum region could produce depolarizing potentials of extremely long duration. No hyperpolarizing inhibitory synaptic responses were recorded during good penetrations. Electronmicroscopic examination of the pyramidal cell region showed some synaptic profiles, even in our youngest fetuses. Such infrequent profiles occurred on dendritic processes and were all of the asymmetric type.

These experiments indicate that electrophysiological investigation of fetal CNS structures can be carried out in parallel with morphological studies of development.

288.15 STIMULATION-INDUCED CHANGES IN DIMENSIONS OF STALKS OF DENDRITIC SPINES IN THE DENTATE MOLECULAR LAYER. E. Fifková and C. L. Anderson*. Dept. of Psychology, Univ. of Colorado, Boulder, CO 80309.

Tetanic stimulation of the entorhinal area has been shown to induce lasting enlargement of the average dendritic spine area and perimeter in the termination zone of the perforant path in the outer two-thirds of the dentate molecular layer. These data were derived from measuring heads of dendritic spines. Since changes in the spine stalk may have a considerable impact on electrical properties of synaptic potentials, we studied the dimensions of the spine stalk and spine head following tetanic stimulation. The lack of identification criteria makes it difficult to sample spine stalks separately. However, a spine stalk can be easily recognized when it connects the spine head with its parent dendrite. Therefore, all measurements were done on spines which satisfied this criterion. Forty mice were divided into four groups. Out of these, two groups were stimulated and sacrificed 4 min and 90 min later. The other two groups were sham controls with survival periods similar to those of the stimulated ones. Tetanic stimulation of the entorhinal area induces in the 4 min poststimulation interval a statistically significant increase in the width of the spine head (21.6%) and spine stalk (42.2%) in the middle third; in the distal third, the enlargements of the head and stalk are 23.2% and 61.7%, respectively. Ninety min after stimulation, both spine head and spine stalk were wider in the distal third by 34.7% and 61.2%, respectively. In the middle third, the stalk width returned to control values; however, the head remained enlarged by 33.6%. The spine stalk was found to be significantly shorter in the distal third of both poststimulation intervals. It, thus, appears that changes in dimensions of the stalk and the head need not persist simultaneously. Given that the spine stalk can be viewed as an avenue communicating electrical and biochemical signals from the synaptic region of the spine head to the parent dendrite and vice versa, such a dissociation may have far-reaching functional implications. In this context, it seems to be noteworthy that the lateral perforant path which terminates in the distal third of the dentate molecular layer shows significantly more long-term potentiation than the medial pathway which terminates in the middle third (McNaughton, et al., Brain Res., 157:277). If the spine stalk enlargement is the substrate of this type of synaptic plasticity, then the earlier return of the stalk width to prestimulation conditions would be in agreement with the observed lower capacity of the medial pathway to generate long-term potentiation. (Supported by NIMH Grant MH 27240-06.)

288.16 SINE WAVE ELECTRICAL STIMULATION AFFECTS SYNAPTIC FUNCTION IN THE HIPPOCAMPAL SLICE. S. M. Bawin, W. R. Adey, M. D. Mahoney* and A. R. Sheppard*. Research Service, VA Hospital, Loma Linda, CA 92357 and Dept. of Physiology, Loma Linda University, Loma Linda, CA 92350.

The effects of sinusoidal currents on field potentials were studied in the CA_1 pyramidal cell layer of rat hippocampal slices.

Transverse, 400 μm slices were continuously perfused (2 ml/min) at 32-35C with a physiological solution saturated with 95% O_2 - 5% CO_2. Warmed gas flowed above the solution in the recording chamber. The extracellular response to test pulses (monophasic square wave, 100-200 μsec, 20-100 μA) in strata radiatum et oriens was monitored using standard microelectrode techniques and recorded on magnetic tape for later analysis.

Focal sinusoidal currents (20-40 μA p-p, 60 Hz, 5-15 sec) through the stimulating electrodes (Pt-Ir, teflon coated, 0.2 mm tip separation, 30-50 Kohm) in either pathway induced short (2-20 sec) and long-term (minimum 5 min) potentiation of the field potential evoked by test pulses in the same pathway. Heterosynaptic depression, lasting for 5-30 sec, was observed concurrently in the non-potentiated synaptic input. Currents of higher intensities (50-100 μA p-p) induced temporary (5-90 sec) mono- and heterosynaptic depression of the evoked response.

Non-focal stimulation of the slices was achieved by sinusoidal currents (200-700 $\mu A/cm^2$) via agar electrodes in contact with the perfusing solution. The electric gradients in the bath were of the order of 10 to 40 mV/cm. Depression of the responses to test pulses in either afferent fiber system was seen occasionally during and shortly after bath current epochs (5-30 sec) in otherwise nonstimulated slices. However, after potentiation of the response either by focal 60 Hz sine waves or trains of pulses (3-10 sec), bath currents induced short lasting depression followed by further enhancement of the population spike amplitude and the number of peaks in the field potential evoked by test pulse stimulation in the potentiated synaptic input. Thus, the sensitivity of the response in the CA_1 pyramidal cell layer to extracellular weak currents appears to be increased by prior repetitive focal stimulation in afferent fibers. (Supported by Dept. of Energy Contract No. DE-AI01-79ET29078 and Southern California Edison Company.)

288.17 LATE DEVELOPMENT OF INHIBITION IN NEONATAL PIRIFORM CORTEX. J.E. Schwob, L.B. Haberly and J.L. Price, Dept. Anatomy, Washington Univ. Sch. of Med., St. Louis, MO 63110.

The piriform cortex of rats is a good system for analyzing the development of neuronal connectivity from either an anatomical or physiological point of view. This study reports in vivo observations on unit activity and field potentials evoked by single and paired shock of the lateral olfactory tract (LOT) in adult rats and in neonatal rats ranging in age from PO (the day of birth) through P17. The rats were anesthetized with sodium pentobarbital (30 mg/kg) and nitrous oxide. The LOT and the cortex were exposed via an orbital approach, and the LOT was stimulated by a bipolar tungsten electrode. Conventional extracellular recording techniques with glass (1-8 M Ω) and tungsten (3-5 M Ω) microelectrodes were employed.

In adult rats units were reliably activated by a single low threshold stimulation of the LOT. In contrast, unit response to a second LOT shock was inhibited for up to 300 msec after the conditioning shock: with intershock intervals (ISI's) less than 100-150 msec single units could not be activated by stimulation up to 8x threshold and multiple unit activity was abolished. These results are similar to previously published observations in opossums (Haberly, J. Neurophysiol., 36: 762) and cats (Biedenbach and Stevens, J. Neurophysiol., 32: 193).

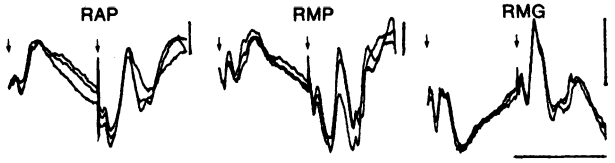
In newborns, units in piriform cortex were activated by stimulation of the LOT, but displayed relatively higher threshold and more variable latency. Inhibition following paired shock was markedly weaker in young pups and was apparent only with long ISI's. For example, from P0-P3 unit activity was actually facilitated with ISI's up to 150-200 msec, as demonstrated by a decrease in single unit threshold, an increase in multiple unit activity and an increase in the size of the population spike component of the field potential. Inhibition of evoked activity was apparent with ISI's of 250-750 msec. At P7 and P10, inhibition was evident shortly after the conditioning shock, but was generally stronger with ISI's exceeding 200 msec. At P15 and P17, a more adult-like pattern was observed. Field potential analysis in neonates demonstrated that paired shock increases the amplitude of the component generated by synaptic excitation of cortical dendrites by the LOT fibers; the timing of this facilitation is coincident with the period of unit facilitation at P0-P3 and weak inhibition at P6-P10. Therefore, the trend in neonates toward stronger inhibition with longer ISI's probably reflects the balance between synaptic facilitation and a weak inhibitory process; inhibition increases in strength as rats mature. (Supported by NIH grants NS09518 and GM 07200).

288.18 PAIRED PULSE FACILITATION IN HUMAN HIPPOCAMPAL FORMATION. Charles L. Wilson, Michael Wang*, Thomas L. Babb and Paul H. Crandall. Brain Res. Inst., Reed Neurological Res. Center, and Dept. of Surg./Neurol., Sch. Med., UCLA, Los Angeles, CA 90024

The excitatory synapses in the hippocampal formation are well known for their sensitivity to potentiation in the several forms in which it has been described (frequency, post-tetanic and long-term). In the present study we obtained preliminary evidence suggesting that synaptic mechanisms similar to those described in animals are present in the human hippocampal formation.

Testing was carried out in epileptic patients who had chronic temporal lobe depth electrodes for localization of seizure foci. Each electrode contained a bundle of platinum 40uM microwires (capable of resolving unit activity), which extended 5 mm beyond the macroelectrode tip. In order to assess the level of excitability of the hippocampus (anterior pes hippocampi, AP, and middle pes hippocampi, MP), entorhinal cortex (middle gyrus hippocampi, MG) and other limbic sites, pairs of conditioning (C) and test (T) pulses were delivered bipolarly to the macroelectrodes and bipolar responses recorded from the microwires (Lomo, Exp. Br. Res., 1973). Pulses were biphasic, symmetrical, 100 usec square waves, and pulse pairs were presented at rates below 0.1 Hz in order to avoid frequency potentiation.

An example of responses to stimulation of the right anterior pes (RAP) at an interstimulus interval of 200 msec is shown below. Recordings are from microwires 5 mm from the stimulating electrode in the same structure (RAP), 12 mm posterior in hippocampus (RMP), and 14 mm posterio-medially in entorhinal cortex (RMG). Three repetitions of the pulse pairs evoked stable C responses followed by prominent facilitation of the field potentials evoked by the T pulse (calibs: 100uV, 200 msec; charge density: 15 uC/cm^2/ph). Declining levels of facilitation (not shown) occurred as C + T intervals were increased to 400 and 800 msec. At 1600 msec intervals there was no evidence of potentiation. Note the facilitation in entorhinal cortex (RMG). Two other patients showed similar paired pulse facilitation of hippocampal field potentials at C + T intervals between 50 msec and 200 msec.



Supported by NIH Grant NS 02808

288.19 TRIMETHYLTIN: EARLY POSTNATAL ADMINISTRATION AFFECTS BEHAVIOR IN JUVENILE AND ADULT RATS. Diane B. Miller* and Robert S. Dyer (Spon: L. D. Grant). Neurotoxicology Division, US Environmental Protection Agency, Research Triangle Park, NC 27711.

Trimethyltin (TMT), when administered in a single dose to adult rats, produces hippocampal damage and a unique behavioral syndrome (Dyer et al., Neurobehav Toxicol. 1981). Since some compounds (e.g. triethyltin) produce more severe and lasting neurotoxicity when administered on postnatal day 5 than during adulthood, we characterized the effects of day 5 administration of TMT on learning and locomotor activity in juvenile and adult rats. On the day of parturition litters of Long-Evans hooded rats were cross-fostered and reduced to 4 male and 4 female pups each. On day 5 (birth = 0) one male and one female from each litter received 0, 5, 6 or 7 mg/kg TMT by intubation. Females were tested for their ability to acquire and perform a radial-arm maze learning task as juveniles (beginning at day 23). In this task efficient performance is characterized by a single entrance into each of 8 arms radiating from a center platform to obtain a food pellet. Both males and females were tested for locomotor activity in a figure-eight maze (Reiter et al., Envir. Hlth. Persp., 1975) as adults. TMT-treated rats acquired the radial arm maze task (i.e. entered and ate in all 8 arms) more slowly than controls. Although treated rats eventually acquired the task, there was a dose-related decrease in the efficiency with which they performed. Treated rats required substantially longer session times to obtain the same number of pellets as controls. This poor efficiency was not due to low activity since the treated rats tended to be hyperactive in the radial arm maze task. In the figure-eight maze TMT-treated males were more active than control males, but the TMT-treated females, perhaps because of their prior experience in the radial arm maze, were not more active than control females. These data indicate that postnatally administered TMT produces long-lasting changes in learning and activity. However, a single acute dose of TMT in the adult rat produces a more severe learning deficit (Walsh et al., SON, 1981) and greater hyperactivity (Ruppert et al., SON, 1981) than was found here. Thus the profile of behavioral toxicity for TMT differs as a function of age with adults being more sensitive to this toxicant than neonates.

288.20 A SPATIAL ANALYSIS OF POTENTIAL SPREAD IN HIPPOCAMPAL SLICES USING OPTICAL PROBES. M. Segal* and A. Grinvald (Spon:G.M.Gilad) Center of Neurosciences, The Weizmann Institute of Science, Rehovot, Israel.

A voltage sensitive membrane-bound merocyanine dye (WW 401) was used for optical monitoring of pre-and postsynaptic potential changes, evoked by electrical stimulation of an excitatory afferent pathway in CA1 region of 300 um thick rat hippocampal slices. The dye binds to neural membranes and changes its optical properties in response to changes in membrane potential. The optical signals thus reflect the averaged intracellular potential changes in a population of neural elements. An array of 10 x 10 photodetectors, positioned in the microscope image-plane was used to record electrical activity in a square area of 450 x 450 um. Conventional extra-cellular recording was performed in the stratum pyramidale with a glass micropipette. Stimulation of the stratum radiatum evoked fast signals (2-3 msec) in a strip of 90-180 um. This wave of fast signals travelled at a velocity of 0.2 m/sec along the Schaffer collateral system. These optical signals probably represent action potentials in the Schaffer collaterals, because (a) the signals were evoked in regions containing the Schaffer collaterals; (b) they persisted in low Ca^{2+} medium; (c) they were blocked by TTX; (d) the conduction velocity was similar to values reported previously for central unmyelinated fibers. A second wave of excitation followed the fast spikes with a delay of 2-10 msec. This wave travelled from the activation zone towards the stratum pyramidale and oriens. These slower responses were identified as EPSPs in the apical dendrites of CA1 pyramids. They were abolished by low Ca^{2+} and were enhanced by tetanic stimulation, TEA, picrotoxin, or low chloride. The changes in the optical signals were correlated with the changes in the electrical signals recorded with the microelectrode. Picrotoxin sensitive inhibitory potentials were also detected and had the largest amplitude in the border zone between stratum radiatum and pyramidale. On the other hand, the largest enhancement of excitation, in low chloride medium, was observed in the remote segments of the apical dendrites. Areas with similar morphology frequently showed different patterns of electrical responses. These experiments illustrate the potential usefulness of optical probes in the pharmacological and morphological analysis of local circuits in the mammalian brain. The resolution of optical method can probably be improved to allow recording from single cells. (as in invertebrate ganglia).

Supported by an NIH grant NS 14176 and a grant from the Muscular Dystrophy Association.

288.21 THE BENZODIAZEPINES AND THE HIPPOCAMPUS: MODE OF ACTION. K.H. Taber, R.W. Sikes and J.F. DeFrance. Dept. Neurobiology and Anatomy, U Texas Med. Sch., Houston, TX 77025.

The hippocampus has been widely used in the study of experimental epilepsy and antiepileptic compounds. As with many limbic system areas, it has been implicated in emotional functioning. Recently, benzodiazepine (BZD) receptors have been found within the hippocampus. The hippocampus is therefore an appropriate area in which to investigate the mechanisms of action of the BZDs.

Microstimulation and microrecording techniques were used in conjunction with microiontophoresis of the BZDs diazepam and chlordiazepoxide into specific layers of area CA_1 of dorsal hippocampus of the rabbit. Careful placement of the stimulating electrode allowed selective activation of either the Schaeffer Collateral pathway which terminates on the apical dendrites of the pyramidal cells (stratum radiatum) or the Commissural pathway which terminates on the basilar dendrites of the pyramidal cells (stratum oreins). Stimulation of these hippocampal paths at low frequencies (0.5 Hz) evokes very stable field potentials. Stimulation at higher frequencies (6.0 Hz) evokes a field potential which increases with each succeeding stimulation (tetanic potentiation, TP). Following the tetanus there is a period of increased excitability to 0.5 Hz stimulation (post-tetanic potentiation, PTP).

We have found that iontophoresis of BZDs into the area of Schaeffer Collateral termination in stratum radiatum has no effect on the field potentials evoked by 0.5 Hz stimulation, indicating that the BZDs have no effect on normal synaptic transmission in this pathway. However, both TP and PTP were altered. TP was slightly depressed. PTP was severely reduced both in amplitude and in duration. Iontophoresis of BZDs into the pyramidal cell body layer (stratum pyramidale) caused a decrease in the size of the population spike both at 0.5 and 6.0 Hz, reflecting a decrease in the excitability of the pyramidal cells. Neither TP nor PTP were significantly altered. Preliminary evidence supports the hypothesis that the decrease in the population spike is due to a BZD-mediated enhancement of GABAergic recurrent inhibition of the pyramidal cell body. We are currently investigating the possibility that the decrease in PTP is mediated by a BZD-induced decrease in cGMP in stratum radiatum.

288.22 THE EFFECT OF IONTOPHORETICALLY APPLIED VASOPRESSIN UPON LATERAL SEPTAL NEURONS. J.E. Marchand and N. Hagino, Dept. of Anatomy, Univ. of Texas Health Sci. Ctr., San Antonio, TX 78284.

Recent immunocytochemical studies have localized axonal fibers and terminals of vasopressinergic neurons in the lateral septal nucleus. In the present study electrophysiological techniques were used to examine the effects of iontophoretically applied vasopressin upon lateral septal neurons.

Field and unitary responses were recorded in the lateral septum of twenty-four urethane anesthetized rats after stimulation of the ipsilateral fimbria. In twelve of these rats, part of the cortex was removed, and stimulating and recording electrodes were positioned visually; in the remaining rats, electrodes were positioned stereotaxically. Iontophoresis electrodes, one or two barrels, were filled with 0.15 mM arginine-vasopressin (AVP) (Boehinger-Mannheim) dissolved in 0.15 M NaCl, pH 4.2, or with the NaCl solution alone, and glued to a glass recording pipette, tip separation 25-50 µm. AVP was ejected as a cation, with currents ranging from 15-80 nA.

Fimbria stimulation (100-200 µsec pulse, 100-400 µA) evoked a negative field potential, 5-8 msec latency, in the lateral septum. In twelve of the twenty-three field responses studied, AVP iontophoresis effected a 30% or greater suppression of the negativity. Iontophoresis of the control solution produced no changes greater than 20% of the control response.

For the unitary studies, fimbria stimulation intensities were adjusted to threshold levels, such that action potentials (5-9 msec latency) arising out of the negative field potential were evoked with an average frequency of 70%. Iontophoresis of AVP onto thirty-eight neurons produced a decrease of fimbria evoked action potentials of 30% or greater in twenty-eight of these cells. No increases were seen. Suppression of evoked responses by AVP developed within an average time of two minutes after iontophoretical application. Iontophoresis of the conrol solution on fourteen neurons produced no changes of the fimbria evoked action potentials greater than 30%.

Recent studies have shown that intracerebroventricularly injected vasopressin can increase theta frequencies of the hippocampus. Since theta frequencies are controlled in part by the reciprocal connections between the septum and hippocampus, the present study indicates that the effects of vasopressin on theta frequencies might be mediated by its ability to modulate hippocampal inputs to the lateral septum and, consequently, the lateral septal input to the medial septum. (Supported by NIH Grant NICHD-1007).

288.23 FETAL BRAIN TRANSPLANTS IN HIPPOCAMPAL-LESIONED RATS: PRELIMINARY FINDINGS. D. P. Kimble, R. BreMiller* and C. R. Stickrod*.

Fragments of posterior telencephalic tissue from fetal rats, aged 15 to 19 days (of gestation) were transplanted into the brains of 20 young adult host rats which had just received small bilateral aspiration lesions of the hippocampal formation. All animals used were from the Charles River Sprague-Dawley strain (CD, random bred). The fetal tissue was placed directly into the cavity produced by the lesion. Both donors and hosts were male. Hosts were from 75-90 days of age at time of operation. Six control animals received bilateral hippocampal lesions but no implant (Group HC). Following uneventful recovery from surgery, all animals were trained and retested twice on a battery of three complex spatial mazes in the Hebb-Williams apparatus across a 6 months period. Following sacrifice, sequential cryostat sections from each brain were stained with cresyl violet and luxol fast blue for Nissl material and myelin, with reduced silver for nerve fibers, and with histofluorescent techniques for monoamines. Examination of the host brains revealed that 6/20 (30%) showed little or no surviving graft tissue (Group I). The remaining 14 (70%) all showed some viable tissue with neurons clearly visible in the transplanted material. These were divided into two groups: Group II (N=7) showed viable transplant tissue, but connections between transplant neuropil and host tissue were absent or marginal. Group III (N=7) showed considerable viable transplant tissue and appeared to share a common neuropil with the host tissue. No behavioral differences were seen on maze acquisition scores between any groups. On the two retests, Group III scored from 13 to 28% fewer errors than any other group, but these were not statistically significant differences. A comparison of Groups II and III (some viable transplant tissue) vs. Groups I and HC (no viable transplant tissue) on the two retention tests narrowly missed the .05 level of significance (Mann-Whitney U=55), with the Groups II and III making an average of 141 total errors (6 mazes) as compared with 163 total errors for Groups I and HC. A rank-order correlation of lesion size and error score among Groups I, II and III was not significant (r_s = -0.13).

288.24 ACQUISITION OF HIPPOCAMPAL SELF-STIMULATION IS FACILITATED BY EITHER KINDLING OR BY A SMALL CONTRALATERAL LESION. Kenneth A. Campbell and N. W. Milgram. Dept. of Psychology, University of Toronto, Scarborough College, West Hill, Ontario, Canada.

We have previously reported that while rats are usually very slow to learn to lever-press for hippocampal (HPC) stimulation, acquisition is markedly facilitated by pretreatment with a program of single daily electrical stimuli to the HPC electrode, leading to a gradual development of convulsions (kindling)(Brain Res.,159,458). Facilitation is also obtained if acquisition for self-stimulation (SS) is tested at the homotopic site contralateral to the kindled site, suggesting that the effect is not due to a known local increase in excitability at the kindled site (Neurosci. Abstr.,5,271). However, to rule out such excitability effects at the kindled site, acquisition must be tested at the contralateral locus, following a lesion to the kindled site.

Forty-eight rats were implanted bilaterally with electrodes in CA3 of the dorsal HPC: 32 rats received a 20-day kindling pretreatment; 16 control rats were handled identically but not stimulated. At the end of the kindling phase, half of the rats in each group received a small lesion in one HPC: half of the kindled rats were lesioned at the kindled site and half at the contralateral site. Lesions were $\leq$ 1 mm dia. and were restricted to CA3. After 4 days, the rats were placed in Skinner boxes and allowed to press the lever in daily 30-min sessions. Two days of responding without reinforcement preceded SS training: 16 rats were tested for SS at the kindled site (IPSILATERAL), 16 were tested for SS contralateral to the kindled site (TRANSFER), and 16 UNKINDLED controls were tested in one HPC — with half of the animals in each group having received a lesion contralateral to the SS site. Thus, the kindled site was lesioned only in 8 of the TRANSFER animals.

Results for the intact groups replicated the previous findings: kindling significantly facilitated acquisition whether SS was tested at the kindled site or at the contralateral site. Lesions of the kindled site did not affect the facilitation of acquisition in animals tested at the contralateral site.

An unanticipated result was that the lesion alone significantly facilitated acquisition for SS at the contralateral site. After the first 2 days of SS testing, the magnitude of the facilitatory effect of the lesion was not significantly different from the facilitatory effect of the kindling pretreatment. Since unreinforced operant rates were unaffected, a non-specific effect on activity is unlikely. Hypotheses involving the removal of inhibition of the SS substrate, denervation supersensitivity, and sprouting of CA3 projections are currently under investigation.

289.1 THE EFFECTS OF CHLORPROMAZINE ON THE BRANCHING PATTERNS OF DIFFERENTIATING CEREBELLAR PURKINJE CELLS. R.S. Hannah*, A.W. Spira* and S.H. Roth, Departments of Anatomy and Pharmacology and Therapeutics, University of Calgary, Calgary, Canada.

The purpose of this study was to examine the effect of chronic administration of chlorpromazine (CPZ) on the dendritic growth of developing Purkinje cells in the rat cerebellum. CPZ (15 mg/Kg) was administered subcutaneously to timed-pregnant Long-Evans rats beginning on day 18 post-coitus. Maternal drug administration was continued daily until the litters were killed at various time intervals up to 21 days postnatal (pn). The cerebella were removed and processed using a conventional rapid Golgi technique. Camera lucida drawings were utilized to quantitate the following parameters - order of branching, length of branches and numbers of branches.

The branches were ranked by order according to the reversed Strahler method. By day 9 pn most of the Purkinje cells from the treated animals had established one additional order of branches over that observed in controls. This condition persisted at three weeks pn.

Comparing the lengths of individual orders of branches demonstrated that by two weeks pn the length of the second and subsequent orders of branches in treated animals were significantly greater ($p < .01$) than in control animals.

The mean number of branches per order with the exception of first order dendrites were significantly reduced ($p < .01$) in the treated groups at two weeks pn. By day 21 pn, there was no significant difference in branch number per order, for orders one to four. However, fifth and higher order branches in the treated groups were significantly increased ($p < .01$) over control values.

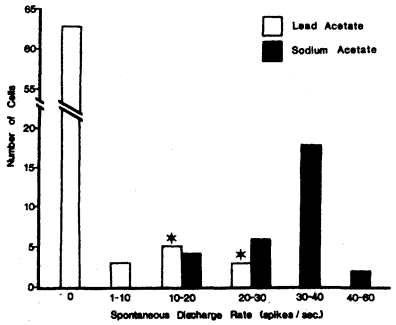
Over the three week pn period examined, the general reaction of Purkinje cells from CPZ treated animals was increased dendritic growth. The increased growth may reflect an attempt by the Purkinje cells to compensate for the depletion in total Purkinje cell number and reduction of dendritic spine densities reported in previous studies from this laboratory.
(Supported by Alberta Mental Health).

289.2 EFFECTS OF NEONATAL THYROID DEFICIENCY ON RAT BRAIN CATECHOLAMINES, SEROTONIN AND PEPTIDE LEVELS. T. Di Paolo*, A. Dupont*, P. Savard*, Y. Mérand*, N. Barden* and J.H. Dussault* (SPON: G. Pelletier), Dept. d'Endocrinologie Moléculaire and Lab. en Biologie du Développement, Centre Hosp. de l'Univ. Laval, Québec G1V 4G2, Canada.

It is well known that neonatal thyroidectomy results in a marked impairement of brain differentiation and leads to the "cretinoid" condition. Since thyroid disorders can influence biochemical specialization of cells as reflected by changes in the development of neurotransmitter receptors in CNS, we studied the effect of neonatal hypothyroidism on the content of norepinephrine (NE), dopamine (DA), serotonin (SER), substance P (SP) and thyrotropin-releasing hormone (TRH) in various brain nuclei dissected by a micro-punch technique. Rats were made hypothyroid by injection of 125 µCi of carrier-free ^{131}I on the first day after birth. Half of this group of animals were subsequently treated with thyroxine (T_4). A third group of littermates were permitted to mature normally. Rats were sacrificed after 45 days by decapitation and subsequently peptide, catecholamine and serotonin contents were measured by RIA, radioenzymatic and LCEC assays, respectively. Few changes in NE and DA distribution were observed in hypothyroid animals. TRH content was decreased by 75% in median eminence, while little increases were seen in medial preoptic nuclei. In contrast to the very localized effects of hypothyroidism on TRH distribution, the effect on central nervous system SP and SER content were both widespread and stimulatory. Indeed, more than half of the 32 brain nuclei assayed displayed significant increase in SP: among them are substantia nigra, nucleus interpeduncularis, nucleus habenulae lateralis, striatum, tractus diagonalis, nuclei of amygdala and preoptic nuclei. Serotonin was increased in nuclei of the raphe, substantia nigra, area ventralis tegmenti, nucleus interpeduncularis, nucleus mamillaris, amygdaloid nuclei, medial forebrain bundle and several hypothalamus nuclei. While thyroxine replacement therapy corrected all the peptides anomalies, T_4 failed to decrease serotonin to control levels in several of the nuclei studied. Evidently, more information is required to establish the influence of neonatal thyroid deficiency on the development of catecholaminergic, serotonergic and peptidergic systems in the CNS. These significant alterations of SP and SER content lend support for an important participation of the substance Pergic system in brain neurotransmission and its possible interrelationship with serotonergic system.

289.3 ELECTROPHYSIOLOGICAL CHANGES OF CEREBELLAR BRAIN GRAFTS EXPOSED TO CHRONIC LOW LEVEL LEAD DURING DEVELOPMENT. L. Olson*†, H. Björklund*†, Å. Seiger*†, J. Marwaha, M.R. Palmer, D.A. Taylor*, R. Freedman*, and B.J. Hoffer*. (SPON: Dr. S.M. Wuerthele.) Depts. of Pharmacology and Psychiatry, Univ. of Colorado Health Sciences Center, Denver, CO 80262; †Dept. of Histology, Karolinska Institutet, Stockholm, Sweden.

The effects of perinatal lead administration on developing rat brain was studied using cerebellar grafts in oculo. Using 1% lead acetate in the drinking water, little change was seen in the survival, growth or histological organization of the graft. In marked contrast, virtually all Purkinje cell spontaneous discharge was absent in these grafts. This was seen even though 4-5 months had elapsed between recording and the cessation of lead treatment. There was no alteration in electrophysiological properties of transplant Purkinje cells from sodium acetate-treated animals.



Moreover, host in situ cerebellar Purkinje cells in both groups of animals discharged normally. These data indicate that lead administration, eliciting blood levels of 450-550 µg/l, produces a long-lasting selective electrophysiological deficit in developing brain in oculo.

Supported by the Swedish Council for Planning and Coordination of Research (79/1027, Al-5/251), the Swedish Medical Research Council (14X-03185, 14P-5867), and USPHS (ES-02011 and MH-00289).

289.4 BRAIN-DAMAGED NEONATAL RATS: RELATIONSHIP BETWEEN DOPAMINE, NOREPINEPHRINE, AND SEROTONIN AND THE DEVELOPMENT OF OPEN-FIELD LOCOMOTOR ACTIVITY. C. Robert Almli, Mark A. Henault,* Craig A. Velozo,* Patricia A. McGinley,* and Edward B. Truitt, Jr.* Washington University School of Medicine, St. Louis, Missouri 63110 and Northeastern Ohio Universities College of Medicine, Rootstown, Ohio 44272.

Brain dopamine depletion in developing rats is suggested as an experimental model of minimal brain dysfunction (Shaywitz et al Science, 1976, 191, 305). The present investigation determined if electrolytic brain damage resulted in developmental activity alteration, and determined the relationship between levels of locomotor activity and brain dopamine (DA), norepinephrine (NE), and serotonin (5-HT).

Forty-eight albino rats were randomly assigned to lateral hypothalamic area (LHA, N=9), ventral tegmental area (VTA, N=11), or control (N=28) groups. At 4 days of age, the LHA and VTA rats sustained bilateral electrolytic destruction.

Body weight and open-field locomotor activity were measured daily from 4 days of age. At 50-60 days of age the straitum and frontal cortex were dissected. Measurement of DA, NE, and 5-HT used Liquid Chromatography with Electrochemical Detection (LC-EC).

LHA rats weighed less than control from the first few days post-surgery. LHA rats displayed higher activity scores than control immediately postsurgery and later during development. Striatal DA (-63%), frontal cortex DA (-82%), and striatal 5-HT (-44%) were depleted. VTA rats did not differ from control for body weight, however, they displayed higher activity scores than control after 16-17 days of age. These rats displayed depletion of striatal DA (-80%) and frontal cortex DA (-87%). A negative correlation (r=-.55) was found for striatal DA and activity level.

Results show that electrolytic lesions of the LHA and VTA of neonatal rats are associated with elevated locomotor activity, and this hyperactivity is associated with DA depletion in striatum and frontal cortex. Hyperactivity was found with (LHA), and without (VTA), body weight reduction. While this supports Shaywitz, it is interesting to note the association of early hyperactivity for LHA rats with striatal 5-HT depletion; and the near-total (>90% depletion) striatal DA depletion for VTA rats with low locomotor activity.

289.5 CHRONIC HALOPERIDOL-INDUCED HYPERACTIVITY IN DEVELOPING RATS: EFFECTS OF LITHIUM AND STIMULANTS. J.T. Concannon and M.D. Schechter. Dept. Pharmacology, Northeastern Ohio Universities College of Medicine, Rootstown, Ohio 44272.

Chronic administration of neuroleptic drugs has been shown to produce behavioral hyperactivity in neonatal and adult rats and an augmented response to catecholaminergic agonists. Supposedly, these effects result from chronic blockade of central dopamine (DA) receptors which is inevitably followed by DA receptor supersensitivity, as indexed by an increased number of DA receptors. In addition, neuroleptic-induced supersensitivity is generally reduced in adult rats by coadministration of lithium with haloperidol, whether the supersensitivity has a pre- or post-synaptic origin.

In the present experimentation, we investigated if chronic neonatal administration of haloperidol would produce hyperactivity in developing rats in a manner mimicking the human situation. More specifically, we were concerned with whether this model would mirror the temporal onset, duration, and offset of the hyperactivity in humans and whether hyperactivity could be prevented by lithium coadministration or be attenuated by effective doses of psychostimulants. To test these notions, neonatal rats were chronically administered haloperidol subcutaneously at doses of 0, 0.25 or 2.5 mg/kg daily from days 5 to 14 of life. Furthermore, one-half of these pups were raised by mothers exposed to a lithium diet (Pert et al, Science, 201:171, 1978) while the other half were raised by normal-diet mothers. Pup activity was observed using a time-sampling technique at 15, 20, 25 and 30 days of age, 30 minutes after an intraperitoneal injection of either saline, d-amphetamine (0.20 or 0.50 mg/kg), or methylphenidate (0.20 mg/kg) Results showed that high-dose haloperidol produced hyperactivity at 25 and 30 days of age in pups of mothers receiving the normal diet. This hyperactivity was manifest as a failure of the haloperidol group to decrease their activity in early adulthood. Surprisingly, not only did administration of lithium not prevent hyperactivity in haloperidol-treated pups, but rather it too produced overactivity in developing pups. Lastly, neither stimulant was able to reduce activity in haloperidol-or lithium-treated over active rats, although the stimulants did increase activity in control pups receiving neither haloperidol nor lithium. These results cast considerable doubt upon the notion that chronic neuroleptic-induced DA receptor blockade provides a viable animal model of the human hyperkinetic syndrome, since the temporal course is not sufficiently similar to humans and since clinically-useful drug regimens are virtually ineffective in reducing hyperactivity. (Supported by NIMH grant No. 33636-02).

289.6 Neonatal Kepone Exposure Impairs Learning/Retention Abilities in the Rat. K.L. Unger*, C.F. Mactutus, and H.A. Tilson (SPON: C.L. Mitchell). Lab. of Behav. Neurol. Toxicol., NIEHS, Research Triangle Park, NC 27709.

The neurobehavioral consequences of Kepone in animals have been studied following adult, prenatal, and combined pre- and postnatal exposures. The present study examined the consequences of a neonatal exposure to Kepone, during a period of sexual differentiation of the brain, on neurobehavioral development prior to weaning. Learning/retention abilities were evaluated as the use of "cognitive" behaviors are particularly sensitive indices of neurotoxicity (Neurosci. Abst. 6:740, 1980).

Neonatal Fischer 344 rats were given a single s.c. injection (20 µl) of either dimethylsulfoxide (DMSO) or 1 mg/pup of Kepone dissolved in DMSO on postnatal day 4. Body weights were slightly, but significantly, depressed for Kepone exposed males and females by day 14 (11% and 10%, respectively) and remained so through 21 days of age (8%). Other neurotoxic effects included a delay in eye opening for the male pups and precocial vaginal opening for the female pups. Passive avoidance acquisition occurred within 2 trials for all pups, but a significantly greater proportion of Kepone exposed pups required the second trial to attain a 60 sec response withholding criteria (12/48 Kepone vs 3/47 DMSO). This impairment was most pronounced, but not restricted to, the male pups. Independent groups tested 24 or 72 hr post-training indicated a significant loss of memory over time for the Kepone relative to the DMSO males, with no effect in the females. When the male pups originally tested at 24 hr were retested at 72 hr significant extinction had occurred for the DMSO pups, but not for the Kepone pups. This latter "protection of memory" was attributed to increased emotionality expressed by a freezing response as both plasma and adrenal steroid levels were elevated (39% and 45%, respectively) in Kepone relative to DMSO pups sacrificed 5 min after testing.

The highlights of the present data are 1) a significant deficit in learning/retention ability consequent to neonatal Kepone exposure, and 2) the greater resistance of female over male pups to the neurobiological consequences of Kepone exposure on nonsexual behaviors. This latter conclusion stands in marked contrast to the well known alterations induced by the estrogenic qualities of Kepone on female sexual development and behavior.

289.7 GENE EXPRESSION IN BEHAVIORAL SYSTEMS. B. E. Ginsburg, Dept. of Biobehavioral Sciences, Univ. of Connecticut, Storrs, CT 06268.

Complex behavioral phenotypes have often been shown to have a simple genetic basis. In some instances, this is demonstrably because a structural gene produces a network of effects, all radiating from a single defect produced at a particular time in development (e.g., Grüneberg's "pedigrees of causes"). In others, the genetic differences appear to act as regulators of the expression of gene assemblies, either by shifting thresholds for their expression, or through selective de-repression as, for example, by hormonal mechanisms. Data will be presented from three categories in which the behavioral expression of a complex phenotype is apparently controlled by means of genetic regulation of alternative gene assemblies that are physically present, but depend upon a triggering mechanism for selective activation. These are: brain laterality studies in humans, where a single genetic threshold model has been investigated; heterosomal determination of thresholds to environmental input in the expression of aggression in mice; and the selective expression of species-specific threat behavior in coyote X beagle hybrids. In the latter, the preweaning expression of the behavioral pattern appears to be genetically independent of its post-pubertal expression, which may be hormone dependent. Implications of these data and concepts will be discussed in relation to canid speciation, where the karyotypes, serum proteins, red cell antigens, and tissue antigens are virtually identical, yet the phenotypic differences are profound enough to have resulted in speciation.

This research was supported by USPHS grants MH28021 and MH35908, and by grants from The Harry Frank Guggenheim Foundation and the Connecticut Research Foundation.

289.8 TISSUE-SPECIFIC REGULATION OF SOME GLYCOSIDASES IN CEREBRAL CORTEX AND CEREBELLUM OF WILDTYPE AND MUTANT MICE. W. Wille* and E. Trenkner. Inst. F. Genetik, Univ. of Cologne, Fed. Rep. Germany and Dept. of Pharmacology, NYU Med. Ctr., New York 10016.

Cell surface carbohydrate (CHO) patterns change during development of normal mouse cerebellum (1). These changes are significantly altered in the neurological mutant staggerer (sg/sg) (2). The membrane bound neuraminidase, possibly involved in cell surface CHO-metabolism, is also regulated during development of cerebral cortex and cerebellum; peak activities are observed at postnatal day 3 (P3) and P27 in normal cerebrum and at P3 alone in cerebellum. However, sg/sg cerebellum a comparable peak activity is delayed until P27 and this might be responsible for the aberrant patern of surface CHO also observed (3).

In this study 5 other glycosidase activities were determined during development of cerebellum and cerebrum of +/+ (C57 Bl), sg/sg and weaver (wv/wv) mice. α-glucosidase (pH 3.7) showed no obvious regulation while α glucosidase (pH 6.0) and β-galactosidase decrease continuously with age in both tissues and all strains tested. Significant differences, however, were expressed in N-acetyl-β-hexosaminidase (NAc-β-glucosaminidase and NAc-β-galactosaminidase) in cerebellum compared to cerebrum. While this enzyme in +/+ cerebellum showed a brief peak of activity at P7, cerebral activity increased approx. 2.5 fold between P3 and P16 and maintained this high activity level through adulthood. In sg/sg cerebellum activity of this enzyme was abnormal and corresponded more to +/+ and sg/sg cerebrum than to +/+ cerebellum. Strikingly in wv/wv, no such variation from +/+ was observed even though wv/wv granule cells degenerate to the same extent as in sg/sg.

We conclude that 1. of the enzymes studied only NAc- -hexosaminidase is regulated, 2. that the age-dependent pattern of regulation of this enzyme is different from cerebrum to cerebellum, and 3. that the cerebellum-specific regulation of this enzyme is significantly altered sg/sg but not in wv/wv.

(1) Trenkner, E. & Sarkar, S. (1977) J. Supramolec. Struc. 6:465.
(2) Trenkner, E. (1979) Nature 277: 566.
(3) Wille, W. & Trenkner, E. (1981) J. Neurochem in press.
Supported by National Science Foundation grant # BNS - 7922673 to E.T.

289.9 CORTICAL ORGANIZATION AND CYTOLOGICAL ABNORMALITIES OBSERVED IN SCLEROSIS TUBEROSA. A Golgi Study. R. Armada*, J. Espinosa* and J.P. Machado-Salas. Div. de Neurociencias, Neuromorfología, ENEP Iztacala, UNAM. and Div. de Patología, Depto. Inv. Cient. CMN. IMSS. México D.F

It shall be described the structural changes that were observed in a cortical biopsy from a case of sclerosis tuberosa. The tissue was taken from a 12 year-old female patient, who showed intractable seizures and severe mental retardation. Her father and two other siblings also showed clinical features of epiloia. The biopsy was performed, under written permission, for diagnostic, pronostic and counseling purposes.

Thus far, it has been established a close correlation between the degree of maturation of normal psycho motor functions and the anatomical complexity shown by the neuropile; nevertheless, the opposite has not yet been demonstrated, i.e.: that a given degree of mental retardation has, as a counterpart, a given degree of abnormal neuronal ciruitry. The data here shown aim to stregth this hypothesis.

The tissue was promptly obtained and fixed, and processed with the rapid-Golgi modification. Our observations are: a) Astrocytic proliferation at the superficial and deeper layers of a rather compact cerebral cortex. b) Presence of atypical neurons, with few branches, without spines, and somatodendritic distortion. These cells were spreaded throughout the cortical layers. c) Unusual neuroglial contacts, and d) Bizarre and and abnormal patterns shown by dendritic spines, from long, hairy-ones up to "megaspines".

Our findings have shown: I. The presence of abnormally developed nerve-cells in other places than "tuberes or heterotopies. II. The presence of peculiar neural and glial anatomical abnormalities, and, in some cases, interactions, which may bear some functional significance. And, III. A putative correlation between structure and function, under pathological conditions.

289.10 MORPHOMETRIC ALTERATIONS IN THE HIPPOCAMPAL FORMATION OF DEVELOPING AND ADULT RATS FOLLOWING EARLY POSTNATAL LEAD EXPOSURE. J.B. Campbell*, D.E. Woolley*, V.K. Vijayan and S.R. Overmann* (SPON: G. P. Moberg). Dept. of Animal Physiology, Univ. of California, Davis, CA 95616.

Neurogenesis and differentiation in the hippocampal formation in rodents and man continue during the early postnatal period, and may be influenced by environmental toxicants. Hippocampal malfunction is thought to underlie symptoms of childhood lead excephalopathy such as seizures and behavioral changes. Therefore we examined the structure of the hippocampal formation by light microscopy and analyzed several subfields of the suprapyramidal mossy fiber zone by electron microscopy in lead-exposed and control rats at 15 days and 3 months of age.

In this study 0.2% lead acetate (1090 µg lead/g solution) was substituted for drinking water of dams from the day of parturition until weaning at postnatal day 20 (P20). Litters were culled to 6 females on the day of birth and were maintained at 4-6 pups until weaning. A single pup from 8-10 litters of each treatment group was perfused with aldehydes at P15 and between P90 and P97 for light and electron microscopic analysis of the hippocampal formation.

Lead-exposed rats showed no weight reduction, paraplegia, gross vascular damage, pyknosis or other signs of encephalopathy or overt cytotoxicity. At P15 the areas of some neuropil layers in the dentate gyrus were reduced and the number of neurons/unit area in some areas of the hippocampal formation was increased. In these same regions the number of neurons/unit area in lead-exposed rats at P90-97 was reduced. At P90-97 the area of these neuropil layers in lead-exposed rats was equal to that in controls. At both ages a part of the suprapyramidal mossy fiber zone which is supplied by the infrapyramidal limb of the dentate gyrus showed fewer mossy fiber synaptic profiles per unit area in lead-treated than in control rats.

These data show that neonatal exposure to low levels of lead caused both transitory and long-term alterations in hippocampal neuropil. These effects were limited to late-developing regions, especially those related to the infrapyramidal limb of the dentate gyrus. (Supported by NIH grant #ES 01503.

289.11 NORMALIZATION OF WEIGHTS OF VARIOUS BRAIN REGIONS AFTER TREATMENT OF GRAFT-VERSUS-HOST-DISEASE (GVHD). S. Duncan, J.R. Head, and W.S.T. Griffin (SPON: R.M. Stewart). Dept. of Cell Biology, The Univ. Tx. Hlth. Sci. Ctr., Dallas, TX 75235.

The potential for regrowth and plasticity of the CNS was investigated in 14-day-old rats that received effective immunotherapy for a neonatally induced immunological syndrome, graft-versus-host-disease (GVHD). Previously, we showed that there are a number of alterations in cerebellar development in this disease which is characterized by, among other symptoms, an ataxic gait. For example, cerebellar weight, DNA, RNA and protein contents, DNA and RNA synthesis and the relative abundance of specific messenger RNAs are all significantly altered by GVHD. Further, we have shown that each of these parameters of cerebellar growth are amenable to regeneration. Here, we hypothesized that GVHD would similarly effect the growth of other brain areas. The experimental groups were as follows: One group contained one-third of the pups and received no treatment at birth (C). The other group contained two-thirds of the pups, and each of these pups was grafted by an intravenous injection of 20×10^6 lymph node cells from an allogeneic donor, viz., a DA adult rat (E). This latter group was further subdivided so that one-half of these pups received 3 injections, on postnatal days 11, 12 and 13, of syngeneic serum containing high titers of antibodies to their allogeneic graft, i.e., FI anti-DA serum (I). To test our hypothesis, we weighed hypothalamus, thalamus, pons, midbrain, cortex and hippocampus expecting that the latter two, because of their continued postnatal growth, might be particularly susceptible to a growth retarding disease such as GVHD. Further, we sought to determine whether or not treatment of GVHD with an alloantiserum, which reverses deleterious effects on neuronal cell growth in cerebellum, would be effective in reversing GVHD-induced cerebral growth retardation. We found that similar to cerebellar weight changes, both cortex and hippocampus weights were significantly less in 14 day old diseased animals and that treatment with alloantiserum begun at day 11 permitted a significant weight gain in these areas. It will be interesting to study the concomitant changes in cell acquisition and function which likely accompany the weight changes noted in cortex and hippocampus during and after GVHD.

This work was supported by NIH AI 14663, AI 10678, and a grant from the Biological Humanics Foundation.

289.12 NORMALIZATION OF DNA SYNTHESIS AFTER TREATMENT OF GRAFT-VERSUS-HOST-DISEASE (GVHD). C. Pevetoe, J.R. Head and W.S.T. Griffin. Dept. Cell Biology, The Univ. Tx. Hlth. Sci. Ctr., Dallas, TX 75235.

Previously, we showed that as early as postnatal day 11 an immunological disease (GVHD) induced by grafting allogeneic lymph node cells (LNC) into an immunoincompetent neonatal rat, significantly decreases cerebellar histogenesis, i.e., DNA synthesis and the number of newly formed neurons. However, subsequent to immunotherapy, there is a reversal of the deleterious, GVHD-induced alterations. Immunotherapy involves injecting the diseased rats on postnatal days 11, 12 and 13 with alloantiserum specifically directed against the grafted LNC. In order to determine whether or not the time course of the accumulation of DNA was affected in a reversible way by GVHD, we utilized autoradiographic techniques which showed that there were differences not only in the number of cells entering the synthesis phase (S) of the cell cycle, but there was also less synthesis per cell in rats with GVHD. Conversely, alloantiserum treated rats were synthesizing cerebellar DNA more nearly like littermate controls. On postnatal day 14, diseased, serum treated and control littermates were injected with ^3H thymidine and fifteen minutes later, the cerebella were excised and processed. The external granular layer (EGL) at the midsagittal section in the fissure between VIa and VIb was examined in each of the animals. The presence of 6 or more autoradiographic grains above a cell was used to denote active synthesis of DNA. A segment of EGL 0.72 mm in length was searched for labeled cells. GVHD rats had few labeled cells ($\bar{x}=3.8 \pm 2.3$) and the number of grains per cell was low ($\bar{x}=6.6 \pm 0.6$) compared to serum treated rats which had a greater number of labeled cells ($\bar{x}=26 \pm 8.2$) and grains per cell ($\bar{x}=7.4 \pm 0.2$). The control group had the greatest number of labeled cells ($\bar{x}=43 \pm 3.8$) and the greatest number of grains per cell ($\bar{x}=9.5 \pm 0.2$). These results indicate that the decreases in total DNA content and synthesis previously noted in GVHD rats is due to a decreased ability to enter S phase coupled with a slowing of the cell cycle shown by less incorporation of label into the DNA of individual mitotically active EGL cells. Treatment with alloantiserum allowed more cells to enter synthesis, and the cell cycle returned to near the control level. We, therefore, conclude that a systemic immunological process which does not result in cerebellar inflammation can slow the cell cycle. Conversely, alloantiserum treatment reverses the deleterious effect, thus attesting to the plastic nature of neuronal cell acquisition in the developing cerebellum.

Supported by NIH AI14663 and the Biological Humanics Foundation.

289.13 NORMALIZATION OF CEREBELLAR RNA SYNTHESIS AND TRANSLATION AFTER TREATMENT OF GRAFT-VERSUS-HOST DISEASE (GVHD). W.S.T. Griffin, J. Snider and M. Morrison. Depts. of Cell Biology and Neurology, The Univ. Tx. Hlth. Sci. Ctr., Dallas, TX 75235.

Analysis of 2d gels (two dimensional gel electrophoresis) of specific abundant proteins synthesized both in vivo and in vitro by rat cerebellar RNAs, shows that the amounts of such messenger RNAs (mRNAs) and their protein products vary with age (2 to 90 days). In addition, the quantities of certain of these mRNAs and proteins, and, indeed the presence of some, are related to the immunological status of the individual. For example, we have shown that neonatal rats with graft-versus-host-disease (GVHD) 1) synthesize significantly less cerebellar RNA, 2) have RNA which is less translationally active and 3) have changes in the proportions of different mRNAs including the induction of one coding for protein "r" which is neither present in control cerebella at any age nor in other brain regions. This systemic disease, induced by intravenous injection of 20 X 10^6 allogeneic (DA) lymph node cells into a newborn Fischer (FI) rat, is characterized by a number of systemic manifestations including an ataxic gait and dramatic alterations in cerebellar cell acquisition and function. Here, we report on the ability of the cerebellum to recover from the previously-reported, GVHD-induced changes in the synthesis of total RNAs and the relative abundance of specific mRNAs. In order to halt the disease, 11-day-old diseased animals were injected with 0.2 ml of hyperimmune serum (FI anti DA) daily for 3 days. By the eleventh postnatal day there are significant decreases in DNA and RNA synthesis and in the number of newly formed neurons. On postnatal day 14, cytoplasmic RNAs were isolated from the cerebella of such serum treated animals, their diseased littermates which were not treated with serum, and littermate controls. After the three days of serum treatment, DNA and RNA synthesis and the acquisition of neurons had returned to near control levels. Comparison, by 2d gel analysis, of in vitro synthesized mRNA translation products showed that "r" was not synthesized by cerebellar RNAs isolated from serum treated animals, indicating that the disease is arrested at the molecular level. This study supports the idea that there are periods in cerebellar development during which deleterious effects can be reversed and suggests that such reversal is accompanied by alterations in the levels of specific mRNAs and their translation products.

Support for this work was provided by NIH AI 14663 and grants from the Leland Fikes Foundation and the Biological Humanics Foundation.

289.14 PERSISTENT ALTERATIONS IN BRAIN MONOAMINES FOLLOWING FRONTAL CORTEX LESIONS IN NEONATAL AND ADULT RATS. M. K. Shellenberger and A. S. Kimes, Dept. of Pharmacology & Smith Center for Mental Retardation, Kans. Univ. Med. Ctr., Kansas City, Ks. 66103.

While there exists a fairly large literature concerning the persistent behavioral and morphologic consequences of perinatal brain trauma, there is little information concerning the residual effects on neurotransmitter systems. This study compares the long-term effects of trauma in neonates and adults.

Adult Sprague-Dawley rats were purchased at 12 wks of age and maintained until fully adult (16-20 wks). Infants were bred in our animal facilities. Litters were cross-fostered and reduced to 8 pups within 48 hrs. At 5 days pups were randomly assigned to one of the following treatments: 1) handled but untreated, 2) anesthetized and sham operated, 3) anesthetized and the cortex exposed from the coronal suture to the olfactory bulbs. Frontal cortex was removed bilaterally leaving the poles, olfactory structures and saggital sinus intact. Groups of adult rats were treated in an analogous fashion. Adults were sacrificed 6 to 8 wks later while infants were raised to adulthood and sacrificed at 16 to 20 wks. The brains were sampled and assays performed for dopamine (DA), norepinephrine (NE) and 5-hydroxytryptamine (5-HT).

Neonatal sham surgery caused an increase in cortical DA and decreases in NE in the brainstem regions. These effects were greater in males. The lesion also resulted in a decrease in midbrain NE and in addition significantly decreased striatal DA. In females this was accompanied by increases in both NE and 5-HT. Males were found to have increases in NE and 5-HT in the pons-medulla. In the areas of cortex posterior and lateral to the lesion, 5-HT increased moderately while DA increased several fold.

The sham procedures produced permanent reductions in 5-HT in adult cortex and brainstem. Lesioning produced a decline in striatal DA, as in infants, and decreases in brainstem NE. Lesioning increased 5-HT in males in all areas. Increases in striatum of both sexes were particularly remarkable.

There are marked differences between the manner in which the infant and adult brain monoamine systems respond to trauma and some sexual dimorphism. The effects resemble those seen with carbon monoxide-induced hypoxia. Further exploration of these similarities may shed some light on the common pathways by which various forms of perinatal trauma produce similar behavioral abnormalities. Supported by USPHS Grants MH-27739 and BRSG-S07 RR05373.

289.15 LEAD EFFECTS ON NEONATAL ACTIVITY AND HIPPOCAMPAL AFTERDISCHARGE CHARACTERISTICS. M. McCarren* and C.U. Eccles* (SPON: N. Khazan). Dept. of Pharmacology and Toxicology, Univ. of Md. Sch. of Pharmacy, Baltimore, MD 21201.

Attempts have been made to link neural deficits in children with chronic low level lead (Pb) poisoning. The present study was designed to expose developing rats to low Pb levels and assess spontaneous locomotor activity during that stage of development in which activity levels are characterized by a maturational peak at 15 days of age. An additional approach to studying the neurotoxic effects of Pb is to examine the functional integrity of the hippocampus, a limbic structure that is known to be vulnerable to damage by several neurotoxins, including Pb. We hypothesized that hippocampal abnormalities in rats exposed to Pb during development could be manifested as alterations in the characteristics of the electrically induced hippocampal afterdischarges (ADs).

Sprague-Dawley rats were exposed postnatally to Pb indirectly via the dam's milk. Upon parturition, litters were culled to 6, and water containing 2.5 mg/ml (HL) or 1.0 mg/ml (LL) lead acetate was substituted as drinking water. Controls received a solution of sodium acetate. Such exposure continued until weaning at 22 days. On days 12, 14, 16, 18, 20, and 22, locomotor activity of each pup was recorded for 30 min in a photoactometer. At 3 mos. of age, adult males were implanted with hippocampal and cortical electrodes for chronic electrical stimulation and recording. Following recovery from surgery, AD characteristics produced by stimulation at 25% above threshold were evaluated first in the drug-free state and following treatment with 0, 10, 20, and 40 mg/kg phenytoin.

HL pups exhibited heightened activity on all test days, relative to controls or LL, although a maturational peak was still evident on day 16. In contrast, LL animals demonstrated hypoactivity at 16 days, but by 18 days the level exceeded that of controls. Activity subsequently declined but remained slightly higher than controls. This delay in peak activity indicates that the age of testing is indeed critical to the effect observed. Preliminary results in AD testing indicate that in the absence of phenytoin, Pb effects are seen as subtle changes, most obvious in the cortical response to hippocampal stimulation. The use of phenytoin served to increase the sensitivity of the model. In all groups, phenytoin blocked the AD in a dose-dependent manner. However, in animals in which an AD did occur, Pb exposed rats responded with a dose-related increase in duration of the AD, while in control rats durations remained fairly stable.

289.16 HIPPOCAMPAL AFTERDISCHARGE CHARACTERISTICS IN ADULT RATS PRENATALLY EXPOSED TO METHYLMERCURY. C.U. Eccles*, K. Rauen* and M. McCarren* (SPON: G. Young). Dept. of Pharmacology and Toxicology, Univ. of Maryland Sch. of Pharmacy, Baltimore, MD 21201.

The hippocampal formation is known to be sensitive to the effects of a variety of neurotoxic agents. In the present study, the characteristics of hippocampal afterdischarges (AD) were examined in adult rats exposed to a low dose of methylmercury chloride (CH_3HgCl) during a period of rapid growth and development of the hippocampus, i.e., the third week of gestation.

On day 15 of gestation, 4 pregnant Sprague-Dawley rats were given 5 mg/kg CH_3Hg as CH_3HgCl by means of intragastric intubation. Vehicle alone was given to 4 rats that served as controls. On postnatal day 1 litters were culled to a standard size. At 16 weeks of age, 10 males from each treatment group were prepared for chronic recording of the hippocampal and cortical EEG.

After a two-week recovery period, AD thresholds were determined by the method described by Pinel (Pinel et al, Epilepsia 17:197-206, 1976). A second AD at 3 times threshold intensity was produced 20 minutes after the threshold AD. EEG records were scored according to rules previously described (Dyer et al, Neurobehav. Tox. 1:5-19, 1979). The following parameters were measured: AD threshold, AD durations in cortex and hippocampus, latency to rebound AD in hippocampus, and duration of rebound AD. One month after the initial testing period, a baseline AD was produced at 3 times threshold intensity followed by administration of subsequent stimuli at varying intervals in order to obtain a measure of the duration of the postictal period during which the hippocampus is refractory to additional AD's.

AD thresholds, cortical AD duration, and hippocampal AD duration were not significantly different in control and experimental animals. The duration of the rebound AD was longer in experimental group after the threshold AD but not after the AD produced at 3 times threshold. In both the threshold AD and the second AD the latency to rebound AD was increased in the CH_3Hg-treated rats (116.4 vs. 84.5 sec; 120.8 vs. 81.9 sec). In the second phase of testing, the postictal refractory period was found to be significantly longer in the CH_3Hg rats as compared to control (13.3 vs. 4.9 min).

The origin, significance, and factors that influence the onset of the rebound AD are unknown. Since existing evidence indicates that it is associated, at least in a temporal manner, with recovery from the postictal depression, the increased latency to the rebound AD in the CH_3Hg-exposed rats may reflect a delayed recovery process in this group. The increased length of the postictal refractory period in experimental animals provides additional support for this hypothesis.

289.17 ETHANOL VS. TERTIARY BUTANOL INDUCED MICROCEPHALY IN NEONATAL RATS: DIFFERENTIAL EFFECTS ON BRAIN COMPONENTS. K. GRANT*, H. SAMSON*, J. DIAZ (SPON: G. CLARK). DEPT. OF PSYCHOLOGY, UNIV. OF WASHINGTON, SEATTLE, WA 98105.

A prominent diagnostic feature of the fetal alcohol syndrome (FAS) is microcephaly. However, neither the mechanisms by which ethanol interferes with brain development nor the brain components effected are known. Using an artificial rearing procedure, ethanol administered during the brain growth spurt has been shown to selectively impair brain development in neonatal rats. Tertiary butanol administered under the same conditions also produces microcephaly. Tertiary butanol is another short chain alcohol, not metabolized by the same enzyme systems as ethanol, and is considered to be a metabolic control for the metabolites of ethanol oxidation. The tertiary butanol was given in a dose equivalent to that of ethanol based upon their membrane to buffer partition coefficients. Thus the relative contribution of membrane solubilizing agents on brain development was explored.

To ascertain whether the microcephaly produced by both alcohols represent similar deficits in brain parameters, biochemical assays of several brain indices were performed. Ten days after alcohol exposure the animals were sacrificed, their brains removed, weighed, and sectioned into cerebral and cerebellar portions by a trans-collicular cut. Each brain sample was assayed for total DNA, protein and cholesterol. Total DNA was used as an index of the amount of cells present and DNA per tissue weight represented cellular density. Total protein per total DNA was used as an average protein per cell measure. Finally, total cholesterol to total DNA represented the degree of cellular arborization and myelination.

Compared to controls, neither ethanol nor tertiary butanol appeared to inhibit cellular proliferation in the cerebral samples. However, average protein per cell was decreased in the ethanol group. The DNA levels of the cerebellar samples were decreased in both ethanol and tertiary butanol groups, with no differences in average protein per cell. Compared to either the control or tertiary butanol brain samples, the ethanol group had lower levels of cholesterol per cell in both the cerebrum and the cerebellum. The results indicate that although both alcohols induce microcephaly, they differentially effect brain development. Therefore, FAS microcephaly may be a combination of both the membrane solubilizing and metabolic consequences of ethanol exposure.

289.18 HYPOXIA AND STUNTED PURKINJE CELL DEVELOPMENT: GOLGI AND ELECTRON MICROSCOPIC STUDIES. M.C. Yu*; D. Spero* and F. Hackett* (SPON: H. Edinger). Dept. of Anatomy, New Jersey Medical School, Newark, NJ 07103

We reported previously that hypoxia (10% O_2; 90% N_2) imposed on neonatal rats from postnatal day 5 to day 11 consecutively caused severe stunting in cerebellar development (Yu, M.C. and Yu, W.H.A., Exp. Neurol. 70:652-664, 1980). The reduction in cerebellar size was reflected at day 12 in a smaller number of proliferative and postmitotic cells in the external granular layer (EGL) and a reduction in the width of the molecular layer. At day 22, the cerebellum of the control rat (kept in ambient air) had attained the adult cytoarchitecture but the development of the hypoxic rat cerebellum was still lagging, with the persistence of the EGL cells incorporating tritiated thymidine. The present study was to examine morphologically the effect of hypoxia on the maturation of the Purkinje cells, and the effect of a smaller population of microneurons on the development of Purkinje cell dendritic field. Electron microscopic and Golgi impregnation preparations were made from the control and hypoxic rats at postnatal days 12 and 22, respectively.

At day 12 the Purkinje cells of the hypoxic rats showed a preponderance of free ribosomes and few Nissl bodies. In contrast the Purkinje cell perikarya of the control rats showed well developed Nissl bodies and scattered free ribosomes. Golgi preparations revealed greatly reduced dendritic arborization and spine formation in the Purkinje cells of the hypoxic rats when compared with the control. At day 22, the Purkinje cell perikarya of the hypoxic rats possessed all the cell organelles; however, the number of Nissl bodies was much less than that of the control. Golgi preparations revealed that the field of dendritic branching of the hypoxic rats continued to lag behind the age-matched control. The present studies, in conjunction with that reported previously, indicated that the reduction in size of the cerebellum during the 12- and 22-day periods was the result of hypoxic inhibition on the proliferative activity of the stem cells in the EGL, and on the stunting of development of neuronal processes of the Purkinje cells.

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289.19 VISUAL SYSTEM DAMAGE, AND AUDITORY AND ELECTRO-TACTILE STARTLE-REFLEX IMPAIRMENTS IN ADULT RATS FOLLOWING NEONATAL VIRAL INFECTION. A.B. Campo*[1], J.R. Ison[2,3], J.L. Orr*[3], S.J. Mestman*[1], D.A. Grover*[4], A.A. Monjan[5], M. del Cerro[1,4]. 1) Center for Brain Research, Univ. of Rochester Med. Ctr., Rochester, NY 14642. 2) Dept. of Psychology, Univ. of Rochester, Rochester, NY 14627. 3) Dept. of Radiation Biology and Biophysics, Univ. of Rochester Med. Ctr., Rochester, NY 14642. 4) Dept. of Ophthalmology, Univ. of Rochester Med. Ctr., Rochester, NY 14642. 5) Dept. of Epidemiology, School of Hygiene and Public Health, The Johns Hopkins Univ., Baltimore, MD 21205.

Albino and hooded rats infected at birth with lymphocytic choriomeningitis virus (LCMV) develop a cell-mediated immune retinitis, with well documented clinical, electroretinographic, and histological damage to the retina. This begins about 2 weeks after infection and progresses relentlessly throughout the life of the animal (del Cerro et al., in review). To further evaluate the functional status of their nervous system we tested their responsiveness to a graded series of acoustic stimuli (85 to 125 dB, 10 kHz tones, 20 msec in duration) and electro-tactile stimuli (.1 to .9 mAmp, single square wave shocks, 20 msec in duration). Five levels of intensity in intermixed order (a total of 50 trials in each modality) were presented at 20 sec intervals. The force of the animal's reaction was detected with an accelerometer, its output amplified and integrated over a 100 msec period immediately following the stimulus. Normal animals (n=5) showed a monotone increasing response to these stimuli; infected animals (n=5) showed a small and insignificant increase in responding, and their overall level was less than 10% that of the normal subjects. The locus of this reflex impairment (whether, e.g., it is afferent, central integrative, or efferent) is currently under investigation.

It is apparent that perinatal LCMV infection produces deficits involving both visual and nonvisual neural systems. The LCMV infection is a useful and important model for the study of neural-immune system interactions in the developing neonate.

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290.1 TOPOGRAPHIC ANATOMY OF TIBIALIS ANTERIOR (TA) MOTONEURONS (MNs) AND THEIR MUSCLE FIBERS IN CAT. A.R. Iliya* and R.P. Dum, Dept. Neurosurgery, Upstate Medical Center, Syracuse, NY 13210.

Burke et al. (J. Neurophysiol. 40:667, 1977) suggested that a topographic relation may exist between the location of a MN within its motor nucleus and the position of its innervated muscle fibers within the muscle. We tested this hypothesis directly in TA muscle. By selective sectioning of either the anterior (ANT) or posterior (POST) branch of the TA nerve, we determined: 1) the relative nuclear location of the remaining branch's MNs in comparison to the contralateral nucleus by using bilateral intramuscular injection of horseradish peroxidase and 2) the intramuscular position of each branch's innervated muscle fibers by using glycogen depletion.

The labeled MNs occupied bilaterally symmetrical fusiform columns (0.5 mm dia; 6.3 $\pm$ 0.6 mm length) within the L6-L7 ventral horn. Although MNs of the ANT or POST branch intermingled within the same spinal cord region, the MNs of the POST branch in two cats were relatively segregated toward the caudal part of the nucleus in contrast to MNs of the ANT branch which had a rostral bias. This trend was not evident in the third cat whose POST branch contained a much higher proportion of the total MN population (48% vs. 26% & 33%). The distribution of soma diameters of labeled MNs was bimodal, allowing a presumptive division into γ(small) andα(large) MNs. On the average, the POST branch contained 51% of the γ MNs but only 26% of theα MNs.

We determined that the POST branch innervated posteriomedial TA while the ANT innervated anterolateral TA with only a little intermingling of innervation at the border. Hence the muscle fibers innervated by each branch were strictly segregated within each muscle. Using myofibrillar ATPase staining at pH 4.4, we determined the proportions of the three fiber types (I, IIA, IIB) for each branch. The POST branch tended to innervate a high proportion of oxidative muscle fibers (54% types I and IIA) while the ANT branch innervated a high proportion of glycolytic fibers (62% type IIB). Since the POST branch contained 26% of theα MNs and innervated roughly 20% of all TA muscle fibers, the POST branch MNs must, on the average, have lower innervation ratios (hence smaller muscle units) than their ANT branch counterparts.

In conclusion, we observed only a weak topography between the nuclear location of MNs and the intramuscular location of their innervated muscle fibers. Thus, the clear segregation of half the γ MNs and ofα MNs innervating mostly small, oxidative muscle units into the POST nerve which innervated a discrete region of the TA muscle cannot be explained solely on the basis of the anatomical position of these MNs within the TA nucleus.

290.2 LOCALIZATION AND CELL SIZE IN SELECTED MOTONEURON POOLS IN THE OPOSSUM LUMBOSACRAL SPINAL CORD. D.A. Thomas*, J.L. Culberson*. (SPON: G.W. Patrick). Dept. of Anatomy, West Virginia University, Morgantown, West Virginia 26506.

The location of motoneurons that supply specific muscles or muscle groups has been studied previously in several mammals using a variety of methods. By using horseradish peroxidase (HRP) as a retrograde label, it is now possible to identify with confidence those cells that supply individual muscles and to examine morphology and size of these cells. The present study, part of a more extensive study of opossum neuromuscular organization, includes our observations on localization and size of motoneurons that supply the tibialis anterior (TA) and medial gastrocnemius (MG) (antagonists) muscles in the opossum, a generalized mammal. Multiple injections of 2-6% HRP (Sigma - Type VI) were made into the belly of the TA (total volume .25 - .5 ml.). For the MG, the muscle nerve was exposed, cut and soaked for 3 hours in a solution of 20% HRP. Animals were killed after 2-4 days by perfusion with a paraformaldehyde-glutaraldehyde fixative. Spinal cord segments were carefully identified and blocked, then frozen sectioned at 40 μm and the sections reacted using a tetramethyl benzidine (TMB) procedure. Cells were identified by dark field microscopy and traced using a drawing tube. Tracings were measured using a semi-automated image analysis system so that area, diameter and circumference data were available. All motor cells were localized within the L_4-L_6 segments of the spinal cord. Both nuclei were in the centrolateral part of the ventral horn with MG cells slightly more peripheral in position. TA motoneurons were consistantly rostral (in L_4-L_5) to MG cells (L_5-L_6). Cells were typical multipolar cells with size (cross-sectional area) ranging from 100 μm^2 to 2800 μm^2. The mean number of neurons labelled was 54 for TA and 93 for MG. Both the localization and the size range for these motor neurons are comparable to those published from other laboratories for the cells that supply the same muscles in cat and rat, however, the distribution of subpopulations (i.e., alpha and gammas motoneuron pools) does not seem to be so clearly separated in the opossum as in these other species.

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290.3 LOCALIZATION OF THE MOTOR NUCLEI AND SENSORY PROJECTIONS OF THE GLOSSOPHARYNGEAL, VAGUS AND HYPOGLOSSAL NERVES IN THE BRAIN OF THE COCKATOO (CACATUA ROSEICAPILLA). J. Martin Wild. Dept. of Behavioural Biology, Research School of Biological Sciences, Australian National University, Canberra, A.C.T. Australia.

The avian caudal cranial nerves are involved in a variety of species specific behaviours, two of the most important of which are feeding and phonation. In parrots these behaviours are distinctive and probably involve the use of some of the same muscles, including those of the tongue. In order to locate the central motor and sensory components of these nerves, horseradish peroxidase (HRP) was applied to their proximal severed ends, and sectioned brains and ganglia were later processed using Hanker-Yates reagent as the chromagen.

Application of HRP to N IX labelled four rhombencephalic nuclei: (1) a large-celled, retrofacial nucleus supplying M. geniohyoideus, the major tongue extensor; (2) a dorsal nucleus composed of medium-sized cells, projecting to most branches of N IX; (3) a ventrolateral nucleus supplying, amongst other structures, the floor of the pharynx and larynx; (4) a ventral portion of the dorsal motor nucleus of the vagus.

Neurons labelled by application of HRP to the cervical vagus comprise the classically defined dorsal motor nucleus, and a ventrolateral medullary nucleus which is coextensive with that of the glossopharyngeus: Together they probably constitute a nucleus ambiguus.

Application of HRP to hypoglossal branches labelled a large nucleus intermedius (IM) and neurons ventral, ventrolateral and caudal to it. The rostral third supplies most of the tongue muscles, the caudal two thirds the tracheal and syringeal muscles. In addition, neurons of the ventral hypoglossal nucleus (nucleus supraspinalis) were labelled from injections of HRP into upper neck muscles, some of which are innervated by hypoglossal nerve branches.

Many labelled neurons were found in the "jugular" ganglion following HRP treatment of each of the three nerves, especially N IX and N XII, which innervate the tongue. Central projections of these neurons are to nuclei of the descending trigeminus and to largely non-overlapping portions of the principal trigeminal nucleus. It is hypothesized that these afferents provide sensory information necessary for the efficient processing and passage of food in the mouth.

290.4 INNERVATION OF THE FACIAL MUSCLES BY THE BRAINSTEM FACIAL NUCLEUS. EveLynn McGuinness* (SPON: John Allman) Division of Biology 216-76, California Institute of Technology, Pasadena, CA 91125

We determined the pattern of innervation of the facial muscles from the brainstem nucleus of the facial nerve in rats by injecting the peripheral muscles with 2-5 μl of 20% horseradish peroxidase (HRP) and examining the facial nucleus for labeled neurons. Morphologically using coronal sections we could distinguish four different subnuclei, dorsal, lateral, intermediate, and medial. In some sections the intermediate group could be further subdivided into an intermediate and a ventromedial group but this boundary was not morphologically distinct and did not correspond to a recognizable border between neurons innervating different muscles so we have included both in the intermediate group. The vibrissae are innervated by the lateral subnucleus. There appears to be a degree of topography in the vibrissal representation. Injections into levator labii superoris which in the rat extends from above the eye to the upper lip normally produced labeling only in the dorsal subnucleus. If the injection included any part of the muscle underlying the vibrissae, labeling was also seen in the lateral group. Injections in orbicularis oris produced labeled neurons in the ventral portion of the intermediate subnucleus. We saw no sharp distinction between injections to the upper lip or the lower lip. Injections into zygomaticus resulted in labeling the more dorsal portion of the intermediate subnucleus and injections in the platysma produced rather scattered labeling in the more medial extent of the intermediate subnucleus. In making the later injections the skin and attached layer of platysma were separated from the underlying muscles to prevent spread. Both frontalis and the extrinsic ear muscles were represented in the medial subnucleus. Frontalis was limited to the dorsal portion of the subnucleus, levator auris longus tended to be in the more ventral portion. Injections in the anterior belly of the diagastricus muscle resulted in labeled neurons only in the motor nucleus of the trigeminal nerve as did injections in the massater muscle.

A curious feature emerged in that each innervation site appears to be represented throughout the rostral caudal extent of the facial nucleus. Any given injection will produce labeled neurons in most sections for which the appropriate subnucleus is present. Smaller injections produce fewer labeled neurons per section and/or less densely labeled neurons but the extent of the rostral caudal extent of the distribution of labeled neurons is not changed.

290.5 MASTICATORY REPRESENTATION IN THE MOTOR NUCLEUS OF THE TRIGEMINAL NERVE OF THE CRAB-EATING MACAQUE (Macaca fascicularis). Marguerite Carlton*, R. Dom*, and E. Rawe* (SPON: James Stevenson). Dept. of Anatomy, Medical University of South Carolina, Charleston, S.C. 29425.

The major masticatory divisions and subdivisions of the trigeminal motor nucleus were determined by injecting horseradish peroxidase (HRP) into each of the jaw-opening and jaw-closing muscles and identifying neurons which were HRP positive. The best results were obtained in animals 48-72 hours after injection of HRP. Cross-sections of the trigeminal motor nucleus show the nucleus to be divided into a large figure 8 shaped lateral division and a smaller and less distinct medial division. This lateral division can be further divided into dorsomedial and ventrolateral subdivisions. The lateral division contains the temporalis neurons throughout its dorsomedial subdivision and masseter and medial pterygoid neurons throughout its ventrolateral subdivision. The neuronal pools supplying the masseter and medial pterygoid muscles had considerable, if not complete, overlap. The waist region of the lateral division contains neurons innervating the superior head of the lateral pterygoid muscle. The medial division is divided into a dorsal subdivision which contains neurons innervating the anterior belly of the digastric muscle and a ventral subdivision containing the neurons supplying the mylohyoid muscle and the inferior head of the lateral pterygoid muscle. The caudal portion of this ventral subdivision contains neurons innervating the mylohyoid muscle, whereas the neurons of the rostral part supply the inferior head of the lateral pterygoid muscle. This study indicates that neurons supplying the jaw-closing muscles are situated in its lateral division and the neurons innervating the jaw-opening muscles lie within the medial division. (Supported by NIH grant RR05767)

290.6 SOME CHARACTERISTICS OF THE NEUROMUSCULAR APPARATUS OF THE PELVIC FLOOR IN SPINAL CATS. B. Dubrovsky and P. Pacheco*. Neurophysiology Lab., Allan Memorial Institute, McGill Univ., Montreal, Quebec, H3A 1A1, and U.N.A.M., Mexico.

As S. J. Gould stated, "Bipedalism is no easy accomplishment. It requires a fundamental reconstruction of our anatomy, particularly of the foot and pelvis." Not much data is available, however, on the neural control of pelvic floor musculature The two broad muscle groups of the region, constrictor cloacae: external ani sphincter representative type, and compressor cloacae: levator ani complex muscular group representative type, are assumed to be activated as a mass unit. We studied this proposition. In spinal cats (section of the spinal cord between 1st and 2nd cervical segments under ether anesthesia) we recorded E.M.G. activity from the right and left levator ani and the external ani sphincter muscles. Stimulation of pudendal regions in either side of the body elicited responses of the external ani sphincter; activation of levator ani musculature was lateralized; i.e. cutaneous stimulation (touching or electrical stimulation of the skin of pudendal regions and/or skin of the external surface of the hindlimbs) elicited activation of the ipsilateral levator ani muscle. Section of one pudendal nerve did not alter the level of tonic background activity (1-4/sec) from the recorded muscles. Bilateral section of the pudendal nerve entirely abolished both tonic activity and responsiveness of the external sphincter. Levator ani muscles were not affected by pudendal sections, although bilateralization of responses were observed more frequently after nerve severances. Tactile stimulation of the internal surface of upper hindlimb regions selectively activated the external sphincter of the anus without detectable changes in the activity pattern of the levator ani muscles. Moderate pressure applied on the region of the knee joint or electrical stimulation of the region selectively activated the corresponding (ipsilateral) levator ani muscle, without noticeable change in the activity pattern of the external ani sphincter E.M.G. Pressure on the uterine cervix significantly decreased the level of tonic activity and phasic twitch responses in both levator ani and external ani sphincter muscles. Gentle tapping on perivaginal skin elicited contraction of the external ani sphincter which was frequently, although not always, accompanied by arrest of the tonic activity in the levator ani muscles. These results indicate that muscles of the pelvic floor can be selectively activated and/or inhibited by discrete sets of stimuli. It will be a serious mistake then to record from one muscle and take the results of this functional exploration as representative of the state of all muscles in the region. This research was supported by NICOLO.

290.7 MOTONEURONE SYNCHRONIZATION AND ITS POSSIBLE ORIGINS P.A. Kirkwood*, T.A. Sears* and R.H. Westgaard*(SPON: A. Taylor). Sobell Dept. Neurophysiology, Institute of Neurology, Queen Square, London WC1N 3BG, England.

Intercostal motoneurones demonstrate short-term synchronization, as shown by narrow central peaks (± 3msec) in cross-correlation histograms between the discharges of paired groups of motoneurones, recorded as inspiratory efferent discharges in the cut central ends of external intercostal nerve filaments of anaesthetized cats (1). In that paper and a later paper (2) this synchronization was interpreted as arising from individual pre-synaptic axons which branched to excite both groups of motoneurones. Quantitative predictions from ref. (2) were recently verified (3), thus confirming the interpretation.

We now report that under some circumstances the synchronization can have a different time course. The circumstances include awake animals (motoneurone activity recorded as e.m.g. signals via fine wires previously implanted aseptically under anaesthesia), lightly anaesthetized animals and animals with spinal cord lesions either rostral or caudal to the motoneurones involved. In place of, or in addition to the narrow central peak, longer duration peaks were present (± 10msec to ± 50msec), which could not be explained by the branched presynaptic axon hypothesis. The effect was present for groups of motoneurones for which narrow central peaks were never observed (e.g. between motoneurones on opposite sides of the cord) and was strongest when the respiratory drive was weakest. We believe that the best explanation for the effect is that in these preparations there were groups of as yet unidentified spinal cord interneurones which provided an input to the motoneurones and were themselves synchronized. The broad peaks represent the time course of that synchronization.

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290.8 *CHRONIC SPINAL KITTEN: 24 HOUR EMG PROFILE OF SLOW AND FAST ANKLE EXTENSORS.* *M.A. Alaimo*, L.A. Smith* and J.L. Smith. Dept. Kinesiology and Brain Research Institute, UCLA,CA. 90024.*

Previous experiments have suggested that the faster contraction times (CT=30-50ms) observed in the normally slow-contracting soleus (SOL) following cord transection were due to "virtual elimination of motoneuron discharge" (Galego, J. Physiol. 281: 1978). When kittens cordotomized(T_{12}) at 2 wks are exercised daily on a treadmill, the SOL exhibits normal EMG activity (Smith Neurosc. Abst.,1979); nevertheless, the SOL-CTs of the exercised kittens are significantly faster than normal (Edgerton, Neurosc. Abst., 1979).

In an attempt to establish an EMG activity profile of SOL and gastrocnemius (GAST) muscles, EMGs were recorded for 10 min intervals each hr for 24 hrs. During the testing day, the 6-8 mths postcordotomy kittens were housed in walking cages (3 sq m), and every attempt was made to duplicate the normal daily routine. Both raw and rectified-averaged (I-EMG) EMGs were quantified.

In general, the 5 cordotomized kittens exhibited 3 peak activity periods, as measured by total I-EMG, around 5-6pm, 11-1am and 1-2pm interspersed with periods of rest or sleep. All behavior was verified by use of video. Each sampling period was characterized by EMG burst number and duration against the total I-EMG. During peak periods of activity, the SOL exhibited 20-30 short-lasting bursts (200-300 ms). In general, this activity resulted from nonweight-supported movement; however, short periods of weight-support were common during both greeting and feeding behaviors. In general the GAST exhibited fewer bursts with shorter durations.

A 10 min treadmill period in which kittens weight-supported during the stance phase, elicited total I-EMG that were 1.2 and 2.2 times greater than the total I-EMG recorded during the entire 24 hr period for SOL and GAST, respectively. The data suggest that although the spontaneous activity of the hindlimb may be reduced in the chronic spinal kitten, 30 min of treadmill exercise daily increases the activity level appreciably. Preliminary findings suggest that normal sedentary kittens, housed in the same cage are not appreciably more active than the chronic spinal kittens. However, normal kittens exhibit more weight-support spontaneous movements and it is possible that the tension produced by the SOL is greater. We are now testing this.

Our results suggest that the SOL of chronic spinal kittens exhibit spontaneous activity as well as normal activity patterns during treadmill locomotion . The conversion of the SOL from a slow to a fast contracting muscle is not due to the "virtual absence" of motoneuron discharge. Supported by NIH grant, NS 16333 and NS 10423.

290.9 THE ORGANIZATION OF STEM DENDRITES OF DORSAL NECK MUSCLE MOTONEURONS IN THE ADULT CAT. P. K. Rose and D. A. S. TWIDDY*, Dept. of Physiology, Queen's University, Kingston, Ontario, Canada, K7L 3N6.

Dendritic trees of dorsal neck muscle motoneurons, biventer cervicis (BC) and complexus (CM), are arranged in remarkedly regular patterns. Each motoneuron has three distinct groups of dendrites. One group projects rostrally and caudally from the soma and is located in the ventromedial nucleus. Another group of dendrites is directed dorsomedially toward the central canal and the third group projects dorsolaterally through the spinal accessory nucleus and into lamina VII. A few scattered dendrites are also found directly dorsal to the soma in lamina VIII. In the present study we have examined the distribution of the branches of individual stem dendrites to determine the contribution of each stem dendrite to the overall dendritic tree. The entire dendritic tree of five BC and CM motoneurons was reconstructed from serial histological sections. Stem dendrites and their branches were extracted from these reconstructions and rotated 90^0 to the plane of section to determine their three-dimensional distribution.

Twenty-four of 47 stem dendrites had branches which were confined to one of the three major dendritic groups characteristic of BC and CM motoneurons. One-half of these dendrites were found in lamina IX, seven were located dorsolaterally to the soma and five projected dorsomedially. All of the remaining 23 stem dendrites contributed branches to more than one dendritic zone. Three stem dendrites distributed branches to all regions of the ventral horn occupied by the complete dendritic tree.

Although there are few detailed anatomical studies of the descending and segmental connections to the upper cervical spinal cord, the available evidence suggests that most of the systems which make monosynaptic connections with BC and CM motoneurons terminate in discrete zones. These zones are much smaller than the territory occupied by the complete dendritic tree of BC and CM motoneurons, but are similar in size to the area occupied by stem dendrites whose branches are restricted to a single dendritic group. These stem dendrites may therefore receive a small, but specific set of inputs. Integration with inputs on other dendrites will occur at the soma. In contrast, stem dendrites whose branches are more widely distributed will receive inputs from a wider variety of sources. Integration of these inputs will occur before reaching the cell body. (Supported by the Canadian MRC.)

290.10 Does the ventral spinocerebellar tract contain cervical collaterals? R. L. McBride, P. R. Lennard, and A. W. English, Emory University, Atlanta, GA 30322

A number of lines of evidence have given rise to speculations that the ventral spinocerebellar tract (VSCT) contains collaterals to other parts of the neuraxis. These speculations led Grillner (Physiol. Rev. 55:247,1975) to hypothesize that the cervical collaterals of VSCT neurons may play a significant role in the coordination of step cycles of the forelimbs and hindlimbs during locomotion in cats. This postulate requires that lumbar neurons projecting directly to the cerebellum via the contralateral VSCT also have terminations in the cervical spinal cord. We now report the results of anatomical experiments designed to test this hypothesis. In separate experiments, the retrograde label horseradish peroxidase (HRP) was injected into the cerebellum and the cervical spinal cord. Special care was taken in the case of cervical injections to use very small quantities and to minimize damage to axons of passage. Comparison of results from these single-label experiments indicates that cells in two lumbar cord regions were labelled in both experiments. These were in the base of the dorsal horn (lamina VI) and the medial parts of the ventral and intermediate grey (laminae VII and VIII). This latter region has been implicated as the origin of cells of the VSCT which receive strong group Ib inputs (Hubbard and Oscarsson, J. Comp. Neurol. 18:199,1962). As a direct test of whether the same VSCT cells contain cervical collaterals, a double retrograde labelling protocol was used. HRP and tritiated, inactivated HRP (H^3 apo HRP) were used as labels - one injected into the cerebellum, the other into the cervical cord. Serial sections of lumbar cord were reacted for HRP and then processed for autoradiography. Results of double labelling experiments confirm those using the single label protocol. Cells were labelled from both injection sites in lamina VI and medial VII and VIII. Dorsal and ventral horn cells were not found to be doubly labelled. Some cells in the intermediate grey showed light double labelling, but others were only singly-labelled. Thus it is concluded that propriospinal-VSCT neurons may exist but, if they do, they form a small subset of either the VSCT or the long ascending propriospinal neurons.

290.11 DISTRIBUTION OF SOMATOSTATIN IN THE GUINEA PIG HINDBRAIN. E. Taber-Pierce, S.C. Feldman and E. Lichtenstein* Dept. of Anatomy, Harvard Medical School, Boston, Mass. 02115; Dept. of Anatomy, CMDNJ-New Jersey Medical School, Newark, N.J. 07103; Hospital for Joint Diseases, New York, N.Y. 10035

Immunoreactive-like somatostatin (SRIF) was localized in neuronal elements of the guinea pig hindbrain, using a highly specific antibody to the peptide and the unlabeled antibody enzyme technique of Sternberger. All staining was found to be specific for SRIF as determined by the absence of immunoprecipitate in neuronal perikarya and fibers in tissue in which the antiserum had been previously adsorbed with synthetic peptide.

Labeled neurons, in which the immunodeposit was present in the perinuclear cytoplasm and in processes extending from the cell body, were present in many areas of the brainstem. In the raphe, large and medium-sized neurons were present in the following nuclei: superior centralis, magnus and obscurous. In the auditory system large numbers of SRIF neurons were found in ventral cochlear n., ventral trapezoid n. and ventral n. of the lateral lemniscus; smaller numbers of neurons also were present in the dorsal medial trapezoid n. In the reticular formation large numbers of large and medium-sized neurons were located in n. gigantocellularis; fewer SRIF neurons were also seen in nuclei pontis centralis oralis and caudalis, and n. parvocellularis. SRIF neurons, primarily medium-sized, were also found scattered throughout the remainder of the brainstem: n. intermedius of the spinal trigeminal tract; n. prepositus hypoglossous; n. Roller; area postrema; and n. tractus solitarius.

Many areas in the brainstem contain a neuropil rich in SRIF fibers, particularly: n. tractus solitarius; area postrema; caudal n. of the spinal trigeminal tract; peribrachial nuclei; interpeduncular n.; and the area of the n. locus coeruleus. In addition to these dense plexuses, bands of SRIF-containing fibers were found throughout the reticular formation at all levels, and were also found ascending and descending in the raphe. From this data, it appears that there is a significant SRIF input to most areas of the brainstem. (Supported by a grant from The Foundation of the College of Medicine and Dentistry of New Jersey to S.C.F.).

290.12 THE FUNCTION OF THE LOCUS COERULEUS IN THE ACTION OF CYCLOBENZAPRINE IN THE RAT. J. Daugherty, R. Pierce*, and C. Barnes. Texas Tech University Health Sciences Center, Department of Physiology, Lubbock, TX 79430.

Early experiments on the cat indicate the main site of action of cyclobenzaprine (CBZ) in producing its centrally acting skeletal muscle relaxation is in the brainstem. Of the brainstem sites explored, both the reticular formation and the locus coeruleus (LC) have been implicated as the main locus of action. Subsequent experiments have demonstrated in the cat that the noradrenergic pathway from the LC to the spinal cord is facilatory and therefore that CBZ's inhibition of LC would produce disfacilitation of motor neurons. A recent report, however, states that in rat, CBZ facilitates LC neuronal activity and explains the muscle relaxor action as an increase in descending inhibition. The present studies were undertaken to determine if there is a species difference in the action of CBZ on LC neurons. Experiments were done on adult rats of either sex anesthetized with α-chloralose. Recordings were made from the LC through glass microelectrodes filled with sodium chloride and saturated with fast Green dye. The electrode placement was initially done by stereotaxic coordinates. Once a cell was isolated with a characteristically slow spontaneous discharge rate, tests were made to determine if it would give the characteristic LC cell response to noxious stimulation of the paw; being an initial high frequency burst followed by inhibition. When such a cell had been functionally identified as LC by this procedure, the spontaneous rate and response to noxious stimuli were repeatedly tested after IV administration of CBZ at 1 mg/kg doses to maximum accumulated doses of 7 mg/kg. At the termination of experiments, fast Green was iontophoresed from the electrode tip for histologic verification that the cell was in the LC. In all cases cells identified as LC cells were seen to decrease their discharge rate, usually following 1 mg/kg and always by a 2 mg/kg accumulated dose, to CBZ. These preliminary studies are indicative of there being no species difference in LC response to CBZ between cat and rat. The criteria for functional determination of the identity of a LC neuron may explain differing results among investigators in this area.

290.13 AN ELECTRON MICROSCOPIC STUDY ON THE NORADRENERGIC AXON TERMINALS IN THE SUBSTANTIA GELATINOSA OF RAT SPINAL CORD. K. Satoh, A. Kashiba, H. Kimura and T. Maeda (SPON: S. C. Sung). Departments of Psychiatry and Anatomy, Shiga University of Medical Science, Shiga, Otsu, Japan.

The presence of noradrenergic (NA) fibers in the dorsal horn of the spinal cord, especially in the substantia gelatinosa (SG), has been known since the early histofluorescent studies (Fuxe, 1965). These NA axons are derived from the descending NA system originating from the lower brain stem. Recently, electrophysiological and pharmacological properties of the descending NA fibers have been extensively studied, and all suggest that the NA terminals are able to alter sensory transmission at the dorsal horn level. In the present study, NA terminals in rat SG of the spinal cord was investigated with the histofluorescence technique and electron microscopic cytochemistry using the glyoxylic acid-KMn04 fixation technique (Kimura et al., 1978).

Many green fluorescent nerve fibers were observed in the superficial part of the dorsal horn from cervical to lumbar cords. They were particularly rich in the outer layer of SG. In accordance with the topographical distribution of fluorescent CA fibers, NA terminals containing small granular vesicles were frequently observed under electron microscope in the outer layer of SG and scarcely in the inner layer. These terminals apposed, most frequently, small caliber dendrites, spine apparatus, and seldomly, large caliber dendrites. In some instances, the NA terminals exhibited synaptic contacts with several types of dendrites. Additionally, the NA terminals apposed other non-NA terminals that are characterized by the content of small agranular synaptic vesicles. Although a synaptic membrane specialization was not observed in such axo-axonic contacts, the agranular vesicles aggregated near to the contact membrane in the cytoplasm of non-NA terminals. In the dorsal root rhizotomized rats, there were no NA terminals which contacted directly with the degenerated axon terminals in SG of the lumbar cord. These findings suggest that the NA afferents to SG may exert their influence on the sensory transmission via the dorsal horn cells.

Fuxe, K. (1965): Acta physiol. scand. 64, Suppl. 24, 39-85.

Kimura, H. et al. (1978): Acta anat. nipponica 53, 62-63.

290.14 PROJECTIONS OF THE LATERAL HYPOTHALAMIC AREA TO BRAINSTEM AND FOREBRAIN IN THE RAT. M. L. Berk and J. A. Finkelstein. Dept. Anat., N. E. Ohio Univs. Col. Med., Rootstown, OH 44272.

The lateral hypothalamic area (LHA) regulates food intake and autonomic functions, but the pathways involved are unclear. In this study, the possible anatomical substrates for these activities are explored. Iontophoretic injections of ^{3}H-leucine were stereotaxically placed in LHA at mid-tuberal levels. After 10-14 days survival, the animals were perfused and the brains processed for autoradiography. The mid-tuberal region of LHA was chosen because it contains numerous labeled cells after horseradish peroxidase injections in the spinal cord.

Efferent projections to numerous neuronal regions are evident. Projections to the forebrain include the following: dorsal part of lateral septum, the region adjacent to diagonal band of Broca, bed nucleus of stria terminalis, substantia innominata, partial encapsulation of central nucleus of amygdala, and lateral habenular nucleus (medial and dorsolateral parts). Within the hypothalamus, connections to many medial nuclei are absent, although a few LHA fibers project to dorsomedial and posterior hypothalamus. Significantly, a narrow band of fibers courses to the borders of the paraventricular nucleus. Labeled fibers ascend and descend in the medial forebrain bundle and cross in the supramammillary decussation to the contralateral LHA.

In the midbrain, numerous fibers enter the central gray (bilateral) and central tegmental field (CTF), while fewer fibers are present in the dorsal raphe nucleus, at the lateral margin of the central superior nucleus and the deeper layers of the superior colliculus. Fibers leave CTF to either join other labeled fibers in central gray or course posteriorly to the medial and lateral parabrachial nuclei in the pons. Other pontine nuclei, such as the dorsolateral tegmental nucleus (bilateral) and the nucleus raphe magnus, also receive LHA projections. At medullary levels, LHA fibers in the lateral reticular formation (medial to spinal nucleus of V) sweep dorsomedially to innervate nucleus solitarius; fibers are seen dorsolateral to the lateral reticular nucleus as far caudal as the pyramidal decussation.

Some of the above regions, which are reciprocally connected to LHA, include lateral septal nucleus, central nucleus of amygdala, lateral habenular nucleus, midbrain central gray, dorsal raphe nucleus, lateral parabrachial nucleus, dorsolateral tegmental nucleus and nucleus of the solitary tract. These efferent and reciprocal afferent connections of LHA interrelate various neuronal systems, such as limbic, autonomic, catecholaminergic and serotonergic. These connections of LHA could participate in regulatory mechanisms. (Supported by NIH NS14344 and NS06186).

290.15 PHARMACOKINETICS OF A SINGLE I.V. METHYLPREDNISOLONE DOSE IN CAT SPINAL CORD: CORRELATION WITH EFFECTS ON SPINAL LIPID PEROXIDATION, $(Na^{+}+K^{+})$-ATPase ACTIVITY AND MOTOR NEURON RESTING POTENTIALS. E.D. Hall and J.M. Braughler* Prog. in Pharmacology, Northeastern Ohio Universities Coll. Med., Rootstown, Ohio 44272

Prior studies from this laboratory have shown that acute i.v. administration of methylprednisolone (MP) sodium succinate (15, 30, or 60 mg/kg) produces a resting hyperpolarization of cat lumbar spinal motor neurons (Hall, E.D. The Pharmacologist 22:297, 1980). The greatest effect is observed with a 30 mg/kg dose. It is possible that the increased resting potential might be due to a glucocorticoid activation of an electrogenic sodium pump. Additional studies have been undertaken which have demonstrated a dose-related increase in $(Na^{+}+K^{+})$-ATPase activity in cat spinal cord synaptosomes that is apparent by 5 min, peaks at 1 hr. and returns to control at 24 hr. after steroid injection (Braughler, J.M. and Hall, E.D., Brain Res., in press). Additionally, experiments have been conducted to examine the acute effects of methylprednisolone on lipid peroxidation using the thiobarbituric acid test. These have shown that a 30 mg/kg dose significantly reduces lipid peroxidation by a third while 90 mg/kg increases spontaneous peroxide formation by over 50% when the cord tissue is removed and assayed at 1 hr. after glucocorticoid administration. (Hall, E.D. and Braughler, J.M., Exp. Neurol. 72:in press).

In order to establish a correlation between the tissue levels of the glucocorticoid and it's neurophysiological and neurochemical effects, studies were done to determine the pharmacokinetics of MP in spinal cord vs. plasma after a single large (15, 30, 60 or 90 mg/kg) i.v. dose. MP levels in lumbar cord (L4 and L5) and plasma were measured by HPLC with UV detection (254nM). As would be expected, the spinal levels of MP are linearly related to dose. After i.v. injection, the peak level is observed at the earliest cord removal time of 5 min. At 1 hr. the amount measured in the cord falls slightly, but not significantly and the half-life of the drug in spinal cord tissue which appears to remain fairly constant, is approximately 3 hr. The plasma disappearance of MP is in general much quicker, and conforms to a multicomponent first order process with a gradual increase in the half-life, characteristic of a highly lipid soluble drug.

The relationship between the pharmacokinetics of a single MP dose and the specific requirements for repeated dosing to maintain the previously observed neurophysiological and neurochemical effects will be presented. Furthermore, the implications of these results for the actions of high dose glucocorticoid administration on the normal and the compromised CNS will be discussed. (Supported by the Amyotrophic Lateral Sclerosis Society of America and by NIMH 34111).

290.16 THE UPTAKE OF [^{3}H]-SEROTONIN, -GABA, AND -GLUTAMATE INTO SYNAPTOSOMES AND THEIR BINDING TO SYNAPTIC MEMBRANES OBTAINED FROM SPINAL CORDS OF NORMAL AND PARAPLEGIC DOGS. P.V. Hall*, J.T. Patrick*, E. Chernet*, W.J. McBride and R.L. Campbell*(SPON: S.L. Morzorati), Depts. of Surg., Psych. and Biochem., Ind. Univ. Sch. of Med., Indianapolis, IN 46223.

Spinal cords were obtained from normal mongrel dogs and from dogs made paraplegic by midthoracic crush. Lumbar regions of the spinal cords were removed either one week (before spasticity begins) or 4 weeks (when spasticity is apparent) after surgery. Spinal cords were removed, stripped of meninges and homogenized in ice-cold 0.32M sucrose with a Potter-Elvehjem homogenizer. A crude synaptosomal fraction (P_2), isolated by standard centrifugation techniques, was then subjected to additional centrifugation steps to yield a myelin-free synaptosomal fraction (MFP_2). A portion of MFP_2 was used to study the high affinity uptake of 30 nM [^{3}H]-serotonin (5-HT), 1.0 µM [^{3}H]-GABA and 1.0 µM [^{3}H]-L-glutamate (Glu). A second portion of the MFP_2 was osmotically lysed; a synaptic plasma membrane (SPM) fraction, isolated by centrifugation, was used to study the Na^{+}-independent binding of 5.0 nM [^{3}H]-Glu and 10 nM [^{3}H]-GABA. The uptake of [^{3}H]-5-HT into MFP_2 from the control group was 0.56 ± 0.05 pmole/mg prot/min (N=10). The uptake of [^{3}H]-5-HT was significantly ($P<0.025$) reduced to 67 ± 9% (N=7) and 22 ± 8% (N=3) of the control level in the one-week and 4-week group, respectively. The uptake of [^{3}H]-GABA and [^{3}H]-Glu into the control group was 64 ± 5 (N=10) and 84 ± 10 (N=10) pmole/mg prot/min, respectively. After one week, the uptake of both amino acids was the same in normal and damaged cords. However, in the 4-week experimental group, the uptake of both [^{3}H]-GABA (143 ± 5% of control; N=3 and -Glu (162 ± 8% of control; N=3) was significantly ($P<0.05$) higher than control levels. The Na^{+}-independent binding of [^{3}H]-Glu (0.055 ± 0.013 pmoles/mg prot for control value; N=11) appeared to be higher in the 1-week (138 ± 14% of control value; N=8) and 4-week (164 ± 3% of control level; N=3) experimental groups and may indicate increased numbers of glutamate receptors or greater receptor affinity. On the other hand, the binding of [^{3}H]-GABA (0.035 ± 0.009 pmoles/mg prot for control value; N=8) appeared to be lower in the 1-week (46 ± 9% of control; N=5) and 4-week (61 ± 7% of control; N=3) experimental groups and could indicate a loss of GABA receptors on descending axon terminals. The increased uptake of both [^{3}H]-GABA and [^{3}H]-Glu in the 4-week experimental group could indicate sprouting of GABAergic and glutaminergic (or aspartergic) neurons which might be related to the spastic condition present at this time. (Supported in part by MH00203).

290.17 EXPERIMENTS ON THERAPEUTIC DIALYSIS OF CEREBROSPINAL FLUID (C.S.F.) BY EPIDURAL COOLING IN SPINAL CORD INJURIES.
C. Romero-Sierra, R.R. Hansebout*, S. Sohal* and R.B. Marina*. Department of Anatomy, Queen's University, Kingston, Ontario, Canada, and Department of Surgery, McMaster University, Hamilton, Ontario, Canada.

Secondary traumatic paraplegias (S.T.P.) are due to an autodestructive process, the causal agents of which are the subject of debate.

Local epidural cooling has been carried out successfully in the treatment of S.T.P. It was hypothesized that this treatment method among other beneficial effects (e.g., reduction in: metabolic demands, edema, swelling, vasospasm; and normalization of local blood pressure) in some way trigger C.S.F. dialysis.

By analytical mathematical modeling of this method of hypothermia, it was concluded and reported last year that the cold source in local epidural cooling is an effective mechanism for the generation of C.S.F. flow.

To further explore this hypothesis, an experimental approach was devised. The strategy was to inject a dye into the subarachnoid space of dogs and observe C.S.F. movement in the following conditions: (a) "normal"; (b) local epidural cooling; (c) compression lesion; and (d) compression lesion and cooling.

Eight mongrel dogs, weighing between 45 and 65 lbs., were anesthetized intravenously with pentobarbital. Laminectomy of the upper and lower lumbar vertebrae was performed.

A compression lesion was made by introducing into the epidural space of the middle lumbar vertebrae a thin-walled silastic balloon measuring 10 mm x 12 mm and inflating it to 160 mm of Hg for one hour.

Local cooling of the cord was achieved by using a miniature epidural heat-exchanger constructed of thin-walled silastic material and shaped to fit over the dorsal dural covering of the spinal cord.

0.1 cc of basic dye was injected into the subarachnoid space via spinal roots to avoid leakage.

The results obtained indicate that local cooling speeds the spread of the dye along the longitudinal axis of the cord. Compression lesion delays or blocks the spread of the dye, an effect that is suppressed by cooling the lesioned area. These findings constitute additional support for the hypothesis of C.S.F. dialysis by epidural cooling.

Further experimental work using radioactive tracers will be carried out to validate the results.

290.18 ACUTE FUNCTIONAL AND STRUCTURAL CHANGES FOLLOWING SPINAL CORD COMPRESSION IN UNANESTHETIZED CATS, AND THE EFFECTS OF DEXAMETHASONE (DM) THEREUPON.
A.C. Nacimiento, F. Loew[X] Research Laboratory, Department of Neurosurgery, Saarland University School of Medicine, 6650 Homburg/Saar, FRG

Following exposure, an intact spinal cord L1 segment was suddenly compressed for about 50 ms by an electromagnetically driven metal rod. Quantification was allowed by high reproducibility of pathophysiological events at different degrees of compression. The following functions were monitored for up to 5 hours after injury: a) polysynaptic reflex discharges (PRD); and b) axonal conduction through the injured segment. Experiments were designed so as to allow a comparison with those made previously under Pentobarbital (PB). PRD in control experiments showed less variability than those under PB. A few minutes following intravenously applied DM (3mg/kg) a PRD decrease of about 30-45% set in and settled at that level. No changes were seen in axonal conduction. A 3mm-compression brought about a PRD decrease of about 60-70%, which remained thereafter unchanged. Axonal conduction was blocked immediately following compression. After a few minutes, however, partial recovery was evident reaching a level of about 30-50% (3mg/kg) of control values. Dexamethasone applied intravenously 30 min following compression, did not change neither the reduced PRD nor the partially blocked axonal conduction. These results are at variance with those obtained under PB, where both functional parameters were clearly influenced, bringing about a clear improvement in both reflex activity and axonal conduction. Clear-cut influence of DM could be observed histopathologically. Intensity, extension and spread of edema were reduced to a significant degree. These results allow the following conclusion: a) Course and degree of acute functional posttraumatic changes may be influenced by PB; and b) the apparent inconsistency between functional and structural changes under DM in the unanesthetized preparation suggest an interaction between PB and DM on the injured spinal cord, which is currently undergoing experimental analysis.

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290.19 ACTION OF 5-HTP ON MOTOR NEURONS EXCITABILITY IN RATS, MONKEYS AND HUMAN WITH SPINAL CORD TRANSSECTION.
H. Barbeau and P. Bédard. Lab. of Neurobiology, Fac. Med., Laval Univ., Québec, Canada, G1K 7P4.

Rats rendered paraplegic by a spinal cord transsection respond to systemic administration of 5-hydroxytryptophan (5-HTP) by spontaneous discharges in the lower limb muscles. This effect becomes more and more evident in the twenty days following the section. Animals having received an intraventricular injection of 5-7 dihydroxytryptamine (5-7 DHT) twenty days previously, show, the day following the section, a response to 5-HTP of the same magnitude as chronically spinalized animals. Nociceptive reflexes however are not increased by 5-7 DHT pretreatment. The effect of 5-HTP can be blocked completely by cyproheptadine. A similar pattern of response to 5-HTP was observed in one spinal monkey. In the chronic spinal monkey, 5-HTP at 100 mg/kg significantly increases the patellar and the nociceptive flexor reflexes. This action of 5-HTP was mimicked by Quipazine 10 mg/kg and was completely blocked by cyproheptadine 10 mg/kg. Cyproheptadine also reduces the reflexes below the control level.

Preliminary observations suggest that the serotonin, antagonist, cyproheptadine is beneficial in patients suffering from spasticity due either to multiple sclerosis or to spinal cord trauma. Cyproheptadine significantly reduced the numbers of spasms, episodes of clonus and hyperreflexia in six spastic patients. Our results suggest that destruction of descending 5-HT pathways is followed by a denervation supersensitivity of lumbar neurons. This mechanism may play a role in spasticity. The use of serotonin antagonists may be potentially beneficial in spastic patients.

291.1 ACTIVITY DEPENDENT K^+ ACCUMULATION IN RAT OPTIC NERVE: A DEVELOPMENTAL STUDY. B.R. Ransom*, B.W. Connors, & David Kunis*. (SPON: M.W. Siegel). Dept. Neurology, Stanford Univ. Sch. of Med., Stanford, CA 94305.

Potassium specific microelectrodes were used to study the extent and kinetics of increases in $[K^+]_o$ resulting from stimulation of rat optic nerves of different postnatal ages (1 day-adulthood). Nerves were dissected free and bathed in physiological saline with $[K^+]_o$ of 5 mM. Supramaximal stimuli (with regard to evoked field potentials) were delivered to the nerves at different frequencies by suction electrodes. The maximum level to which $[K^+]_o$ rose with repetitive stimulation was strongly dependent upon the age of the animal: 1-3 days old, 17.2± 2.4mM (S.D.); 4-12 days old, 12.0± 1.5 mM; 13-21 days old, 10.8± 1.1 and older than 22 days, 9.8± 1mM. Mechanisms which might underlie this striking sequence include developmental changes in 1) K^+ reuptake kinetics, 2) absolute size and activity-dependent variations in extracellular space, 3) the amount of K^+ secreted per nerve impulse, 4) the sensitivity of K^+ secretion to increases in ambient $[K^+]_o$, or 5) the anatomy of this structure. Some of these possibilities were tested experimentally. Evidence of active K^+ reuptake following activity-dependent K^+ accumulation was obtained in optic nerves of all ages. Our data indicate that, if anything, younger nerves dissipate excess K^+ more rapidly than older ones. Stimulation-dependent reductions in the size of the extracellular space, as measured by changes in the concentration of an impermeant ion (choline), were much more prominent in the optic nerves from older animals. This result is contrary to that expected if enhanced K^+ accumulation in young nerves is to be explained on this basis. The amount of K^+ secreted per single nerve impulse declined with age in a manner similar to the age-dependent reduction in maximum K^+ accumulation. This phenomenon may be related to the longer duration of action potentials seen in young nerves and has a developmental time course which is substantially different from that of the development of myelination. Furthermore, stimulation induced K^+ secretion was less inhibited by high levels of ambient $[K^+]_o$ in younger than older nerves. The uniquely massive K^+ accumulation seen in immature nerves could have profound functional consequences, since these levels of $[K^+]_o$ are capable of partially blocking impulse transmission when introduced exogenously. The data so far available suggest that developmental changes in K^+ accumulation may be related to changes in the amount of K^+ secreted per nerve impulse, coupled with an altered sensitivity of K^+ secretion to ambient $[K^+]_o$. Thus, high levels of K^+ accumulation may result when the rate of K^+ release is relatively enhanced compared to that of reuptake.

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291.2 IONIC MECHANISM OF POST-TETANIC HYPERPOLARIZATION IN MYENTERIC NEURONS. K. Morita*, T. Tokimasa* and R.A. North (SPON: A.G. Karczmar). Neurophysiology Laboratory, Department of Pharmacology, Loyola University Stritch School of Medicine, Maywood, Illinois 60153.

Intracellular recordings were made from myenteric neurons removed from the ileum of guinea-pigs. Action potentials were evoked by passing depolarizing currents across the soma membrane, or by focal stimulation of a cell process. Repeated action potentials (1-30 Hz, 1-5 s) were followed by a post-tetanic hyperpolarization (PTH). The PTH required extracellular calcium ions, was accompanied by an increased membrane conductance and reversed its polarity at the potassium equilibrium potential. The peak amplitude of the PTH was dependent on the number of action potentials used to evoke it. The peak amplitude, corrected for non-linear summation of voltage, or the peak conductance increase, were both linearly related to the logarithm of the number of action potentials (range 1 to 60). The time course of decay of the PTH, corrected for non-linear summation, or of the underlying conductance change, was double exponential with time constants of approximately 6 and 15 s. Conditioning hyperpolarization increased the amplitude and duration of the conductance change underlying the PTH, without marked change in the time course of decay. This could result from an increased calcium entry and/or a voltage sensitivity of the calcium-dependent potassium conductance. TEA (1 mM) caffeine (1 μM) or doubling the extracellular calcium concentration each prolonged the PTH without significant effect on resting membrane properties. The duration of the PTH may be a useful indicator of the ability of the neuron to reduce its intracellular calcium concentration. The prolongation of the PTH by low concentrations of clonidine and morphine suggests an effect of these drugs on the intracellular calcium concentrations.

291.3 A QUALITATIVE DESCRIPTION OF THE CURRENTS SEEN UNDER VOLTAGE CLAMP OF RAT SYMPATHETIC NEURONS IN CULTURE. Joseph E. Freschi, Physiology Dept., Armed Forces Radiobiol. Res. Inst., Bethesda, MD 20014

Cultured sympathetic neurons in HEPES-buffered Hank's saline were voltage-clamped with 2 microelectrodes (20-50 MΩ). The cells were held at the resting membrane potential (RP), generally -50 to -60 mV. Depolarizing step commands of 10-20 mV from RP evoked a rapid transient inward current that peaked within 1 msec. This current was blocked by tetrodotoxin (TTX). Electrode resistances imposed a technical constraint on quantitative study of the rapid inward current. Depolarizing steps from RP also gave rise to an outward current that comprised 3 components. Small depolarizing steps produced a slowly activating current with a time constant (τ) of 50-100 msec. This current was better studied with hyperpolarizing steps. Such steps caused a small inward relaxation that reversed in direction beyond about -80 mV. This slow current appears analogous to the "M"-current of Brown and Adams (Nature 283: 673, 1980) in frog sympathetic neurons. The other 2 components of the outward current were seen after depolarizing steps 20 mV positive to the RP. Analysis of tail currents showed that one component decayed rapidly (τ about 5 msec) and was blocked by tetraethylammonium (TEA). The other component decayed at least 10 times more slowly and was inhibited by cobalt (Co). The tail currents of the outward currents reversed at about -80 mV. In the presence of TTX, onset of outward current activation occurred within 2 msec. Repolarization from hyperpolarizing steps beyond about -70 mV, and depolarizing steps from a holding potential more negative than -60 mV elicited a transient outward current that peaked within 5-10 msec and decayed over about 50 msec. This current is similar to the "A"-current of Connor and Stevens (J. Physiol. 213: 21, 1971). In the presence of TTX and TEA, a slow inward current was produced by depolarizing steps from RP. The current appeared to peak within 5 msec and inactivated slowly over about 50 msec. Analysis of the activation and inactivation of this slow inward current was complicated by the coactivation of outward currents. The slow inward current was blocked by Co. The overlap of the voltage sensitivities of many of these currents requires pharmacological separation for a more detailed analysis.

In summary, it appears that in these cultured mammalian sympathetic neurons, voltage-sensitive conductances may produce the following currents: A fast inward sodium current, a slow inward calcium current, a fast outward potassium current (delayed rectifier), a slow calcium-dependent outward potassium current, a slow outward potassium "M"-current, and a transient outward potassium "A"-current.

291.4 ANALYSIS OF THE ROLE OF CALCIUM IN RHYTHMIC HYPERPOLARIZATIONS INDUCED BY CITRATE IONS IN BULLFROG SYMPATHETIC NEURONS. Stephen M. McCort* and Forrest F. Weight. (SPON: I. Tasaki). Laboratory of Preclinical Studies, National Institute on Alcohol Abuse and Alcoholism, Rockville, MD 20852.

Previous investigations have shown that methylxanthines (theophylline or caffeine) initiate spontaneous rhythmic hyperpolarizations (SRH) in bullfrog sympathetic neurons (McCort, S.M. and Weight, F.F., Neurosci. Abstr., 5:47, 1979; Kuba, K., J. Physiol., 298:251, 1980). Morita et al. (Nature, 283:264, 1980) have reported the occurrence of SRH in these neurons without methylxanthines, using potassium citrate filled microelectrodes. In this study, we have investigated the role of calcium ions in the SRH induced by citrate ions. Type B neurons in the IXth or Xth paravertebral sympathetic ganglia of the bullfrog were studied in vitro. The superfusing Ringer's solution had the following composition (mM): NaCl 112, KCl 2.0, $CaCl_2$ 1.8, $NaHCO_3$ 2.4, and glucose 1 gm/l. Intracellular recordings were obtained using microelectrodes filled with 1 M potassium citrate (electrical resistance 20 to 60 megohms). SRH were observed in 20% of the neurons studied, occurring either spontaneously or resulting from passing a continuous inward (negative) current. The SRH occured approximately every 5 min, had an amplitude of 5-8 mV, and a duration of 1-3 min. By contrast, the methylxanthine induced SRH have an amplitude of 15-20 mV, a duration of 20-30 sec, and they usually occur approximately once a minute. Membrane resistance was decreased during the citrate induced SRH. Passing hyperpolarizing currents of progressively greater magnitude at first increased and then decreased both the frequency and the amplitude of the SRH. The SRH were increased in frequency by Ringer's solutions containing increased calcium (8 mM), A23187 (10 μM) or increased potassium (8 mM). On the other hand, the SRH were decreased in frequency or abolished by a Ca-free Ringer (containing 1.8 mM Mg^{2+}) or by the addition of dantrolene (43 μM) to the Ringer. These data indicate that the mechanism of generation of the citrate induced spontaneous hyperpolarizations appears to be similar to that of the methylxanthine induced events. The results suggest that the spontaneous hyperpolarizations are generated by a calcium-sensitive potassium conductance that involves a periodic intracellular release of calcium.

291.5 THE MACROSCOPIC SPATIAL DISTRIBUTION OF VOLTAGE-DEPENDENT TRANSMEMBRANE CONDUCTANCES ON THE SOMA MEMBRANE OF THE SNAIL NEURON. Carl Scheffey. Dept. of Physiology-Anatomy, Univ. of Calif., Berkeley, CA 94720.

Two extracellular recording techniques were used to examine the distribution of ionic currents on the somata of voltage-clamped Helix aspersa identified giant neurons. The fast vibrating probe technique (*Smith and Scheffey, 1980, Fed. Proc. 39:2129*) was used to measure extracellular current density just outside different areas of the soma surface during voltage clamp programs that activated the various conductances. This vibrating probe measurement represented local membrane current density with a spatial resolution of 30 microns or better. Measurements made at different probe positions were scaled to make equal the jumps in the scaled vibrating probe signals at the beginnings of hyperpolarizing voltage ramps under clamp. The scaled signals were then proportional to current density per unit membrane capacity, and hence (presumably) per unit membrane area. Most conductances examined were uniformly distributed in the soma membrane, with a typical measurement uncertainty of 15%. These included (1) Ca^{++}-independent potassium current activated by depolarization from -40 mV to +20 mV for 200 msec in Co^{++}-for-Ca^{++} ringer, (2) inward calcium current as represented by the peak inward current during a depolarization to +10 mV in a ringer which substituted Ba^{++} for Ca^{++}, Cs^{+} for K^{+}, and tetraethylammonium^{+} for Na^{+}, and (3) Ca^{++}-dependent potassium current activated by a depolarization. Fast inward sodium current was measured as the difference between inward current peaks during depolarizations with and without an inactivating prepulse. In contrast to the other currents, sodium current was not uniformly distributed, but was denser in the axon hillock area than in the area on the soma opposite to the hillock.

Patch recording was done by sealing a 20 micron internal diameter suction pipette to the soma surface. The pipette was connected to a virtual ground-type circuit that held the voltage inside the tip (extracellular to the membrane patch) to that measured by a reference electrode just outside the seal. Patch experiments confirmed that sodium current was denser near the hillock.

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291.6 ISOLATION OF THREE NEUROTOXINS FROM CENTRUROIDES SCORPION VENOM AND THEIR ACTION ON SODIUM CHANNELS. G.K. Wang* and G.R. Strichartz, Dept. of Physiology and Biophysics, SUNY, Stony Brook, N.Y. 11794.

Three neurotoxins which modify sodium channel kinetics were isolated from Centruroides sculpturatus venom by cation exchange (Bio-Rex 70, and CM-52) and gel filtration (G-50) methods. The sucrose-gap method on frog sciatic nerve was used to monitor the pharmacological activity during the purification procedure. All toxins had a molecular mass of about 7,500 daltons under SDS-gel electrophoresis. In the presence of 12mM TEA frog Ringer the neurotoxins produced multiple compound action potentials followed by a long plateau of depolarization in response to a single stimulus.

Neurotoxin actions were further characterized on single myelinated fibers isolated from frog or toad sciatic nerve. Each toxin could generate repetitive action potentials following a single 0.3 msec stimulus, and occasionally spontaneous A.P. were observed. Under voltage-clamp these toxins produced an induced inward sodium current upon repolarization from a depolarizing pulse, as previously reported by Cahalan (1975) for Centruroides venom. Each toxin thus selectively modifies the activation function of sodium channels. The concentrations of the three purified toxins required to produce the induced-current were different, ranging from 140ng to 5ug protein per ml.

When the fiber was exposed to a purified toxin from Leiurus quinquestriatus scorpion venom, which slows normal sodium channel inactivation, following its treatment by Centruroides toxin, the induced-current became much larger and its rate of appearance also increased. This result shows that different binding sites are responsible for the modification of activation and inactivation processes of the sodium channel by these toxins and that these processes can be altered simultaneously in an independent manner.

Cahalan, M.D. (1976). J. Physiol. 244, 511-534.
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291.7 TETRODOTOXIN SENSITIVITY VARIATION AMONG IDENTIFIED LEECH NEURONS. A.L.Kleinhaus, and J.W.Prichard, Dept. of Neurology Yale Univ. Sch. of Med., 333 Cedar Street, New Haven, Ct. 06510

In leech segmental ganglia, the maximum rate of depolarization of the action potentials of Retzius (R) cells and the cells sensitive to touch (T) cells, pressure (P) cells and noxious stimuli (N) cells all depended to a large extent on $[Na]_o$. In the case of the R cell the relationship was linear and passed through the origin. In the three sensory cells other ions may have contributed to the depolarizing phase of the action potential, but heavy dependence on Na was nevertheless evident. Under voltage clamp in all four cells, apparent inward current declined to near zero as external NaCl was replaced by sucrose, LiCl or choline Cl, but the relationship was not linear. Current carried by other ions, artifacts of incomplete space clamp, or some combination of the two may have caused the departure from linearity.

Tetrodotoxin in doses 20-100 micromolar had a minimal effect on the action potential of R cells. In contrast, the depolarizing phases of the N, P and T action potentials, as measured by electronic differentiation of the voltage signal, slowed in a dose-dependent fashion above 20 micromolar. Under voltage clamp the observed differences in TTX sensitivity between cells were maintained; inward current was not reduced by concentrations up to 100 micromolar in the R cell, but it was quickly, substantially and reversibly depressed in the sensory cells. Measurements of the passive electrical properties of R, N, P and T cells suggested that differences in these could not have been entirely responsible for the observed differences in TTX sensitivity. The differences in sensitivity were also unaffected by duration of exposure.

These data imply the existence of two kinds of Na channels in mature leech neurons. The R cell appears to have Na channels which are distinctly less sensitive to TTX than those of the three sensory cells. Previous studies have shown that there are also calcium conductance variations among these four cells (J.Physiol. 270 : 181, 1977). Careful examination of such variations should lead to better understanding of how particular neurons perform different functions.

291.8 CHARACTERISTICS OF SINGLE SODIUM CHANNEL CURRENTS AND THEIR MODIFICATION BY BATRACHOTOXIN IN NEUROBLASTOMA CELLS. Fred N. Quandt* and Toshio Narahashi. Dept. of Pharmacol., Northwestern Univ. Med. Sch., Chicago, IL 60611.

Currents through single channels which open in response to membrane depolarization were observed in recordings from N1E-115 neuroblastoma cells. The effects of the sodium channel blocker pancuronium and the sodium channel modulator batrachotoxin (BTX) on these currents were examined. The methods of Sigworth and Neher (Nature 287, 447, 1980) and Horn and Patlak (PNAS 77, 6930, 1980) developed for recording from muscle membrane patches were utilized. Cultured neuroblastoma cells appear to be a good preparation for this application since there is free access to the membrane, the cells can be voltage clamped and perfused internally, and the sodium channel density is low (maximum of 20 per μm^2). Patch pipette openings were $<1\mu^2$ and resistances were 2 to 10 megohms. The pipette-membrane seal resistance was 1 to 10 gigohms. Depolarizations to -40 mV were utilized in these studies since this potential is at the foot of the sodium conductance-voltage curve for these cells. This procedure lowers the probability of opening during a depolarization and permits the measurement of individual openings.

In a typical experiment, inward current jumps having a variable latency from the onset of the pulse were recorded. These currents were then measured for a number of pulses. The population had an amplitude of 1.4±0.5 pA and an open time of 1.7±0.6 msec (mean ± s.d., n = 112, 16°C). These currents appear to be due to the opening of sodium channels for two reasons. First, addition of 135 µM pancuronium to the cytoplasmic side of the excised membrane preparation produces a reversible block of these currents. Second, with well differentiated cells, larger pipette openings, and depolarizations to +10 mV, inward currents having a similar time course to those for sodium currents recorded by conventional voltage clamp methods are observed. The mean sodium channel conductance is estimated to be 15 pS for the typical experiment cited above. The effects of BTX were examined by incubating the cells in a low Na solution having 4 µM BTX. The patch pipette had normal Na (145 mM). Inward currents in response to membrane depolarizations to -40 mV appeared to have a normal amplitude but a prolonged open time. In a typical experiment, a single channel current was 1.3±0.3 pA and an open time was 16.7±9.1 msec (mean ± s.d., n = 133, 22°C). This latter value is consistant with the slower kinetics of the sodium channel in the presence of BTX (Supported by NIH grants ES 02330 and NS 14144).

291.9 DOES MAMMALIAN FAST-TWITCH MUSCLE POSSESS TWO DELAYED-RECTIFIER CHANNELS? P.L. Donaldson & K.G Beam*, Department of Physiology & Biophysics, University of Iowa, Iowa City, IA. 52242.

Based on tail current experiments, Adrian, et al., (J. Physiol. 208: 645-668, 1970) have suggested that voltage activates both fast and slow delayed-rectifier channels in amphibian twitch muscle. We have used the three-microelectrode technique of Adrian et al., (1970) to measure the delayed-rectifier currents in fast-twitch, skeletal muscle of rats at 15° C. The external solution contained (mM): Na+ (151), K+(5), Ca++(10), Mg++(1), HEPES (10) pH=7.4, methylsulfate (146), acetate (27), sucrose (400), and 1 μM TTX. Tail currents were measured at clamp potentials of -120 to -40 mV, which were applied to the muscle following a 25 ms conditioning pulse to either -10 or +10 mV. The reversal potential (E_{rev}) for the tail currents ranged from -90 to -60 mV. The outward tail currents could in most cases be adequately fit with a single exponential, whereas the inward tail currents showed both a fast and a slow phase of decay (τ≈5.0 & 250 ms, respectively, at -90 mV). Increasing the duration of the conditioning pulse caused a depolarizing shift in E_{rev} and a roughly proportional increase in both components of the inward tail currents, suggesting that both are affected by accumulation of potassium within a restricted extracellular space. In experiments where the conditioning pulse elicited relatively small currents, exponential fits of the tail currents revealed that the conductance of either the outward tails or of the inward fast phase agreed reasonably well with the conductance at the end of the conditioning pulse. When the conditioning pulse elicited large currents the agreement was not as close, especially for the rapid inward tails, which tended to meld with the capacity transient. Replacement of external K+ with 5 mM Rb+ abolished inward rectifier currents (over the range -90 to -120 mV). Rb+ also eliminated the slow component of the inward tail currents, after both 25 and 100 ms conditioning pulses. Thus, it appears that the slow phase of the inward tail current is predominantly carried through the inward rectifier.

Rb+ caused the delayed-rectifier channels to rectify: the conductance at the beginning of the outward current tails was approximately equal to the conductance at the end of the conditioning pulse; in contrast, the conductance of inward tail currents was reduced. Compared to tail currents in K+, the decay of inward, but not outward, tail currents in Rb+ was considerably slowed. Activation kinetics were unaffected by Rb+.

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291.10 HYPERPOLARIZING REGENERATIVE RESPONSES IN DISSOCIATED FROG ADULT MUSCLE FIBRES AFTER PROLONGED CULTURING. M.I. Glavinovic[1], R. Miledi[2], Y. Nakajima[3], [1] Dept. Anaesth. Res. & Physiol., McGill Univ., Montréal, Canada, [2] Dept. Biophysics, Univ. College, London, UK, [3] Dept. Biological Sci., Purdue Univ., Lafayette, IN, U.S.A.

If a depolarizing current pulse is given to a normal fibre of the frog's lumbricalis muscle or to dissociated muscle fibre kept in tissue culture for several days, an action potential is generated when the membrane potential reaches the threshold. After prolonged culturing of the muscle fibres (4-7 weeks) the action potential can still be elicited although it does not overshoot to as high a membrane potential as in normal muscle fibre and its time course is slower.

Moreover, in some but not all muscle fibres that were dissociated and cultured for a long period of time, a hyperpolarizing regenerative response (hyperpolarizing action potentials (h.a.p.)) could be elicited by hyperpolarizing current pulses when the membrane potential reached a threshold of ≈120 mV. Before this threshold value was attained the hyperpolarization had the form expected for an electrotonic change in membrane potential. After reaching the threshold the membrane potential increased slowly at first, and then more rapidly, reaching a peak, of as low as -240 mV, and then - though the current is still applied - it declined and stabilized close to the threshold, producing a characteristic spike-like appearance, that however lasted hundreds of milliseconds.

The time course as well as amplitude of h.a.p. depended on the intensity of the applied current. With stronger currents the rate of rise and decay were faster and the peak was at a more negative membrane potential. When the polarizing current ceased, the membrane potential quickly returned to the initial preset value (-90 mV). The shape of the h.a.p. was quite labile and depended not only on the current intensity but also on the frequency of application of current pulses, showing refractoriness that lasted tens of seconds.

The above experiments indicate that the regenerative hyperpolarizing responses that occur during continued applications of a constant current, were accompanied and perhaps resulted from an increase in membrane resistance, followed with a subsequent decrease in resistance. Further experiments are necessary in order to determine the ionic mechanism of the hyperpolarizing responses.

291.11 PRODUCTION OF DEPOLARIZING AFTERPOTENTIALS BY HIGH INTERNAL AND EXTERNAL CALCIUM CONCENTRATIONS. Michael Merickel, Richard Gray and R. Blaine Moore*, Department of Neurology, Baylor College of Medicine, Houston, Texas 77030.

We have been interested in studying the involvement of various ionic events underlying the action potential (AP) afterhyperpolarization and repetitive firing behavior of myotubes in primary cultures prepared from adult human biopsy specimens. These studies are motivated by previous work demonstrating that myotubes from patients with Myotonic Muscular Dystrophy often exhibit depolarizing afterpotentials (DAP's) as well as an increased tendency to repetitively fire AP's (Merickel et al, PNAS 78:648-652, 1981).

Initial experiments suggested that the AP hyperpolarization (AHP), typically approx. 10 mV negative to the resting potential (RMP), is not primarily dependent on a Ca^{++}-dependent K^+ current: 1) Substitution of Ba^{++} for Ca^{++} only slightly reduced the AHP amplitude, increased input resistance but did not significantly alter AP duration; 2) Attempts to inject EGTA to chelate $[Ca]_i$ had little or no effect on the AHP amplitude, suggesting the importance of a voltage dependent K^+ current. Unexpectedly, increasing $[Ca]_o$ from 2 to 10 mM caused the AHP to become depolarizing (i.e., producing a DAP). Single electrode voltage clamp experiments demonstrated that the 10 mM $[Ca]_o$ treatment produces a reduction in amplitude and slower time course of activation of net outward current which is probably responsible for the reversal of the AHP. Rapid reversal of the AHP could also be obtained by raising $[Ca]_i$ by inserting an electrode containing 0.5 M $CaCl_2$ and 0.5 M KCl (to permit simultaneous recording) for less than a minute. The reversal of the AHP could be observed after removal of the Ca^{++}-containing electrode by reimpalement with a "normal" KCl electrode. Continued recording with a KCl electrode demonstrated that the afterpotential of the myotube gradually becomes hyperpolarizing, returning to a pre-Ca^{++} shape within 30 to 60 minutes. Neither the reversal of the AHP by increased $[Ca]_o$ or $[Ca]_i$ was associated with a significant change in RMP, indicating that the appearance of the DAP's is not due to a shift in RMP. These experiments suggest that higher than normal levels of internal Ca^{++} are able to reduce the net outward current resulting in DAP's by an unknown mechanism.

The production of DAP's by high internal Ca^{++} is being explored as a possible mechanism accounting for the DAP's and increased repetitive firing activity observed in myotubes from patients with Myotonic Muscular Dystrophy. (Support from the Muscular Dystrophy Association and Kleberg Foundation)

291.12 MODULATION OF THE ACTION POTENTIAL AND IONIC CURRENTS IN PRIMARY CULTURED RAT MUSCLE. R. Gray & M. Merickel. Dept. Neurology and Prog. in Neurosci., Baylor Col. of Med., Houston, TX 77030.

The ionic basis of the action potential (AP) in cultured embryonic rat muscle was studied by comparing changes in ionic currents with changes in the AP in response to ion substitutions and ion channel blocking agents. Ionic currents were measured in isopotential myoballs with a two electrode voltage clamp. Step depolarizations from -60 mV produced a transient inward current which developed into a net outward current, followed by an outward tail current after return to -60 mV. The AP is followed by an afterhyperpolarization which is due to a conductance increase to K and reverses near -78 mV, similar to the reversal potential for the tail current. The AP and all observable rapid inward current were blocked by 1 μM TTX, indicating a Na component of the spike.

Additionally, the AP contains an inward Ca component which activates a slow outward K current based on the following evidence: 1) When $[Ca]_o$ was increased from 2 to more than 10 mM in the presence of TTX an all-or-none AP could be elicited and the AHP was increased in amplitude and duration. 2) 10 mM $[Ca]_o$ caused a small increase in outward currents during clamp commands more depolarized than -30 mV and a larger increase in the outward tail current. 3) Blockage of calcium channels by substitution of Ni for Ca or by addition of verapamil reduced AP and AHP amplitudes as well as outward and tail currents. 4) Substitution of Ba for Ca resulted in membrane depolarization and repetitive firing of APs with long plateau phases. The net outward current was markedly decreased and the tail current became inward after large depolarizing commands.

Reduction of K current by tetraethylammonium ions (TEA) caused a broadening of the spike, a decreased AHP and repetitive AP generation. The net outward current during step commands and the tail current were also reduced. Little change was seen in outward currents during commands in TEA + 10 mM $[Ca]_o$ from that with TEA alone, but the tail current became inward after large depolarizations. These results suggest that the AP in cultured rat muscle contains a Ca component which normally plays a small role compared to Na during the rapid phase of the spike, but a larger role as a modulator of a slow K conductance most easily seen in the AHP and tail current measurements. Support is acknowledged from Muscular Dystrophy Association and the Kleberg Foundation.

291.13 CYCLIC NUCLEOTIDE MODULATION OF NA^+ AND K^+ CURRENTS IN PERIPHERAL NERVE NODE OF RANVIER. T.L. Seelig* and J.J. Kendig. Dept. of Anesthesia, Stanford Univ. Sch. of Med., Stanford, CA 94305.

There is evidence that cyclic nucleotides, and the enzymes necessary to metabolize them, exist within the nodal region of peripheral nerve axons. An important question is whether their localization within the axon is functionally significant in affecting nerve conduction. This possibility was explored electrophysiologically in the sciatic nerves of Xenopus laevis by examining their responses to dibutyryl adenosine 3':5' cyclic monophosphate (d-cAMP).

In 6 preliminary experiments using sucrose gap recording of whole nerve there was a consistent and marked increase in the amplitude of the compound action potential, and a hyperpolarizing drift in the baseline, in response to d-cAMP, or SQ20009, a phosphodiesterase inhibitor.

Specific parameters of this effect were studied using the voltage clamped node of Ranvier. Perfusion of the node with (2mM) d-cAMP resulted, in 4 out of 5 preparations, in (1) a marked increase in the amplitude of the early inward current, (2) an apparent increase in steady state inactivation (h), and (3) a longer time constant for the development of the late outward current.

There is evidence that cAMP may act in some neuronal cell bodies to modify K^+ conductance. To determine whether the action of d-cAMP at the node is dependent upon K^+ conductance changes, K^+ channels were blocked by addition of TEA to the normal frog Ringer's solution. In 3 nodes studied under these conditions d-cAMP had no effect on the amplitude of the Na^+ current, h, or the time constant of inactivation (T_h).

To look specifically at the K^+ conductance changes initiated by d-cAMP, Na^+ currents were blocked by replacing NaCl by choline Cl in the extracellular solution. Preliminary results in 2 nodes indicate that the voltage dependent K^+ current amplitude is increased as a result of d-cAMP treatment. Non-voltage dependent membrane conductance does not change as indicated by an unchanged leakage conductance throughout the experiments.

These findings suggest a role for intra-axonal cAMP in the modulation of action potential parameters. Although the exact mechanism for these effects can not yet be fully explained, it enables one to view the action potential as a much more plastic response than it is traditionally considered. This metabolic modulation of action potential parameters could be physiologically important in increasing the efficacy of specific synaptic connections. Supported by NIH Grants NS13108, GM22113, and ONR contract N00014-75-1021

291.14 TEA ENHANCES THE DEPOLARIZING AFTERPOTENTIAL IN SINGLE MYELINATED AXONS: Karen A. Scappaticci* and Ellen F. Barrett. Dept. of Physiology and Biophysics, Univ. of Miami Med. Sch., Miami, FLA. 33101.

Action potentials and afterpotentials were recorded intracellularly from peripheral myelinated axons, either motor axons innervating the dewlap extensor muscle of the lizard (Anolis sagrei) or axons dissected from the isolated sciatic nerve of the frog (Rana pipiens). All the solutions used with the lizard neuromuscular preparation contained 150 μM carbachol to block muscle contraction and all control solutions contained 2 mM calcium. Axons selected for study had resting potentials of -60 to -80 mV, and action potentials with peak amplitudes of 60 to 90 mV. Most axons showed a depolarizing afterpotential with a peak amplitude of 5-20 mV at the resting potential which decayed with a half time of 20-100 msec. Previous work by Barrett and Barrett suggested that this depolarizing afterpotential represents a passive capacitative discharge of the axon membrane through an internodal leakage pathway. In both preparations Tetraethylammonium (TEA, 5 mM) increased the duration of the action potential and markedly increased the amplitude and the time course of the depolarizing afterpotential. Increasing concentrations of TEA (10 and 25 mM) progressively increased the peak amplitude of the depolarizing afterpotential to up to 350% of control and increased its duration to up to 1 sec. These effects of TEA were slowly reversible. The fact that TEA enhances the depolarizing afterpotential in sciatic nerve axons lacking central and peripheral terminals indicates that TEA acts on the myelinated axon itself. These effects are due at least in part to blockade of potassium channels in the resting and depolarized axon, which would increase current flow through the depolarizing afterpotential leakage pathway and increase the passive time constant of the axonal membrane.

In 25 mM TEA motor axons in the lizard neuromuscular preparation showed a prolonged hump superimposed on the depolarizing afterpotential. This hump was blocked by 1 mM manganese, suggesting that the hump reflects a TEA-enhanced calcium action potential reflected electrotonically into the axon from the motor nerve terminal. Concentrations of TEA greater than 25 mM also produced repetitive activity in the lizard motor axons. Supported by the Muscular Dystrophy Association and USPHS grant NS-12404.

291.15 REPETITIVE FIRING CHARACTERISTICS OF MAMMALIAN MYELINATED AXONS: AN INTRA-AXONAL ANALYSIS. J. A. Ruiz*, J. D. Kocsis, and R. J. Preston*. (Spon. M. E. Smith) Dept. of Neurology, Stanford Sch of Med., and V. A. Medical Center, Palo Alto, CA 94304.

Intra-axonal recordings were obtained in vitro with glass microelectrodes from rat ventral root and sciatic nerve fibers. The excised nerves or roots were positioned across bipolar stimulating and recording electrodes in a nerve chamber. Axon impalements with resting potentials of at least -60 mV and action potential amplitudes of 60 mV or greater were selected for analysis. Inflections were occasionally observed on the rising phase of the action potentials. Depolarizing current passage through the recording microelectrode accentuated the inflections and fractionation of the impulse could occur at these points. The amplitude of the action potential was increased by passage of hyperpolarizing current and decreased by depolarizing current. Subthreshold depolarization often induced local responses. Most action potentials were associated with either depolarizing or hyperpolarizing afterpotentials. The amplitude of the hyperpolarizing afterpotential was increased by depolarizing current and decreased by hyperpolarizing current. Current-voltage curves were nearly linear and average input resistance was 18 megohms (N=12).

Current levels necessary for direct spike elicitation varied following either subthreshold depolarizing or hyperpolarizing constant current prepulses. Depolarizing prepulses were followed by a period of increased threshold; and hyperpolarizing prepulses were followed by a period of decreased threshold. The decrease in threshold immediately following hyperpolarizing prepulses was often sufficient to elicit anode break excitation. These threshold changes were both time- and voltage-dependent and may represent changes in sodium inactivation elicited by the prepulse.

Action potentials reliably followed nerve stimulation or intra-axonal pulses (dur.<2 msec) repeated at frequencies of 300 Hz or more. In contrast a long duration depolarization pulse typically produced only one action potential near the onset of the depolarization pulse.

These results suggest that action potentials at mammalian nodal membrane are readily elicited by discrete depolarizing steps. But, sustained depolarization does not elicit repetitive firing as occurs for many excitable membranes. (Supported in part by the National Multiple Sclerosis Society (RG 1365) and by the Veterans Administration.

291.16 CURRENT-VOLTAGE HYSTERESIS AND REPETITIVE FIRING IN MOLLUSCAN NEURONS. L. Donald Partridge. Dept. of Physiology, Univ. of New Mexico, Albuquerque, New Mexico, 87131

Molluscan neurons are capable of maintained repetitive firing at low frequencies, often at less than one spike per second. Such regularly firing neurons, when voltage clamped, exhibit a marked hysteresis in the semi-stationary current-voltage relationship. The study reported here was an investigation of the relationship between the current hysteresis and low frequency repetitive firing.

The currents responsible for this current hysteresis were isolated by stopping the triangle wave clamp command signal at a given voltage during either the depolarizing or the hyperpolarizing phase of the current-voltage curve. Currents measured on the rising phase were subtracted from those measured at the same voltage on the falling phase. This difference current was fitted by the sum of two exponentials, the slower of which had a time constant of 1 - 2 sec. The reversal potential of the slow component of the difference current suggests a potassium mechanism. Both this current and the hysteresis were greatly reduced by procedures known to block calcium currents.

A computer model for repetitive firing was extended to include a calcium activated potassium conductance using published values for calcium diffusion, sequestration, and exchange at the membrane. When the voltage in the model was varied as in a triangle wave voltage clamp, a current-voltage hysteresis was observed. This model exhibited a much more linear frequency vs. stimulus intensity relationship at low current intensities than did a similar model without calcium activated potassium conductance. Neurons with a pronounced hysteresis exhibited a similar linear range of the frequency vs. stimulus intensity relationship.

Other studies have related spike afterhyperpolarization and regulation of repetitive firing to a calcium activated potassium conductance. In the molluscan neurons studied here such a current which has a pronounced influence on the current-voltage relationship is seen to influence very low frequencies of repetitive firing.

291.17 IDENTIFICATION OF THE IMPULSE-RESPONSE FUNCTIONS OF A NEURON AND OF ITS PARTS. C.I. Valenzuela. Neural and Behavioral Biology Program, University of Illinois, Urbana, IL, 61801.

The observable behavior of a linear, time-invariant system is completely characterized by the system impulse-response function. In a previous report (C.I.Valenzuela, Soc. Neurosci. Abstr.,#36.8, 1980) the impulse-response functions (as seen from the soma) of a neuron and of its parts are identified when the neuron consists of a spherical soma concatenated to a finite-length cylindrical dendrite (SS-FCD). The present work develops a method for the more common case where the cylindrical dendrite (or equivalent cable) has a length greater than 3 space-constants. In such case the neuron can be modeled accurately by a spherical soma with an infinite-length cylindrical dendrite (SS-ICD) system.

The identification method was based on the following :

1. Proof that regardless neuron geometry :

$$\int_{t=0}^{t=\infty} H_N(t)\, dt = R_N$$

where : $H_N(t)$ is the neuron impulse-response function, and R_N is the neuron input resistance, both as seen from the soma.

However, for all practical purposes this integral converges to R_N when the upper integration limit equals $5\tau_m$, thereby providing a means to estimate τ_m.

2. Proof that for the SS-ICD system :

$$\int_{t=0}^{t=\infty} +I_{clamp}(t)\, dt = -V_0\tau_m/2R_{dn}$$

where : $I_{clamp}(t)$ is the clamping current (after the soma capacitive discharge) that results from voltage clamping the soma to the resting potential following the steady-state caused by step current, I_{step}, injection at the soma,
V_0 is the soma steady-state voltage prior to voltage clamping ($V_0 = R_N I_{step}$), and
R_{dn} is the dendrite input resistance as seen from the soma.

Thus this integral provides a means to estimate R_{dn}.

3. An algorithm developed to estimate (in the time-domain) the impulse-response function of a linear, time-invariant system given an arbitrary set of system input-output data.

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291.18 DELTA MODULATION THEORY CAN BE USED TO OPTIMIZE THRESHOLD HUNTING. L. Richard Carley* and S. A. Raymond (SPON: L. S. Frishkopf). Research Laboratory of Electronics, MIT, Cambridge MA 02139.

It is difficult to measure the firing threshold of nerve and muscle as a single valued function of time. To bound the threshold within a certain range requires that both a successful stimulus and an unsuccessful stimulus be applied to the system. The time interval between the two stimuli must be brief with respect to the rate of change in threshold. Since the first test stimulus will cause an immediate, lasting, and significant change in the threshold, such a measurement scheme fails because successive samples, spaced closely enough to capture all natural fluctuations in the threshold, would perturb the variable.

The french physiologist Pecher and others studied probability density function (PDF) of threshold in resting nerve and observed that outcomes of successive test stimuli were not significantly correlated, in frog sciatic nerve, if stimuli were applied $\simeq$2 s. apart. The most familiar way to use test stimuli to measure the PDF of the threshold is to count the successful fraction of repeated test stimuli of fixed strength. This method does not use information gained from previous trials and therefore takes more samples than necessary and is highly sensitive to drift. Threshold hunting refers to automated methods that use each response and failure of individual fibers to converge on threshold. A threshold hunter tracks changes in stimulus required to hold the firing probability at a fixed percentage (a fixed point on the cumulative distribution function (CDF)) as threshold changes.

Delta Modulation theory applies to threshold hunters that increment (Δ_{up}) or decrement (Δ_{dn}) the test stimulus after each trial. The fixed point on the CDF that the hunter tracks is *i)* $\Delta_{up}/(\Delta_{up}+\Delta_{dn})$. Using the theory we have defined 3 fundamental types of errors: (1) quantization error — occurs because the hunter's output is quantized into steps and is related to the size of the steps by *ii)* $[(\Delta_{up}+\Delta_{dn})^2/16]$, (2) fluctuation error — caused by the imperfect filtering of the natural fluctuations in the running threshold is *iii)* $[\sigma_T(\Delta_{up}+\Delta_{dn})/3.5]$, where σ_T is the standard deviation of the threshold fluctuations, and (3) tracking error — is inversely proportional to step size and is *iv)* $[\delta S/(\Delta_{up}+\Delta_{dn})]$, where δS is the difference between successive stimuli when tracking a fixed point on a moving CDF.

The speed of a threshold hunter can be greatly increased by any approach which reduces the information to be extracted from the nerve. For example, by predicting the derivative of the hunter's output, which can be done throughout an experiment by extrapolating from the accumulated data, the step sized can be adjusted dynamically. Thus the hunter's tracking rate can be matched to the rate of threshold changes, δS. Step sizes are adjusted so that their sum, Δ, is held constant (which keeps the quantization and fluctuation errors fixed). The predicted derivative, $\delta S'$, is used to predict the step sizes as in equations *v)* $\Delta_{up} = P\Delta + \delta S'$ and *vi)* $\Delta_{dn} = (1-P)\Delta - \delta S'$, where P is the firing probability being tracked.

By using several hunters in rotation, each set to converge on a different fixed point on the CDF, the actual shape of the CDF can be tracked.

291.19 NORMAL AND ABNORMAL SIGNAL PATTERNS IN NERVE CELLS. Gail A. Carpenter. Dept. of Mathematics, Northeastern University, Boston, MA 02115.

A fundamental problem of psychophysics is an inverse problem: induce underlying cellular or network mechanisms from signal patterns. The patterns may be observed at various levels: from EEG, extracellular, or intracellular recordings or from psychological experiments. In each case the recorded pattern is an ensemble of events taking place at a finer, and usually unobservable, level. At the center of the present work is the following simple question: which intracellular signal patterns are the result of basic single-cell membrane mechanisms and how are these patterns altered as membrane parameters change? In other words, we consider the inverse problem at the level of the single cell.

A generalized Hodgkin-Huxley (HH) model is defined and mathematical analysis leads to many predictions about the patterns of action potentials. "Prediction" here means a property of solutions of the model in question. If the prediction fails to hold in a particular case, then the observed effect must be due to mechanisms not embodied in the model. For example, the effect could be due to additional ionic currents, slow parameter shifts, or network interactions. Examples are given in which the predictions of the basic model fail, forcing modifications for those cases. With the properties of the core model established, it is relatively easy to see how changes in the model change the solution patterns. (The simplified FitzHugh-Nagumo model is also discussed from the point of view of the present analysis.) The results show that generalized Hodgkin-Huxley models with only the usual Na^+ and K^+ current variables predict complex phenomena previously ascribed to more intricate mechanisms. Many of the predictions focus on burst and regular (evenly-spaced) periodic signal patterns. It is shown, for example, that the HH model predicts the existence of two types of regular periodic patterns or action potentials. One pattern is, in fact, a sequence of "bursts with one spike per burst". The two classes of regular periodic spikes are predicted to behave differently under parametric shifts, and once this fact is observed, examples of the two types are found in the experimental literature.

291.20 EXCITABILITY IN SIMULATED PROTOCELLS FROM PROTEINOID, Aleksander Przybylski* and Sidney W. Fox. Institute for Molecular and Cellular Evolution, University of Miami, Coral Gables, FL 33134.

Vesicles of proteinoid (thermal copolyamino acid) and lecithin display such properties as compartmentation, the abilities to proliferate and to communicate upon conjugation, enzymic activities including the ability to synthesize peptides, and permselective membranes (Fox and Dose, *Molecular Evolution and the Origin of Life*, rev. ed., Dekker, 1977; Nakashima and Fox, *J. Mol. Evol.* 15 161-168, 1980). These results prompted a search for excitability, which has been reported (Ishima and Fox, Abstract Third Annual Meeting, Society for Neuroscience, 17.10, 1973). The studies have been extended (Ishima, Przybylski, and Fox, *BioSystems*, in press).

The ability to form vesicles and to undergo electrical discharge varies with the identity of the proteinoid. Spiking is usually stimulated by shifts in ion concentration in the solution bathing the vesicles. In some cases, spiking is spontaneous; this latter phenomenon is explained by compartments, which are in turn induced by reaction of Ca^{++} with proteinoid. Vesicles are self-healing, and are capable of microencapsulating a number of substances. The microsystems thus permit modelling cellular events, especially those of the neuronal type.

A variety of action patterns has been observed (Fig. 1); some, for example, resemble discharge in *Aplysia* neurons (Chen, von Baumgarten, and Harth, *Pflügers Arch.* 345 179-193, 1973).

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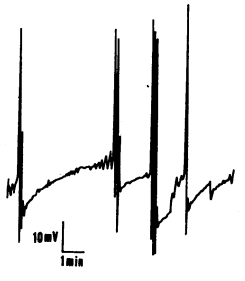

Fig. 1. Discharge of simulated protocell.

292.1 A DISTINCT ACTIN BINDING PROTEIN IN BRAIN. Robert L. Beach, E. Edward Mena and Carl W. Cotman. Dept Psychobiology, Univ. of California, Irvine, Ca. 92717.

Neuronal cytoskeletal proteins may play important roles in the development and function of the brain. Since actin has been found to be a major component of isolated synapses, and has been implicated in processes such as axon elongation and cellular movements, we have attempted to identify other proteins in brain which may interact with actin. We recently described the presence of a brain myosin, which was distinct from muscle myosins, in synaptic plasma membranes (SPM) and synaptic junctions (SJ).

In the present study we have isolated from brain a high molecular weight protein which binds reversibly to actin filaments. Purification was acheived by ammonium sulfate fractionation, coprecipitation with actin filaments and agarose gel filtration. This protein is a doublet of M.W. about 240,000 and 250,000 daltons on SDS PAGE in the presence of reducing agents, and it comigrates with macrophage actin binding protein. The binding of this protein to actin is reversed in the presence of elevated concentrations of KCL. This actin binding protein is widely distributed in subcellular fractions of brain, and is a signifigant component of SPMs and SJs. However it is solubilized along with the membranes when either of these fractions are treated with n-lauryl sarcosinate to prepare postsynaptic densities. This suggests that this protein may be associated with the membrane component of the former fractions.

We have compared the properties of this protein with other similar high molecular weight proteins. Fibronectin, which has recently been reported to bind actin, nearly comigrates with the actin binding protein doublet, but is distinguishable from this protein by other criteria. Fibronectin is a glycoprotein and it binds ^{125}I-Concanavalin A after gel electrophoresis, however the brain actin binding protein doublet does not. Furthermore two dimensional peptide maps of fibronectin and the brain protein show that the two proteins are distinct. Filamin is a high molecular weight actin binding protein, which was originally found in smooth muscle, but it has been identified in other cells. We have labelled filamin following SDS PAGE using antibody to filamin and ^{125}I-Protein A. The brain actin binding protein doublet does not bind antibodies to filamin. We have also compared this protein with brain myosin heavy chain (M.W.=200,000). The actin binding protein does not bind antibodies to myosin, and the two dimensional peptide maps of these proteins are distinct. Furthermore, unlike myosin, the association of brain actin binding protein is not reversed by ATP. Thus it appears a distinct actin binding protein is present in brain and is associated with subcellular fractions including SPMs and SJs.

292.2 CALCIUM-BINDING PROTEIN IN THE RAT BRAIN. S.C. Feldman and S. Christakos* Depts. of Anatomy and Biochemistry, CMDNJ-New Jersey Medical School, Newark, N.J. 07103.

A vitamin-D dependent calcium-binding protein (CaBP), molecular weight 28,000, has been localized in chick tissues, including CNS. In the rat, a calcium-binding protein with the same molecular weight has been identified in the CNS and shown to cross-react with antisera to the chick CaBP by Ouchterlony double-diffusion analysis (Wasserman, etal., 1977). The present study was undertaken to determine the concentration and distribution of this 28,000 molecular weight CaBP in the rat CNS, using a highly specific antiserum to chick intestinal CaBP (Christakos and Norman, 1980).

The concentration of CaBP in 4 areas of the rat brain was determined by radioimmunoassay (RIA): forebrain, hypothalamus, cerebellum and brainstem. The mean concentration of CaBP was 4.6 $\pm$ 0.8 ng/mg protein and did not vary significantly as a function of area. To determine if CaBP was present in neurons, the protein was localized by immunocytochemistry in Bouin's fixed section of rat forebrain, using the same antiserum as for the RIA. All staining in neuronal elements was judged specific as determined by the absence of staining in sections in which the antiserum had been previously adsorbed with CaBP. CaBP was present in neuronal elements, i.e., neurons and fibers in several areas of the rat forebrain. CaBP-containing neurons were found in: neocortex, primarily in layers II-IV; hippocampal formation, including a small number of cells in the granule layer of the dentate gyrus and pyramidal neurons of CA_1 but not CA_3 or CA_4; basal ganglia, including caudate/putamen and claustrum; hypothalamus, in scattered cells along the midline; thalamus, primarily in the mid-line nuclei; amygdala, in corticomedial and basomedial nuclei; and in substantia nigra, pars compacta. CaBP-containing fibers were present in most major tracts including corpus callosum, optic tract, stria terminalis, mammillothalamic tract and medial lemniscus.

In the chick, a 28,000 molecular weight, vitamin-D dependent protein has been shown to bind calcium. The presence of an immunologically identical molecule in the rat CNS suggests that this protein may have a role in neuronal function in the mammalian CNS. (Supported by a grant to S.C.F. from the Foundation of the College of Medicine and Dentistry of New Jersey).

292.3 CALCIUM-ACTIVATED, PHOSPHOLIPID-DEPENDENT PROTEIN KINASE FROM RAT BRAIN IS INHIBITED BY ACTH. V.J. Aloyo, M.A. Hoogkamer*, H. Zwiers* and W.H. Gispen. Inst. Mol. Biology, Division of Neurobiology, State University of Utrecht, 3508 TB Utrecht, The Netherlands.

Recently a calcium-activated phospholipid-dependent protein kinase (kinase C) from rat brain was described (Inoue et al., J. Biol. Chem., 252:7610, 1977). This enzyme had a molecular weight of 77K and was not activated by cAMP or cGMP. A number of phospholipids, such as phosphatidylserine and phosphatidylinositol, were able to activate this kinase (Takai et al., J. Biol. Chem., 254:3692, 1979).

We have partially purified kinase C according to the method of Inoue et al. After DEAE-cellulose column chromatography and gel filtration on Sephadex G-100, a protein mixture was obtained that was essentially free of substrate phosphoproteins and had a major protein band of 70K as determined by SDS-polyacrylamide gel electrophoresis.

The protein kinase activity present in this preparation was studied using whole histone as the substrate. Phosphorylation of histone was stimulated by the addition of phosphatidylserine and inhibited by chlorpromazine. No effect of cAMP could be detected. These results suggest that this kinase activity closely resembles the one described by Inoue et al. $ACTH_{1-24}$, which is a potent inhibitor of the phosphorylation of certain low molecular weight proteins in synaptosomal plasma membranes from rat brain tissue (Zwiers et al., Neurochem. Res., 1:669, 1976), did not inhibit the phosphorylation of histone by kinase C.

We have tested whether protein kinase C would phosphorylate the ACTH-sensitive phosphorylatable protein B-50 that was purified to homogeneity with isoelectric focussing as the last step in the purification procedure (Zwiers et al., J. Neurochem., 34: 1689, 1980). B-50 appeared to be a good substrate for kinase C, and its phosphorylation was stimulated by phosphatidylserine. No effect of cAMP was detected. But $ACTH_{1-24}$ did inhibit B-50 phosphorylation by kinase C.

Work is in progress to determine the possible identity of protein kinase C and the B-50 protein kinase as isolated by Zwiers et al. (J. Neurochem., 34:1689, 1980).

292.4 ALTERATIONS IN BRAIN mRNA LEVELS FOR SPECIFIC TUBULIN SUBUNITS DURING HUMAN DEVELOPMENT. M. Morrison and W.S.T. Griffin. Depts. Neurology and Cell Biology, The Univ. Tx. Hlth. Sci. Ctr., Dallas, TX 75235.

The ability to isolate undegraded mRNAs from human postmortem brain would facilitate the molecular analysis of various neurological diseases and allow comparisons to be made between prenatal and adult mRNA levels. Our control studies on isolation of cytoplasmic RNAs from rat cerebella showed that 60% of the cytoplasmic mRNA activity was retained after storage of the cerebella for up to 16 hrs at room temperature. Comparison of the abundant mRNA populations by two dimensional gel analysis of their in vitro translation products showed that the mRNAs isolated from stored cerebella were virtually identical to those obtained from fresh tissue. Comparison of mRNAs isolated from two human cerebella, 4 hrs and 16 hrs postmortem, also showed little evidence of selective mRNA degradation postmortem. Similar results were obtained with postmortem human cortex. These experiments show that abundant mRNAs characteristic of the in vivo tissue can be isolated from human postmortem brain. These RNAs can therefore be used to study changes in abundant mRNA populations during human development. The results from human adult cerebellum and cortex show that there is significantly less translatable mRNA for the α_1 and α_2 tubulins and the β_1 tubulin than there is for the major β_2 tubulin subunit. The proportions are therefore similar to those of the different tubulin subunits in adult rat cerebellum (Morrison et al. J. Biol. Chem. 256 (1981) 3550-3556). Analysis of mRNAs from human fetal cortex and cerebellum showed that the levels of translatable mRNAs for the α subunits and the β_1 subunit were dramatically increased relative to those in the adult. These proportions were again similar to the different mRNA levels in the neonatal rat although the levels of the β_1 tubulin subunit mRNA were relatively higher in human fetal cortex than in neonatal rat cortex. Further, the relative amounts of this mRNA were always higher in the cortex than in the corresponding cerebellum. These results suggest that mRNA levels for the minor β subunit are higher in glia-rich brain areas such as cortex than in neuron-rich areas such as cerebellum. The different developmental and regional patterns of tubulin mRNAs may be a reflection of a diversity of roles for the different forms of α and β tubulins during brain development and or within different brain cell populations.

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292.5 ISOLATION OF BRAIN TUBULIN SPECIES FROM IEF GELS. K. von Hungen*, J. Khoury*, R. C. Chin* and C. F. Baxter (SPON: H. Hirsch). Neurochemistry Labs, V.A. Medical Center, Sepulveda, CA 91343.

Brain tubulin is now known to be composed of several closely related species. The relative amounts of these species change during early development for both the alpha and beta subunits (Dahl and Weibel, BBRC, 86:822, 1979), but not during advanced aging (von Hungen et al., J. Neurochem., in press). The species can be separated on isoelectric focusing (IEF) gels (banding between pH 5.2 and 5.4), but the patterns observed are critically dependent upon conditions of the run and on the ampholytes used. It is thus difficult to determine the exact number and relative quantity of species. We have achieved good resolution of species using Bio-Rad pH 4-6 ampholytes. The differences in species which give rise to different isoelectric points are not known, and we have attempted to isolate individual species in order to be able to determine what the differences are. We have found that the tubulin species can be visualized by placing IEF gels in distilled water (or dilute Tris buffer). This permits cutting out of very close bands without staining the gel. Dissected discs from the IEF gels can then be run on SDS slab gels to remove ampholytes. The species are visualized on the SDS gels with KCl solution, cut out, electrophoretically eluted, dialyzed and lyophilized. Using this procedure, we have purified 5 species associated with the alpha subunit and 10 species associated with the beta subunit. When run again on IEF gels, the species run at their original position. Amino acid analyses on these species are in progress. Early data indicate several possible amino acid differences, and notably in one unidentified basic molecule in the protein hydrolysates.

The procedure used for isolation of tubulin species from IEF gels may be of value for isolation of other proteins as well. Tubulin species and several other proteins form white bands in the IEF gel within minutes of being placed in water. These bands are stable indefinitely, although the background increases noticeably after a few days. The gels can be scanned for light scatter (e.g. at 300 nm). Ampholytes, at least those we have tested (Biolytes), do not interfere, while in stained gels interference from ampholytes is frequently a problem. The absorbance appears linear for low concentrations of proteins. Resolution is nearly as good as in stained gels. Sensitivity is good; less than one microgram of many proteins is easily detectable. The sensitivity, however, appears to vary considerably with different proteins. The method works well with tubulin, BSA and alkaline phosphatase, but not with urease. Further characterization of the method with additional proteins will be presented.
(Supported by the Medical Research Service of the V.A.)

292.6 CHANGES OF CYTOSKELETAL PROTEINS IN THE PERIKARYA AND AXONS OF REGENERATING FROG MOTONEURONS. D. V. Sinicropi and D. L. McIlwain. Department of Physiology, University of North Carolina, Chapel Hill, NC 27514

Changes in the amounts of tubulin, actin, and neurofilament proteins were sought in regenerating motoneurons of Rana pipiens. Ventral roots 9 and 10 were unilaterally transected approximately 7 mm from the spinal cord. One to 75 days later, 3 types of samples were taken for biochemical analyses: a) spinal cord segments 9 and 10 divided at the midline into left and right halves, b) left and right ventral roots 9 and 10 from the spinal cord to the site of transection, and c) motoneuronal perikarya isolated from the left and right halves of spinal segments 9 and 10 by a new method employing bulk separation techniques followed by the collection of individual cell bodies with micropipettes. Aliquots of spinal cord and ventral root homogenates were assayed for tubulin by a [^{3}H]colchicine-binding method and for total protein, and another aliquot was analyzed by two-dimensional (2-D) electrophoresis. Proteins extracted from pooled samples of 100-150 isolated motoneuronal perikarya were also analyzed by 2-D electrophoresis. Gels of experimental and control samples were stained with either Coomassie Blue or silver and were compared visually, using ovalbumin as an internal standard. Actin, α and β tubulin, and the lowest molecular weight subunit of neurofilaments (NF1) were tentatively identified on gels by their comigration with purified samples of these proteins.

Data reported below were obtained from animals which were allowed to survive 20-35 days after axotomy, at which time the changes in several proteins became maximal (unpublished) and the tips of regenerating motor axons had exited the vertebral column but had not yet reinnervated muscles. Binding of [^{3}H]colchicine decreased to 50% of control in the transected ventral roots, but no decrease was detected in spinal cord samples. NF1 was markedly decreased, and α and β tubulin also appeared to be decreased in 2-D gels of transected ventral roots. In contrast, the amount of actin was increased in the transected ventral roots. In axotomized perikarya the amount of NF1 was increased compared to controls. Changes in the amounts of actin and tubulin in axotomized perikarya were less obvious and will require quantitative analyses. Quantitative analyses of the 2-D gels by a television-based densitometric method (Aycock et al., 1981, Comput. Biomed. Res., in press) are in progress.

One possible explanation of these data is that the export of neurofilaments and perhaps other cytoskeletal proteins from motoneuronal perikarya is altered after axotomy.

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292.7 COMPOSITIONAL MAKEUP OF REGENERATING GOLDFISH AXONS AND IDENTIFICATION OF LOCALLY SYNTHESIZED PROTEINS. E.Koenig and P.Adams* Div. Neurobiology, State Univ. of NY, Buffalo, NY 14214.

We have been utilizing the goldfish retinal explant system, developed by Landreth and Agranoff (Brain Res., 118:299, 1976) to study compositional makeup and the capacity of regenerating axons to synthesize proteins. The explants, when cultured in the presence of 5-deoxyuridine (Johns, et al., Brain Res., 142: 531, 1978), yield axon fasicles free of periaxonal investment and non neural cells (unpublished observations). After outgrowth, axonal fields can be severed from their attachment to explants and incubated with radioactive amino acids. Using direct quantitative microanalytical methods, we have demonstrated that cycloheximide inhibits local incorporation by 80% (Koenig and Adams; submitted). Present studies are concerned with characterizing compositional makeup of major endogenous proteins in regenerating axons and identification of proteins labelled during in vitro incubation with a mixture of ^{3}H-amino acids. For this purpose a gel microslab system, developed in this laboratory, is being used. SDS gel patterns indicate that tubulin and actin are the most abundant proteins present. The molecular forms of neurofilament polypeptides in mature goldfish axons are different from those reported for mammals. Based on relative mass concentration, the putative neurofilament oligomers in goldfish have nominal molecular weights of 145K, 135K and 120K. While a 68K polypeptide is present, it is a relatively minor component. In regenerating axons, the 145K putative neurofilament polypeptide is readily discernible, but it is uncertain whether the 135K and 120K polypeptides are present in significant amounts. These findings are consistent with electron microscopic studies, which indicate occasional single neurofilaments that are randomly distributed in these growing axons. Fluorograms of regenerating axonal extracts separated in the gel microslab system show three principal peaks, corresponding to tubulin, actin and the 64-67K region of the gel. Microcombustion of excised tubulin, actin and the 64-67K portions of the microelectrophoretograms containing control and cycloheximide-treated extracts show that about 25% of the tubulin labelling is cycloheximide-insensitive. Because ^{3}H-tyrosine is present in the amino acid mixture, it seems likely that posttranslational labelling is due to endogenous tubulin tyrosine ligase activity. These preliminary findings indicate that there is an apparent supplementation by local synthesis of two major classes of structural proteins essential for axonal growth.

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292.8 POST-MORTEM STABILITY OF PROTEINS WITHIN BOVINE SPINAL MOTONEURONS: A TWO-DIMENSIONAL ELECTROPHORETIC ANALYSIS. Thomas O. Brock, III and D. L. McIlwain. Neurobiol. Prog. and Dept. Physiology, *Sch. Med., Univ. N. Carolina, Chapel Hill, N.C. 27514*

An issue in the use of post-mortem nervous tissue for the study of neurological diseases is the quality of specimens necessarily taken several hours after death. The following experiments were performed to evaluate the effect of harsh post-mortem conditions on the proteins of spinal motoneurons. Perikaryal and axonal proteins extracted from motoneurons of bovine spinal tissue that had been stored at room temperature for one day were compared to proteins from motoneuronal cell bodies isolated promptly from the same animal. Cervical and lumbar enlargements were removed from each of four spinal cords which had been placed on ice within 30 min of slaughter. The enlargements were transected and cell bodies of spinal motoneurons were isolated from combined lumbar and cervical halves of each spinal cord by the procedure of Weil et al. (J. Neurochem. 29: 847-852, 1977). The four purified cell body preparations, along with intradural ventral roots from the same enlargements, were stored at -70°C. The remaining cervical and lumbar halves were wrapped in plastic and left at 24° ± 1°C for one day, after which motoneuron cell bodies were isolated and stored at -70°C with ventral roots from the same day-old tissue.

Total protein was extracted from the isolated perikarya and ventral roots, using O'Farrell's lysis buffer (J. Biol. Chem. 250: 4007-4021, 1975) to which 2% SDS had been added. Proteins were separated by 2-D electrophoresis (O'Farrell, supra vide) and stained with Coomassie blue or silver. Based in part upon light and electron microscopic evidence from sections of bovine ventral roots, we believe that most of the proteins extracted from ventral roots originate from the motor axons, rather than from myelin and other sources. Visual comparisons of 150-200 proteins from gels of motoneuronal perikarya and ventral roots revealed no qualitative differences between control and one-day groups. A few quantitative differences were apparent to the eye and were reproducible from animal to animal. The gels are currently being analyzed by computer-assisted densitometry (Aycock et al., Computers and Biomed. Res., 1981, in press).

These results show that even at room temperature the major proteins in bovine spinal motoneurons are remarkably stable during the first day after death.

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292.9 METABOLISM OF γ-GLUTAMYL-POLYAMINES IN THE APLYSIA NERVOUS SYSTEM: EVIDENCE FOR TRANSGLUTAMINASE AND γ-GLUTAMYLAMINE CYCLOTRANSFERASE ACTIVITIES. Leon Kremzner* and Richard Ambron (sponsor J. Correll), Depts. Rehab. Med., Anatomy, Neurology, P&S, Columbia Univ., N.Y., N.Y., 10032.

Recent studies by a number of investigators have shown that intracellular proteins are post-translationally modified by the covalent incorporation of polyamines; a process catalyzed by one or more Ca^{++}-dependent transglutaminase enzymes. These findings are of significance since transglutaminases are able to directly cross-link proteins. Hence, they may be involved in the modification and stablization of membrane properties (Davies et al., 1980). We have now shown that ^{3}H-putrescine, when injected into the cell body of R2, the giant neuron of Aplysia californica, is metabolized to GABA, spermidine, and spermine. One or more of the polyamines are also incorporated into cellular proteins. Incorporation of ^{3}H-putrescine in an amide linkage at the carboxyl group of protein-bound glutamic acid was demonstrated by exhaustive proteolytic digestion, followed by the identification of the covalently bound putrescine as N-(γ-glutamyl)-^{14}C-putrescine, using an automatic ion exchange column chromatographic procedure. Free ^{14}C-putrescine was liberated from this compound after acid hydrolysis. At least two other polyamine derivatives were also formed.

The existence of transglutaminase activity in Aplysia was also shown by incubation of nervous tissue homogenates with ^{14}C-putrescine, followed by proteolytic digestion and isolation of N-(γ-glutamyl)-^{14}C-putrescine. Recent studies (Fink, Chung, and Folk, 1980) in rabbits, have demonstrated a catabolic pathway for γ-glutamylpolyamines, involving the enzyme γ-glutamylamine cyclotransferase. This enzyme acts on γ-glutamylamines to liberate the free polyamine and 5-oxo-L-proline (5-pyrrolidone-2-carboxylic acid). We have now demonstrated the cyclotransferase activity in Aplysia ganglia. The physiological significance of these findings is being investigated by the isolation and identification of the specifically modified neuronal proteins (Ambron and Kremzner these proceedings). (Supported in part by Muscular Dystrophy Foundation, NIH, and a Career Development Award to RA)

292.10 PROTEIN SYNTHESIS IN THE RABBIT RETINA FOLLOWING THE INTRAVENOUS INJECTION OF LSD. Bruce D. Clark* and Ian R. Brown, Dept. of Zoology, Scarborough College, University of Toronto, West Hill, Ont. M1C 1A4, Canada.

Polysomes were isolated from the retinas of young adult rabbits and separated into various size classes by centrifugation on 15-45% sucrose gradients. The intravenous injection of LSD at 100 µg/kg induced a disaggregation of polysomes to monosomes in the retina. This effect was transient with maximal disaggregation 1 hr after drug injection and a return to control polysome levels by 4 hr. The disaggregation of retinal polysomes was not the result of RNase degradation of polysomal mRNA since the 80s monosome peak which accumulated following LSD was dissociable in high salt to 40s and 60s ribosomal subunits. Monosomes generated by digestion of polysomes with RNase were not dissociable under these conditions. Our previous results suggest that LSD-induced hyperthermia is involved in the disaggregation of polysomes in total cerebral hemispheres of the rabbit brain and in the induction of a brain protein which is similar in molecular weight (i.e. 74,000) to one of the major 'heat shock' proteins previously reported to be synthesized in tissue culture systems following elevation of ambient temperature. (Freedman et al., Brain Res. 1981, 207, 129-145; Cosgrove et al., J. Neurochem. 1981, 36, 1037-1045; Heikkila et al., J. Neurochem. 1981, 36, 1229-1238). Our present studies indicate that during LSD-induced polysome disaggregation in retina, induction of synthesis of a retinal protein of molecular weight 74,000 is observed in vivo following intra-vitreal injection of [^{35}S]-methionine 20 min after intravenous administration of LSD at 100 µg/kg. Induction of synthesis of the 74,000 molecular weight retinal protein was also observed following translation in a reticulocyte cell-free system of residual polysomes isolated 1 hr following intravenous injection of LSD at 100 µg/kg. [^{35}S]-methionine labeled products from both the cell-free translation system and the in vivo labeling experiments were analyzed by two-dimensional polyacrylamide gel electrophoresis followed by fluorography. (Supported by the Medical Research Council of Canada).

292.11 EFFECTS OF THE SODIUM IONOPHORE MONENSIN ON SYNTHESIS AND PROCESSING OF NEUROSECRETORY PROTEINS IN APYLSIA BAG CELLS. Michael E. Yates and Robert W. Berry, Dept. of Cell Biology and Anatomy, Northwestern Univ. Med. School, Chicago, IL. 60611.

Inhibition of the cell secretory process by the sodium ionophore monensin, through an alteration in formation of Golgi-derived secretory vesicles, has been documented in several non-neural systems (Tartakoff and Vassalli, J Cell Biol 79:694, 1978). More recently, it has been suggested that monensin is able to reduce in amount, at the Golgi appartaus, all ^{3}H-labeled protein destined for fast axonal transport (Hammerschlag, Stone, and Bolen, Trans Am Soc Neurochem 12:144, 1981). These results led us to an investigation of the effects of monensin on a well defined proteolytic processing sequence leading to the formation of neurosecretory products in the bag cells of Aplysia. These cells produce two ca. 4500 Dalton (4.5K) secretory peptides, ELH and AP, from a 29K precursor via a 6-9K intermediate (Berry, Baylen, and Trump, this volume). An additional non-secreted 12K product is produced concomitant with the 6-9K intermediate. We monitored the effect of 10^{-6}M monensin on this processing sequence using pulse-chase labeling protocols in which members of the sequence were identified by mobility on SDS-PAGE.

This concentration of monensin did not inhibit total protein synthesis, but if present for 2hr prior to and during a 6hr labeing period, increased the amount of label in the 29K precursor by 114%. This was accompanied by a 40% reduction in the labeling of both the 12K product and 6-9K plus 4.5K material, indicating a partial blockade of the initial cleavage of the 29K precursor. Furthermore, while the 4.5K products accounted for 20% of the label in control cells at this time point, none was produced in the presence of monensin, suggesting that cleavage of the 6-9K intermediate is also impaired. This was confirmed by labeling cells for 2hr, then chasing for 4hr in the presence of monensin. This produced a mean increase in amount of 6-9K material of 100%, accompanied by a 50% decrease in each of the 4.5K products. These data suggest that monensin blocks two sites in the precursor-product sequence leading to the production of neurosecretory products in the bag cells: 1) the initial cleavage of the 29K precursor, and 2) the subsequent cleavage of the 6-9K intermediate leading to the final products. It seems likely that these processing blockades may reflect impairment of Golgi apparatus function, but this remains to be determined. Supported by NIH grant NS-11519 (RWB).

292.12 IMMUNOCHEMICAL PURIFICATION OF MESSENGER RNA FOR MYELIN BASIC PROTEIN. A.A. Akowitz* and J.H. Carson*. (Spon: S. Pfeiffer). Dept. of Biochemistry, Univ. of Conn. Health Center, Farmington, CT 06032.

An immunochemical procedure for the isolation of myelin basic protein (MBP) specific polysomes from mouse brain has been developed. Brains from 15 day old C57BL mice were homogenized (10% w/v) using a Polytron homogenizer in medium containing .25M sucrose, .025M NaCl, .05M Tris-HCl, .005M MgAc, .007M Mercaptoethanol, 10 units/ml Heparin, 10mM Vanadyl Ribonucleoside. The homogenate was centrifuged at 1,000xg for 30 minutes to remove myelin. Deoxycholate (1%) and Triton X 100 (1%) were added to the supernatant and the polysomes were centrifuged through a 1.5M sucrose cushion at 100,000xg for 3-1/2 hours. The polysomes were resuspended in buffer containing .1M sucrose, .025M Tris, .05M NaCl, .005M MgAc, .007M Mercaptoethanol, 5 unit/ml Heparin and centrifuged through a cushion of 1M sucrose. This step removed adventitiously bound MBP. The recovery of polysomes was 9.0 OD_{260} units/gram brain wet weight.

MBP specific polysomes were isolated from total polysomes by immunoabsorption chromatography. Affinity purified antibody to mouse 14K MBP (7.6 mg IgG with a capacity to bind 8.4 ug MBP/mg) was bound to a Protein A sepharose column. The total polysome fraction (413 OD_{260} units containing less then 8ug MBP) was applied to the column. The column was washed with resuspension buffer to remove unbound polysomes. MBP specific polysomes were eluted with 1% SDS in resuspension buffer. The MBP specific polysomes isolated in this manner constituted 3% of the total polysome fraction and contained 40ng MBP/OD_{260} unit.

Poly A containing mRNA was isolated from the total polysome fraction, the unbound polysomes and the MBP specific polysomes by phenol chloroform extraction and affinity chromatography on poly U sepharose. The MBP coding potential of the isolated mRNA was analyzed by in vitro translation and immunoprecipitation with antibody to MBP. The total polysomes and the MBP specific polysomes were shown to contain mRNA for MBP. The unbound polysomes contained no detectable mRNA for MBP.

292.13 INHIBITION OF DNA SYNTHESIS BY THE NEUROCARCINOGEN ENU ADMINISTERED IN UTERO OR IN WEANLING RATS. M. J. W. Chang and R. W. Hart*. Dept. of Radiology, The Ohio State Univ. Columbus, OH 43210 and Natl. Ctr. Toxicol. Res. Jefferson, AK 72079.

Ethylnitrosourea (ENU) is a perinatal neurocarcinogen and teratogen in rats. The effects of ENU on DNA content and rate of DNA synthesis were determined in: 1) offspring of Sprague-Dawley rats treated in utero and 2) 30 day old female BD IV rats. At various times after a single dose of ENU (90 mg/kg i.p.) each animal was given 1.7 uCi/g (^{3}H-CH_3)-thymidine (S.A. = 40 mCi/mM and sacrificed 24 hours later. Brains, kidneys, livers and lungs were collected and the DNA content and specific activity determined. Offspring of Sprague-Dawley rats treated with ENU transplacentally (at 20 day of gestation) consistently had reduced body weights compared to control animals over the period of 2 to 30 days of age. Similarly, DNA content of neonatal brains and kidneys were less than controls over the same period. The DNA content of neonatal liver and lung was less in ENU treated rats for the first 12 days in liver and 17 days in lung. The inhibition of DNA synthesis in the treated rats was observed in all of the organs studied. The suppression of the thymidine incorporation lasted for approximately 2 weeks after birth in liver and lung, for approximately 3 weeks after birth in brain and kidney. When ENU was given to 30 day old BD IV rats. DNA content and thymidine incorporation into DNA were reduced in all 4 organs examined. By 7 days post ENU treatment, the suppression of DNA synthesis was reversed with thymidine incorporation exceeding controls in brain, liver and lung but not in kidney. The observed decrease of DNA content may be indicative of cell death while the increase of thymidine incorporation may suggest restorative hyperplasia.
Supported in part by USEPA grant R805008010 to R.W.Hart and the National Center for Toxicological Research.

293.1 INCORPORATION OF EXOGENOUS ^{125}I-TYRAMINE-GANGLIOSIDE-GM$_1$ (ITG) INTO CRUDE MEMBRANES FROM RAT BRAIN. M.A. Walz*, N.E. Klemm* and I. Jeng*. (SPON: J.C. Webster). Biochemistry Dept. and Missouri Inst. Psychiatry, Univ. Missouri-Columbia, Sch. of Med., St. Louis, MO 63139

Exogenous ganglioside GM$_1$ is inserted into plasma membranes under appropriate conditions. To study such insertion, GM$_1$ labelled with ^{125}I-tyramine covalently attached has been prepared with high specific activity according to our newly-developed procedure (manuscript in preparation). The ITG was incubated with membrane sedimented from homogenized rat brain at 11,000 x g, and significant amounts of ITG were incorporated into the membrane fraction. An irreversible nature of the incorporation was indicated by failure of the label to be removed by repeated washings or incubation of the membrane-bound ITG with nonradioactive GM$_1$. In comparison, ^{125}I as the Na salt and ^{125}I-tyramine did not bind significantly, and ^{125}I linked directly to GM$_1$ bound to a lesser degree. Incorporation of ITG into the membrane fraction was time- and temperature-dependent for up to 2 hrs. and from 0 to 37°C. Over the range of ratios tested, binding of ITG was linearly related to the amount added. The modified GM$_1$ was indistinguishable from the unlabelled compound in this study. However, regardless of the amount of GM$_1$ present, only approximately 20% was recovered in the membrane-bound form. Binding of ITG was slightly increased by the addition of unlabelled GM$_1$ to the reaction mixture, and, thus, cooperativity of GM$_1$ incorporation is suggested. The characteristics of ITG binding reported here compare well with those of tritiated-GM$_1$ binding reported elsewhere (Toffano, G. et al., J. Neurochem., 35:861, 1980) and support the conclusion that ITG incorporation into plasma membranes is indistinguishable from incorporation of unlabelled GM$_1$. (Supported by the Missouri Institute of Psychiatry Intramural Funds.)

293.2 INCORPORATION OF 2-^{14}C-MEVALONATE INTO RAT BRAIN LIPIDS: ARE THERE GLYCOSYLSTEROLS IN MAMMALIAN BRAIN? B.L. Hungund*. (SPONS: S. Mahadik). Long Island Research Institute, State University of New York at Stony Brook, Stony Brook, NY 11794.

Glycosylsterols are known to be widely distributed in the plant kingdom. However, there are no studies reported indicating their presence in mammalian systems. Since mammalian brains are known to be highly enriched in glycoconjugates (gangliosides) and in view of the increasing evidence for their involvement in CNS function, this study was undertaken to see if glycosylsterols exist in brain and how much they contribute to the glycolipid content.

Twenty day old rats were injected intracerebrally with 2-^{14}C-mevalonic acid. After 4 hours brains were removed by decapitation and lipids were extracted following the procedure of Fujino and Ohnishi (Biochim Biophys Acta 574, 94-102, 1979) and partitioned according to the Folch procedure. Thin layer chromatographic and radio-autographic results indicate that there are selected radioactive bands in both neutral glycolipid and ganglioside fractions. Chemical characterization of these bands will be presented.

293.3 EFFECTS OF MATERNAL ALCOHOLISM ON MYELIN GANGLIOSIDES (GA) IN OFFSPRING. A.B. Noronha*, J.M. Gnaedinger* and M.J. Druse-Manteuffel. Department of Biochemistry, Loyola University School of Medicine, Maywood, IL 60153

Female Sprague-Dawley rats were pair-fed, using control(C) or 6.6%(v/v) ethanol(E) liquid diets. The diets are a modification of those described by DeCarli & Lieber(J.Nutr. 91:336,1967). Protein, fat & carbohydrate (including ethanol-derived calories) accounted for 21%, 29% &50% of the total calories, respectively. On postnatal days 19,23,26 or 33 E & C offspring were given an intracerebral injection of either ^{3}H- or ^{14}C-N-acetylmannosamine. Rats were sacrificed 18h later and CNS myelin was isolated. Lipids were extracted by the procedure of Folch-Pi et al.(JBC 226:497,1957). GA were extracted by combining the methods of Irwin et al. (Anal.Biochem.94:335,1979) and Vance & Sweeley(J.Lipid Res.8:621,1967), thus enabling the extraction of more monosialogangliosides than would be extracted by conventional procedures. GA were separated by thin layer chromatography (Zanetta et al., Lipids 15:1055,1980). Separated GA were either visualized with resorcinol spray, scanned and quantitated or used for the determination of radioactivity.

At all ages examined in both C & E pups, the monosialogangliosides accounted for >60% of myelin GA sialic acid. The proportion of GM$_1$ in the myelin GA fraction was 2½-3 times higher at 34d than at 20d. In contrast, the proportion of myelin GD$_{1a}$ decreased by ∿70% during this age period. Using the indicated extraction method, the proportion of myelin GM$_4$ was found to be comparable to that of GM$_1$ at 34d. Despite the fact that the monosialogangliosides predominated at all ages, the highest relative specific activity(RSA) obtained after injecting either the ^{3}H or ^{14}C precursor was associated with GT$_1$. The monosialogangliosides rarely were observed to have an RSA >1. The E and C pups had comparable proportions of individual GA and comparable distributions of radioactivity among the GA at all ages examined. The normal content and synthesis of myelin GA in E pups contrasts with their abnormal synthesis of the major myelin-associated glycoprotein(Gnaedinger & Druse, Trans.Am.Soc.Neurochem.11:153,1980).

This research was supported by grants from Loyola BRSG, the USPHS (AA03490) and from the Schweppe Foundation. Mary Druse-Manteuffel is a recipient of a Schweppe Foundation Career Development Award.

293.4 THE USE OF A MONOCLONAL ANTIBODY TO STUDY THE DEVELOPMENT OF MYELINATING CELLS. B.Ranscht, P.A.Clapshaw, J.Price*, M.Noble* and W.Seifert. Friedrich-Miescher-Laboratorium der Max-Planck-Gesellschaft, Spemannstr.37-39, D-7400 Tübingen 1, W-Germany MRC Neuroimmunology Project, University College London, Gower Street, London WC IE, United Kingdom

A hybridoma cell line has been established secreting IgG3 monoclonal antibody that specifically binds to the surfaces of oligodendrocytes and Schwann cells, the cells involved in myelin formation in the central and peripheral nervous systems, respectively. Even though a mixture of antigens was used for immunization (a synaptic plasma membrane fraction from bovine hippocampi) the antigen specificity of the monoclonal antibody could be determined: the antibody reacts strongly against the major myelin sphingolipid, galactocerebroside (GalC). This was demonstrated using both ELISA and micelle absorption with lipids purified by thinlayer chromatography. These techniques also allowed the characterization of various rabbit glycolipid antisera, revealing one to have a strong affinity to GalC and one to sulphatide. These antibodies were used together with the monoclonal antibody to examine the appearance of GalC and sulphatide on developing oligodendrocytes from rat cortex and Schwann cells from sciatic nerve both in vivo and in vitro. Using indirect immunofluorescence techniques, evidence was obtained that GalC is expressed on the surfaces of these cells 2-3 days before sulphatide. This finding was confirmed on an electron microscopic level using ferritin conjugates to visualize GalC and horseradish peroxidase to detect sulphatide on oligodendrocytes in vitro. The monoclonal GalC antibody is currently being used to study the involvement of this galactosphingolipid in myelin formation.

293.5 INCORPORATION OF P_o PROTEIN INTO LIPOSOMES: DEMONSTRATION OF A TWO DOMAIN STRUCTURE BY IMMUNOCHEMICAL AND PAGE ANALYSIS. C.L. Koski* and M.L. Shin* (SPON: M. Rennels). Dept. of Neurology and Pathology, University of Maryland Medical School, Baltimore, Maryland 21201

P_o is the major glycoprotein of periperal nerve myelin and has an approximate molecular weight of 30,000 daltons. The amphiphilic nature of this protein has been suggested previously (Roomi M.W., Biochim. Biophys. Acta., 536: 122, 1978). In the present study, we wanted to characterize this protein further. P_o was purified by Sephadex Chromatography (Kitamura K., et al., Biochim. Biophys. Acta., 455: 806, 1976) and incorporated into an artificial lipid bilayer consisting of synthetic phosphatidylcholine and cholesterol. The liposomes were fractionated on a sucrose gradient. The continued expression of P_o antigenicity by the liposomes was shown by a specific complement consumption assay employing either a multivalent antiserum against P_o or an IgM monoclonal antibody derived from a hybridoma. Both antibodies were shown to recognize P_o expressed on the surface of peripheral nerve myelin. Trypsin treatment of the P_o liposomes removed a surface antigen, as shown by subsequent failure of the monoclonal anti-P_o to activate complement. PAGE analysis of the trypsin treated P_o liposomes revealed a 23,500 dalton protein and suggested that this segment of the molecule was protected from trypsin digestion. The purified P_o protein treated with trypsin in the fluid phase was cleaved into many smaller fragments. Similar results were obtained with chymotrypsin, although a slightly smaller residual liposome-associated fragment was found.

These experimental results suggest that the P_o glycoprotein consists of two domains, a hydrophilic domain that is accesible to either trypsin or chymotrypsin digestion and a hydrophobic domain, that is potentially trypsin sensitive, but is shielded by the lipid bilayer and, therefore, preserved. Since the anti P_o-monoclonal antibody binds to P_o reincorporated into an artificial bilayer, it is evident that the orientation of the protein in the liposome is similar to that in peripheral nerve myelin.

293.6 REGIONAL FATTY ACID UPTAKE INTO RAT BRAIN. A.S. Kimes and S.I. Rapoport. Laboratory of Neurosciences, Gerontology Research Center, National Institute on Aging, Baltimore City Hospitals, Baltimore, Maryland 21224.

Small amounts of fatty acids are normally taken up from the blood and are incorporated into brain lipids, primarily cell membranes. It was of interest to study the uptake and regional distribution of a saturated fatty acid, palmititic acid, in brains of conscious rats. Osborne-Mendel rats (250-350 g) were anesthesized with pentobarbital. Femoral and arterial catheters were implanted. Then the rats were allowed to regain consciousness. ^{14}C palmititic acid, in tracer amounts, was administered i.v. (75 μCi/kg) and the radioactivities in the plasma and brain were followed. At various time points from 1 min to 24 hr, the rats were killed and the brains were removed and dissected into 22 regions. Levels of radioactivity in the regions and in the plasma were measured in a liquid scintillation counter. From the early time points, the (capillary permeability x surface area (PA)) was calculated in the various regions. Radioactivity increased in the brain up to 20 minutes, then remained stable for 40 minutes. There was a 50% loss of radioactivity between 1 hr and 2 hrs., followed by 22 hrs of constancy.

Plasma radioactivity was 100% palmitate for the first 30 min, as tested by thin layer chromatography. After the first 30 min, progressively increasing percentages of the plasma radioactivity were phospholipids and triglycerides.

White matter accumulated and retained consistantly lower amounts of radioactivity than gray matter over the entire time course studied. The inferior colliculus, olfactory tubercule, cerebellar gray and cortical areas accumulated consistantly higher radioactivities than did other gray matter regions. These findings suggest that steady state levels of palmitate, (after 4 hrs), can be used to study the regional functional status of the brain.

293.7 FATTY ACID-INDUCED ALTERATIONS IN ACTION POTENTIALS FROM DISSOCIATED SPINAL CORD NEURONS. D.E. Brenneman* (SPON: B.K. Schrier). Lab. Devel. Neurobiol., N.I.C.H.D., N.I.H., Bethesda, MD 20205.

The relationship between action potential (AP) characteristics and nutrient fatty acid (FA) structure was investigated in mouse spinal cord neurons. Primary dissociated cell cultures were prepared from 12-14 day old fetal mice. After 3 weeks in culture, the medium was supplemented with 100 μM fatty acid bound to bovine serum albumin (molar ratio 4:1). The duration of FA enrichment was either 18-24 hrs or 4 days. Intracellular recordings of large multipolar neurons (30-45 μ somal dia.) were made with 4 M KAc microelectrodes. Action potentials were evoked by intracellular stimulation.

Fatty acid supplementation had no effect on resting membrane potential during either testing period. After 18-24 hrs exposure, myristic acid (14:0) produced a 60% increase in AP rate of rise as compared to control cells. In addition, cells given 14:0 had a 43% increase in AP rate of fall and a significant increase ($p<0.025$) in spike height (11mV). Addition of linolenic acid (18:3) increased the AP rate of rise by 23% but had no effect on the rate of fall or spike height. Supplementation with oleic acid (18:1), linoleic acid (18:2) or arachidonic acid (20:4) had no effect on AP characteristics after 18-24 hrs exposure.

Neurons which had been incubated 4 days with 14:0, 20:4, 18:2 or 18:3 exhibited a 23-50% increase ($p<0.025$) in AP rate of rise as compared to control cells. In contrast, cells given 18:1 had a 22% decrease ($p<0.025$) from control values. Similar relationships were observed between the rate of fall and FA structure. AP height was significantly increased with 20:4, 18:2 or 14:0 but not with 18:3 or 18:1. These data indicate that FA can significantly effect AP characteristics in large spinal cord neurons and that these alterations in electrical activity are influenced by FA structure and duration of FA exposure.

293.8 FURTHER EVIDENCE FOR MYELINATION OF JP, QK, AND JP^{MSD} AXONS BY NORMAL OPTIC NERVE OLIGODENDROGLIA IN COMBINED CULTURES. Susan Billings-Gagliardi, Lori H. Adcock*, Edward D. Lamperti*, Gail B. Schwing*, Merrill K. Wolf. Department of Anatomy, University of Massachusetts Medical Center, Worcester, MA 01605.

The defects of the hypomyelinated mutant mice jp, qk, and jp^{msd} could result from primary abnormalities of axon, oligodendrocyte, or other factors. We previously reported that myelination in jp^{msd} organotypic cerebellar cultures is significantly increased by co-culture with normal optic nerve (Brain Res. 206: 193, 1981). We now have similar results with qk and jp, and have autoradiographic evidence that cells from the normal optic nerve colonize regions of mutant cerebellum in the zone where optic nerve and cerebellum fuse.

About 50% (22/41) of quaking cultures "treated" with normal optic nerve produce myelin visible in the living state. (Myelin in untreated qk cultures is invisible by this technique.) Increases in myelination up to 33-fold have been confirmed by quantitative light microscopic methods; they have been produced by optic nerve from donors at postnatal ages 0, 1, 2, 5, 7, and 9 days. No cultures treated with qk optic nerve or with normal optic nerve displaced 0.5mm from the cerebellar explants have shown any increased myelination. Successful treatments have been obtained by adding optic nerve at 0, 4, and 11 days in vitro (DIV). Myelin in successfully treated qk cultures is concentrated in the fusion zone, and regularly shows 10 or more compacted lamellae (rarely seen in qk myelin).

Previous culture studies of jp have suffered from genetic flaws in various jp stocks. We have now prepared a B6C3H jp stock which is congenic with jp^{msd}, produces jp myelin clusters in about 50% (15/31) of untreated cultures, and produces normal appearing myelin in cultures treated with normal optic nerve.

To label optic nerve cells in treated cultures, optic nerve from normal animals injected with ^{3}H thymidine was co-cultured with jp^{msd} cerebellum and studied by autoradiography. After 21 DIV heavily labeled cells were identified in the optic nerve itself and in the zone of fusion with cerebellum. Less heavily labeled cells were found directly apposed or immediately adjacent to myelin segments in this fusion zone. Labeled cells became less frequent with increasing distance from the optic nerve, even along myelin, partly because of mitotic dilution of the label. These observations provide additional evidence that the introduced optic nerve glia directly myelinate the mutant axons rather than contributing a diffusible factor.

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293.9 ESTABLISHMENT OF HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC METHODS FOR THE QUANTITATIVE DETERMINATION OF TOCOPHEROLS AND UBIQUINONES IN BRAIN, AND ALTERATION AFTER DECAPITATION. K. Abe*[1,2], S. Yoshida*[1], K. Kogure[1], O. Alonso[1], B. Watson*, M. Santiso*[1], and M. Ginsberg*[1]. [1]Cerebral Vascular Disease Research Center, University of Miami, School of Medicine, Miami, FL 33101, [2]Eisai Research Lab., Tokyo, Japan 112.

Tocopherols (Toc) and ubihydroquinones (UQH_2) are known as fat-soluble antioxidants in biological systems and it has been shown that active oxygen species and free radicals decrease levels of these substances. Ubiquinones (UQ) and UQH_2 also participate as members of the respiratory chain and play a role in energy production in mitochondria. Recently, attention has been given to abnormal free radicals and tissue redox states in various types of experimental brain injury. However, the lack of simple and specific analytical methods for the determination of these substances has hindered progress in this reserach field.

We have established high-performance liquid chromatographic (HPLC) methods for the determination of Toc and the simultaneous determination of UQ and UQH_2 in brain. The procedure used was as follows: Toc, UQ, and UQH_2 were extracted from 1 ml of brain homogenate with 8 ml of a mixture of n-hexane and ethanol (5:3) containing internal standards. Four ml of the n-hexane extracts were evaporated under nitrogen gas. The residue was dissolved in ca. 100 µl of n-hexane or isopropanol. 20-40 µl of the solution were injected onto an HPLC column (NUCLEOSIL-NH_2 or C_{18}). The eluted Toc and UQ were detected with an ultraviolet detector, and UQH_2 was monitored with an electrochemical detector.

These techniques were applied to the estimation of Toc, UQ, and UQH_2 levels in rat brain after decapitation. The control values of α-Toc, UQ-9, and UQ-$9H_2$ obtained from brains frozen *in situ* were 14.75 ± 0.88, 19.90 ± 2.76 and 6.79 ± 1.00 µg/wet g tissue respectively. The amount of UQ-$9H_2$ increased by 68% immediately after decapitation, which is consistent with accumulation of reducing equivalents in the respiratory chain. However, at 3 and 15 minutes after decapitation, transformation of UQH_2 to UQ was observed concomitant with decrease (19%) of α-Toc content. This decrease in bioantioxidants may indicate that free radical reactions occur at a low rate during ischemia. Our proposed methods are so simple, sensitive, and specific that the concentration can be determined in extracts from 20-40 mg of brain.

(Supported by PHS Grant NS 05820).

294.1 INFLUENCE OF OPTIC NERVE AND NUCLEUS ISTHMI ON ACETYLCHOLINESTERASE ACTIVITY IN THE TECTUM OF THE FROG RANA PIPIENS. E. R. Gruberg. Res. Lab. of Electronics, M.I.T., Cambridge, MA 02139.

The superficial tectum of the frog has significant levels of acetylcholinesterase (AChE) activity. It receives major input contralaterally from the retina and bilaterally from n. isthmi. Using a two-step staining process, the contributions of these inputs to tectal AChE activity were parsed. 40 µm-thick cryostat sections were studied in control frogs and in frogs where one or both optic nerves were cut and/or one or both n. isthmi were electrolytically ablated 2 to 4 weeks prior to killing.

1) In control frogs the outermost tectal layer (corresponding to layer A of Potter's notation, which is used throughout this study) has high AChE activity, i.e., stains dark. Layers B, C, and D are very lightly stained; layer E is most intensely stained; layer F stains dark; layer 8 is lighter stained and layer G stains dark.

2) In a tectal lobe with no optic fiber input the superficial tectum is reduced in thickness, but significant AChE activity remains. Both layers A and B stain dark; layers C and D remain light; and layer E, or its remnant, shows no staining. The other superficial layers have AChE activity like the controls.

3) In a tectal lobe with no optic fiber input and no ipsilateral n. isthmi input, only layer A shows AChE activity.

4) A tectal lobe with no optic fiber input and no contralateral n. isthmi input has a staining pattern like that of 2).

5) Without ipsilateral n. isthmi input, layer A is darkest; layers B, C, and D are light; layer E stains dark; layer F has light staining; and layers 8 and G show virtually no staining.

6) Without contralateral n. isthmi input, tectal staining looks normal except that layer B is darker.

These results indicate that n. isthmi, in addition to optic fibers, contribute significantly to tectal AChE activity.

In other animals, horseradish peroxidase was injected into one tectal lobe, and nuclear yellow was injected into the other lobe. After retrograde transport, it was found that the two labels were in mutually exclusive regions of n. isthmi. The medio-ventral region of n. isthmi, which projects to the contralateral tectum, shows less AChE activity than the latero-dorsal region which projects to the ipsilateral tectal lobe.

294.2 INTRACELLULAR ACHE IDENTIFIES CHOLINERGIC NEURONS. Bruce G. Wallace and Jean Gillon.* Dept. of Neurobiology, Stanford Univ. Sch. of Med., Stanford CA 94305.

In the central nervous system of the leech Hirudo medicinalis, cholinergic neurons were found to contain significantly more acetylcholinesterase (AChE) activity than non-cholinergic neurons. AChE activity was measured in extracts of individual, identified nerve cells, which can be dissected from leech ganglia relatively free of both neural and glial contamination. All activity associated with isolated cells was acetylcholinesterase. Leech blood was found to contain butyrylcholinesterase; a variable amount of this activity was found in extracts of whole ganglia. Ganglia were pretreated with saline with or without echothiophate, a charged phosphinylthiocholine derivative that irreversibly inhibits AChE. It was assumed that activity remaining after echothiophate pretreatment was intracellular. Total and echothiophate-resistant AChE activities were used to determine the intracellular and surface concentration of AChE associated with isolated cells. Excitatory motor neurons which are though to release acetylcholine (ACh) as a neurotransmitter, had approximately 10-fold higher levels of intracellular AChE than non-cholinergic cells, while all neurons had comparable levels of activity associated with their surface. This non-uniform distribution of intracellular AChE is consistent with the observation that AChE controls the accumulation of ACh in cholinergic neurons.

Based on these findings standard cholinesterase histochemical techniques were modified to stain selectively intracellular AChE. Individual neurons were identified and labelled by intracellular recording and injection of Lucifer Yellow prior to fixation and staining. A fraction of the cells in leech ganglia, including all suspected cholinergic neurons, were stained by this method. Known non-cholinergic cells were not stained. The reaction product appeared as granular deposits within the cytoplasm of stained cells. Thus, although AChE activity is widespread, selective staining of intracellular AChE may provide a useful method for identifying neurons that release ACh as a transmitter, just as high concentrations of extracellular cholinesterase often identify cholinergic junctions.

294.3 CHOLINE ACETYLTRANSFERASE-CONTAINING STRUCTURES OF THE FELINE CNS BY IMMUNOHISTOCHEMISTRY, P. L. McGeer, H. Kimura*, J. H. Peng*, and E. G. McGeer. Kinsmen Laboratory of Neurological Research, Department of Psychiatry, University of B.C., Vancouver, B. C., Canada, V6T 1W5.

Choline acetyltransferase (CAT)-containing cell bodies, fibers and terminals were detected in the feline CNS (1) using purified Fab fragments of rabbit anti-human CAT of high titer (2). Well fixed tissue was obtained by perfusing animals with 4% paraformaldehyde and 0.35% glutaraldehyde. Staining was by standard PAP techniques. Cholinergic cell bodies were observed in more than forty areas and cholinoceptive cells in more than sixt regions of the feline brain.

Major cholinergic cell systems were found to exist in the following five regions: (i) the basal forebrain including the medial septal area, nucleus of the diagonal band of Broca, and substantia innominata complex; (ii) the basal ganglia including the caudate, putamen and nucleus accumbens; (iii) the reticular formation; (iv) motor nuclei of cranial nerves; and (v) the parabrachial complex. Other less prominent systems exist. In all areas the cells were in the large to giant category.

Many cholinoceptive areas were categorized. The staining is in accord with details on cholinergic systems available from other techniques but it is apparent that many cholinergic pathways remain to be defined.

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294.4 LOCALIZATION OF COMPONENTS OF THE CYTOCHEMICAL MAP OF THE CAUDATE NUCLEUS. Marjorie A. Ariano. Anatomy & Neurobiology, Univ. of Vermont College of Medicine, Burlington, VT 05405.

The intrinsic cellular elements of the rat caudate nucleus contain cyclic AMP and cyclic GMP (Ariano, et al, Neuroscience 5: 1269, 1980). The morphological localization of these potential second messenger compounds in relation to other neurochemical substances in the striatum has been assessed. Terminal varicosities containing dopamine from the nigrostriatal input have been visualized with histofluorescence (De la Torre, Neurosci. Lett. 17: 339, 1980). Dopamine terminals are anatomically situated to influence all cellular elements containing substance P and the cyclic nucleotides, as determined through subsequent immunochemical processing and re-examination of the exact area of the caudate nucleus.

Cyclic AMP has been visualized in 1) medium spiny neurons of 12-20 µm diameter, 2) oligodendroglia, and 3) astrocytic processes. The unequivocal identification of these cellular constituents has been accomplished through ultrastructural examination of caudate tissue exposed to anti-cyclic AMP antisera and processed for PAP-immunocytochemistry. Caudate elements containing cyclic GMP immunoreactivity include 1) medium spiny neurons and 2) filamentous processes of fibrous astrocytes. Identification of these structures has been accomplished through examination of anti-GFAP antibody-treated caudate tissue sections, and electron microscopic immunocytochemistry. Cyclic GMP-stained neurons exhibit a marked cytoplasmic staining and characteristically lack nuclear reaction. Astrocytic elements are less numerous than neuronal staining. This is confirmed at ultrastructural resolutions, where predominant subcellular elements positively reacting following application of anti-cyclic GMP antisera are neuronal, e.g. postsynaptic densities of type I terminal boutons and perikarya.

Substance P-containing elements have been visualized by immuno histochemical techniques. This putative neurotransmitter of striatal efferents is restricted to cells of 12-20 µm that qualitatively resemble cyclic GMP-reactive neurons, with prominent cytoplasmic staining and less nuclear immunofluorescence.

This data cytochemically demonstrates the relationship of four substances in a qualitative neurochemical map of the intact rat caudate nucleus.

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294.5 THE DISTRIBUTION OF BOVINE PANCREATIC POLYPEPTIDE-LIKE IMMUNOREACTIVITY IN THE RAT CENTRAL NERVOUS SYSTEM. J. A. Olschowka, T. L. O'Donohue* and D. M. Jacobowitz. Lab. of Clin. Sci., NIMH, Bethesda, MD 20205.

Bovine pancreatic polypeptide (BPP), a 36 amino acid peptide, is produced by a specific type of endocrine cell in the pancreas and GI tract. Recently avian pancreatic polypeptide, a structural homolog of BPP, was reported in nerve fibers and cell bodies of mamallian and avian CNS. The present investigation describes the immunohistochemical localization and detailed mapping of BPP-containing varicose processes and cell bodies in the rat brain.

Normal and colchincine-treated male Sprague-Dawley rats were used. Frozen sections of the brain were stained using the indirect immunofluorescence procedure of Coons. The BPP antiserum was generously provided by Dr. R. E. Chance (Lilly Research Laboratories, Eli Lilly and Co.). Sections incubated with antisera previously preabsorbed with pure antigen served as controls. The antiserum was tested for cross reactivity by adding either VIP, CCK, α-MSH, glucagon, secretin or bombesin (10 μg/ml diluted antiserum).

The BPP staining appeared specific since preabsorption of the antiserum with BPP resulted in a complete loss of staining. The antiserum did not react with any of the other peptides tested. BPP-like immunoreactive cell bodies were observed throughout the cortex (layers II-VI), however, the majority of labeled neurons appeared subcortically. Large numbers of BPP cells were observed in the arcuate nucleus, with scattered cells in the olfactory tubercle, neostriatum, nucleus accumbens, nucleus preopticus medialis, nucleus interstitialis stria terminalis and in the area of the medial forebrain bundle. Caudally BPP cells were found in regions which contain catecholamine cell groups -- the locus coeruleus (A6), the nucleus tractus solitarius (A2) and in the area of the nucleus reticularis lateralis (A1). Delicate, varicose, BPP nerve fibers were widely distributed, but were very numerous in several subcortical areas -- nucleus accumbens, nucleus interstitialis stria terminalis, nucleus preopticus medialis, periventricular thalamic and hypothalamic nuclei, paraventricular nucleus, nucleus dorsomedialis, and caudally in the nucleus parabrachialis dorsalis, nucleus tractus solitarius and the substantia gelatinosa trigemini. This extensive distribution of BPP in the brain suggests that it is involved in significant neuronal circuitry, possibly in a neurotransmitter or neuromodulator role. However, the exact identity of this peptide and its physiological role remain to be determined.

294.6 SUBSTANCE P, ENKEPHALINS IN HUNTINGTON'S DISEASE-IMMUNOCYTOCHEMICAL OBSERVATIONS. L. L. Vacca, J. Hobbs* and D. Washington*. Depts. of Pathology and Anatomy, Medical College of Georgia, Augusta, GA 30912.

Normally, adult Sprague-Dawley and Wister rats exhibit a dense network of immunoreactive processes which contain the peptide substance P (SP) within the substantia gelatinosa (SG) of the dorsal spinal cord and the substantia nigra (SN) of the midbrain. Like the rat, the normal human also exhibits high concentrations of immunoreactive SP within SG and SN. Therefore we have become interested in examining these tissues in certain human diseases. Currently, we have directed our efforts towards Huntington's Disease (HD), a rare inherited disorder characterized in part by the severe degeneration of basal ganglia neurons and their connections. Our immunocytochemical evaluations for SP in SN indicate that the peptide clearly becomes reduced in HD specimens compared with normal (non HD) controls. Conceivably the reduced amounts of SP as an excitatory neuropeptide can influence the firing of the nigral dopamine-containing cells in HD and other movement disorders. We are continuing our immunocytochemical evaluations for SP in human spinal cord, normal and HD, as well.

The probable degeneration of SP neurons in HD suggests a new direction for research in the disease process and a novel role for SP in motor control or disturbances. We are hopeful that the rat will provide us with an experimental "model" in which SP can be manipulated pharmacologically, particularly in the SG and SN regions. To date, our immunocytochemical evaluations for SP have shown that morphine, and probably other opiate substances, can increase intraneuronal SP within SG but not within SN. These divergent data may reflect regional differences in the location of opiate receptors pre-synaptically on SP neurons in SG and perhaps post-synaptically to SP neurons in SN. Other data have shown that opiate receptors in SN occur in close association with nigral dopamine-containing cells and their processes. After morphine, we failed to observe signs of nigral degeneration. Other opiate substances are under investigation. Additionally, we have undertaken an immunocytochemical study of enkephalins (methionine and leucine) in rat and human (normal and HD) tissues.

Many thanks go to the following people: Dr. Susan Leeman and Dr. John Stewart for anti-SP sera; Dr. Eric Naftchi, Dr. Susan Abrahams, Dr. Ken Bonnet for anti-enkephalin sera and morphine treated animals; Dr. Edward Bird and Mr. Tom Stevens (RO 1 MH/NS 31 862), Dr. F. Yaghmai, Dr. P. Farmer for assistance in obtaining appropriately fixed human specimens; the Atlanta Chapter and the National Committee to Combat Huntington's Disease for their support (CCHD 10-12-04-3600-67).

294.7 LOCALIZATION OF NEUROTENSIN IMMUNOREACTIVE NEURONS IN THE SEPTAL NUCLEI OF THE RAT. C. Jenq,* J. Jew and T.H. Williams (SPON: S. Itaya) Dept. of Anatomy, Univ. of Iowa, Coll. of Med., Iowa City, IA 52242.

In previous studies, neurotensin (NT) has been found in the synaptosomal fraction, providing evidence for a neurotransmitter function. It has been localized immunocytochemically in many regions of the CNS including spinal cord, brainstem, hypothalamus and telencephalon. A substantial amount of NT has been found by radioimmunoassy in the septal area. The present study deals with localization of NT-like peptide in septal neurons of the rat using light (LM) and electronmicroscopy (EM).

Female Sprague-Dawley rats (approximately 180-230 gm) were fixed by perfusion with 0.2% glutaraldehyde and 4% paraformaldehyde in Sorensen's phosphate buffer. Some of the animals had received 50-100 μg colchicine intraventricularly 24-48 hours prior to perfusion. Serial 40μm vibratome sections were processed using a modified Sternberger PAP technique. Sections were mounted on slides for LM or flat embedded in Epon for thin sectioning.

In LM sections, the greatest number of NT-immunoreactive neurons were visualized in animals pretreated with 100μg colchicine, fewer with 50μg, and none without colchicine pretreatment. In NT-positive neurons, reaction product was distributed within the cytoplasm of perikarya and processes. NT-immunoreactive perikarya in the septal nuclei were distributed within the rostral half. The majority of these neurons were located in the intermediate portion (Swanson and Cowan nomenclature, J. Comp. Neurol. 186:621-656, 1979) of the lateral septal nucleus. Staining was not observed in controls which were incubated with NT-immunoabsorbed antiserum.

At the EM level, NT-immunoreactive neurons were observed in both colchicine- and noncolchicine-treated animals. In labeled perikarya, reaction product was present in the cytosol but absent from major organelles and the nucleus. It was often associated with microtubules in labeled dendritic profiles and with synaptic vesicle membranes in labeled axon terminals. Asymmetric (Gray Type I) synaptic contacts were seen between unlabeled axon terminals and labeled dendrites. Synaptic contacts between labeled terminals and labeled dendrites were sometimes observed and appeared to be symmetric (Gray Type II). (Supported by NIH grants NS11650 to THW and HL21914 to JJ.)

294.8 LM AND EM EXAMINATION OF SOMATOSTATIN-LIKE IMMUNOREACTIVITY IN THE CENTRAL NUCLEUS OF THE RAT AMYGDALA. T.S. Gray, M.D. Cassell* and T.H. Williams. Dept. Anat. Univ. Iowa, Coll. of Med., Iowa City, IA 52242.

Somatostatin (SS) is a peptide known to inhibit growth hormone release from the anterior pituitary, though neurons immunoreactive for SS have been identified in many parts of the CNS. Although most reports have concentrated upon localization of SS in the hypothalamus or pituitary, SS-immunoreactive neurons have been identified also in many extrahypothalamic sites, including the amygdala. A description of the morphology of SS-immunoreactive neurons in the central nucleus of the amygdala (CNA) and their distribution will be presented.

Brains of Sprague-Dawley rats were fixed by perfusion with buffered 4% paraformaldehyde and 0.2% glutaraldehyde, 24-48h after intraventricular injections of 50ug of colchicine. Vibratome sections were cut at 30-40μm and processed according to a modified Sternberger immunoperoxidase procedure and mounted on slides for LM examination or flat-embedded in Epon for thin sectioning. Control sections were treated with normal rabbit serum or immunoabsorbed antiserum.

Two types of SS-immunoreactive neurons were identified in the CNA: 1) cells with multipolar perikarya, 15-20μm in diameter, with many long, smooth dendrites; 2) others with fusiform perikarya, 10-15um in diameter, with fewer long, spiny dendrites. The majority of SS-immunoreactive neurons were located in the caudal two-thirds of the dorsal part of the CNA. Labeled axons and presumed terminals were distributed throughout the rostrocaudal extent of the CNA and appeared to contact both SS-positive and SS-negative neurons.

EM examination showed many SS-immunoreactive perikarya and dendrites, with reaction product distributed throughout the cytosol. Reaction product was most concentrated in axon terminals where it was found at or near the surfaces of round agranular vesicles. A few large dense-cored vesicles, which appeared to possess labeled vesicular membranes, were observed within SS-positive perikarya, dendrites and axons. Symmetrical (Gray Type II) synaptic contacts were observed between labeled terminals and both labeled and unlabeled perikarya and dendrites. Asymmetrical (Gray Type I) synaptic contacts, with prominent postsynaptic webs, were found between unlabeled terminals and labeled dendrites. (Supported by NIH Grant NS 11650 to T.H.W.)

294.9 COLCHICINE DEPLETES AND KAINIC ACID ENHANCES ENKEPHALIN-LIKE IMMUNOCYTOCHEMICAL REACTIVITY IN THE HIPPOCAMPUS. Jacqueline F. McGinty, Illana Gozes, and Floyd E. Bloom. A.V. Davis Ctr. The Salk Institute, La Jolla, CA 92037.

The unique excitatory action of enkephalin in the rat hippocampus (HPC) has prompted intense interest in the localization of enkephalin immunoreactivity (ir) there but immunocytochemical results have been highly variable. The most extensive distribution of HPC enkephalin ir has been found in apparent mossy fibers arising from dentate granule cells (Gall, et al. Soc. Neurosci. 354, 1980). We have also observed enkephalin ir in mossy fibers but rarely in dentate granule cell bodies (antiserum A206, R. Miller, U. Chicago).

To enhance the enkephalin ir staining in HPC we administered kainic acid (KA) (1 µg/10 µl, icv) to rats several days prior to perfusion. KA destroyed the CA3-CA4 pyramidal cells and concomitantly increased the enkephalin ir in the mossy fibers. A greater number of granule cells were enkephalin positive in KA-treated rats than in controls. However, the number seemed insufficient to account for the dense mossy fiber staining unless it is assumed that the immunoreactive material in the nerve terminals arises from a non-immunoreactive precursor present in dentate perikarya.

Colchicine administration (50 µg/50 µl, icv) failed to enhance enkephalin ir in granule cell bodies, leading us to consider the report that colchicine is preferentially toxic to dentate granule cells (Goldschmidt and Steward, PNAS 77: 3047, 1980). After infusion of colchicine (3 µg/0.6 µl) into the dorsal and ventral dentate, ipsilateral granule cells exhibited a time-dependent degeneration associated with an ipsilateral decrease in enkephalin ir in mossy fibers. The toxic effect of colchicine on granule cells was also observed in preparations stained with a monoclonal anti-tubulin (Barnstable and Gozes, J.C.B. 87: 251a, 1980). Colchicine disrupted the microtubules of dentate granule cells without altering the normal pattern of tubulin ir in CA1 cells or fibers. These results suggest that the tubulin cytoskeleton is organized differently in different classes of neurons. We are using double immunocytochemical staining (McGinty and Bloom, Soc. Neurosci. 6: 354, 1980) to determine the relative changes in enkephalin and tubulin-ir during the expression of colchicine and kainate neurotoxicity.

The staining characteristics of enkephalin-ir in the HPC are sufficiently different from those observed in other brain areas to suggest the presence of some other opioid peptide instead of, or in addition to, enkephalin. The presence of multiple opiate receptor populations in the HPC lends credence to this view.

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294.10 SEX RELATED VARIATION IN OPIOID BINDING DENSITIES IN LEUCOPHAEA MADERAE (BLATTARIA), G.B. Stefano and B. Scharrer. Dept. Natural Sciences Medgar Evers College, C.U.N.Y., Brooklyn, N.Y. 11225 and Dept's Anatomy and Neuroscience Albert Einstein College of Medicine, Bronx, N.Y. 10461.

In recent times there has been a burgeoning of studies concerned with the elucidation of opioid mechanisms in activities that transcend analgesic functions. Binding of (^{3}H)DALA in cerebral ganglia suspensions of Leucophaea was monophasic and saturable and when analyzed by a Scatchard plot a class of high affinity sites was found with affinity constants (Kd's) of 8.7 and 8.5 nM for male and female adults, respectively. However, the densities of the binding sites turned out to be different for the two sexes. The females had a higher affinity site receptor density (Bmax) value of 56.0, whereas the males had a Bmax value of 38.1 (pmol/g of protein). In other words, the same amount of brain tissue from the adult females had 30% more binding sites for DALA. Specific binding of DALA to brain suspensions of last instar nymphs was also found to be monophasic, saturable and of high affinity K_D value's $\simeq$ 8.5 for both males and females and Bmax values of 38.4 and 41.2(pmol/g of protein) for males and females. There appears to be no difference in binding site density per mg protein between males and females in the immature state. Specific opioid binding could not be detected in suspensions of optic lobes removed from the cerebral ganglia, suggesting that opioid receptors are confined to specific areas in the nervous tissue. Of the biogenic amines tested epinephrine and phenylethanolamine appear to be absent in the cerebral ganglion of Leucophaea. The levels of dopamine (15.3, pM/mg tissue wet weight), norepinephrine (2.29) and Octopamine (6.68) were the same in both sexes. The study demonstrates a sex-related difference in receptor density in adult animals but not in nymphs. In this ovoviviparous insect the ovarian cycle depends on activity cycles of the endocrine corpora allata which is known to be under the control of peptidergic neurosecretory elements of the brain. We surmise that an opioid neuroregulator might participate in this neuroendocrine control system.

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294.11 β-ENDORPHIN (END) AND LUTEINIZING HORMONE RELEASING HORMONE (LHRH) IMMUNOREACTIVE NEURONS IN THE MEDIAL BASAL HYPOTHALAMUS (MBH) OF THE RAT. O.K. Rónnekleiv, S. Kaul* and R.L. Eskay*, Department of Psychiatry (OKR), Department of Physiology (S.K.) School of Medicine, University of Pittsburgh, Pittsburgh, PA 15261 and NIMH, Bethesda, MD (RLE).

A wealth of neurophysiological as well as neuroendocrine studies strongly suggested that LHRH neurons are located in the MBH. However, recently there has been some controversy concerning the transmitter identity of fusiform neurons in the arcuate region of the rat brain (C.J. Clayton and G.E. Hoffman, Am. J. Anatomy 155:139, 1979). We, therefore, decided to carefully evaluate the existence of LHRH positive neurons in the rat MBH and to compare its morphology as well as distribution to END.

Young adult Sprague Dawley female rats were perfused intracardially with 4% paraformaldehyde in 0.1M phosphate buffer (pH 7.2). The brains were blocked and kept overnight in phosphate buffer (pH 7.2) containing 10% sucrose. Sagittal and coronal cryostat sections were cut, mounted on gelatinized slides and kept frozen until processed by the PAP method of Sternberger (1974) for light microscopy. The β-endorphin antiserum (B-E-1) used in these experiments recognizes β-endorphin as well as β-lipotrophin on an equimolar basis. Immunoreactive END cells were evenly distributed in the pre-mammilary and the arcuate region. Furthermore, END neurons appeared to be located in the VMN region. The cells were round to fusiform with a large unstained nucleus. Characteristically, the cytoplasm was filled with dark granular golgi-like material. Fusiform LHRH neurons, demonstrated by the WP-1 antiserum, were distributed along the dorsal half of the arcuate nucleus, while the ventral part of the nucleus was filled with LHRH fibers. Cells were also seen in the VMN region often in clusters in the vicinity of blood vessels. All immunoreactive END and LHRH cells and fiber stain were eliminated by pre-absorption of the primary antiserum with synthetic β-endorphin (S-END) and synthetic LHRH respectively. On the contrary, no LHRH stain was eliminated by pre-absorption of the LHRH antiserum with S-END, indicating that the immunoreactivity of the LHRH neurons in the MBH is not due to crossreactivity with an endorphin component of the LHRH antiserum.

In conclusion, END neurons are found evenly distributed in the arcuate region of the rat hypothalamus. LHRH neurons are found mainly in the dorsal half of the arcuate nucleus and the VMN. Endogenous opiates have been found to inhibit LH release, presumably through an action on hypothalamic LHRH. Future experiments will determine the functional interaction between hypothalamic END and LHRH neurons.

294.12 THE MORPHOLOGY OF NEUROTENSIN-IMMUNOREACTIVE NEURONS AND THEIR PROCESSES IN THE CENTRAL NUCLEUS OF THE RAT AMYGDALA. M.D. Cassell*, T.S. Gray and T.H. Williams. Dept. Anat. Univ. of Iowa, Coll. of Med., Iowa City, IA 52242

Neurotensin (NT), a physiologically active tri-decapeptide, has been demonstrated by immunocytochemical methods in neurons in the central nucleus of the amygdala (CNA). Detailed studies of the morphology and distribution of these neurons are a first step towards understanding the possible functional roles of NT in the CNA.

Serial 30-40µm vibratome sections of the rat amygdala were processed by a modified Sternberger PAP-DAB immunocytochemical method (Leranth et al. 1980). All solutions used in the processing of LM sections contained 0·1% Triton-X. Sections for EM examination were post-fixed in OsO_4 and flat-embedded in Epon for thin sectioning. Control sections were incubated with normal rabbit serum and immunoabsorbed anti-NT.

Under LM, neurotensin-immunoreactive neurons were identified by the dark reaction product seen throughout the cytoplasm of perikarya and dendrites. NT-immunoreactive neurons in the CNA appear to be of a single type: multipolar, 15-20µm in diameter, with long, apparently aspinous dendrites. These neurons are located in the dorsal parts of the CNA, principally in its caudal two-thirds. Pretreatment of animals with intraventricular injections of 50-100µg of colchicine increased the observed number of immunoreactive neurons, but did not alter their distribution in the CNA. NT-immunoreactive axons and presumed terminals were observed throughout the rostrocaudal extent of the CNA, though the terminals were most concentrated in the ventrolateral parts of the nucleus surrounding unlabeled perikarya. Many labeled terminals were observed on both labeled and unlabeled neurons in the rest of the CNA. A few labeled axons were seen to pass from the CNA into the stria terminalis.

Electron microscopy confirmed and extended the LM observations. In NT-immunoreactive perikarya and dendrites, reaction product was distributed throughout the cytosol. Occasional large, dense-cored vesicles were observed in the cytosol of labeled perikarya, dendrites and axons. In labeled axon terminals, reaction product was located on or near the surface of round, agranular vesicles. Symmetrical (Gray Type II) synaptic contacts were observed between labeled terminals and both labeled and unlabeled perikarya and dendrites. Asymmetrical (Gray Type I) synaptic contacts with prominent postsynaptic webs were observed between unlabeled terminals and labeled dendrites. Supported by NIH Grant NS 11650 to T.H.W.

294.13 SUBSTANCE P, BUT NOT ENKEPHALIN, IMMUNOREACTIVITY DISTINGUISHES VENTRAL FROM DORSAL PALLIDUM. S.N. Haber and W.J.H. Nauta. Dept. of Psychol., Mass. Inst. of Technology, Cambridge, MA 02139.

The ventral pallidum seems to extend in a rostroventral direction. This has been indicated in sagittal Nissl sections (see Heimer, In Limbic Mechanisms, 1978), as well as in material stained with Perl's method for demonstrating ferric-iron (see Switzer and Hill, Neurosci. Abstr., 1979). Using immunohistochemical techniques, we have demonstrated that the distribution of enkephalin and substance P appears to correspond to this rostral extension of the ventral pallidum. Furthermore, immunoreactivity in this region appears morphologically similar to Golgi impregnations of pallidal neurons as described by Fox et al.(J. fur Hirnforschung, 1973): positive beaded fibers can be seen running longitudinally along the long, thick dendrites. The ventral pallidum is unique in that substance P immunoreactivity is dense here while in the rest of the globus pallidus staining is very light. Enkephalin-positive fibers are very dense throughout the globus pallidus including the ventral pallidum. In coronal sections both enkephalin and substance P immunoreactivity appears beneath the anterior commissure in an S-shaped configuration, flanked by areas of lighter staining: laterally the subcommissural striatum and medially the bed nucleus of the stria terminalis. In more rostral sections, staining continues ventrally with fibers dipping deep into the olfactory tubercle. Enkephalin and substance P staining can be seen capping the islands of Calleja in a fashion similar to that found in Perl-stained material (Hill and Switzer, Neurosci. Abstr., 1979). The continuity of the immunoreactivity for both peptides can be clearly seen in parasagittal sections. Enkephalin staining is observed in the main body of the globus pallidus extending beneath the anterior commissure and continuing in a rostroventral direction into the olfactory tubercle, but, while substance P staining likewise appears in the ventral pallidum, it fades out in the main body of the pallidum.

Preliminary experiments suggest that these peptide fibers originate from cells in the striatum and project in a topographic fashion to the pallidum, so that lesions of the dorsal striatum decrease enkephalin immunoreactivity in the dorsal pallidum, while lesions of the ventral striatum decrease both enkephalin and substance P in the ventral pallidum. The fact that substance P immunoreactivity distinguishes the ventral pallidum from the main body of the globus pallidus may have important implications for the functional role of this subcommissural segment of the pallidum and its relationship to the limbic system.

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294.14 CO-EXISTENCE OF GAD IMMUNOREACTIVITY AND HIGH AFFINITY ^{3}H-GABA UPTAKE IN NEURONS IN DISSOCIATED CELL CULTURES OF CEREBRAL CORTEX. E.A. Neale, W.H. Oertel† and L.M. Bowers*. Lab. Devel. Neurobiol. NICHD and Lab. Clin. Sci., NIMH, NIH, Bethesda, MD 20205

The role of γ-aminobutyric acid (GABA) as a major inhibitory neurotransmitter in the mammalian central nervous system has been documented by biochemical, pharmacologic, electrophysiologic and morphologic studies. GABA-ergic neurons had been morphologically identified by the radioautographic demonstration of high affinity ^{3}H-GABA uptake, and more recently, by the immunohistochemical localization of glutamic acid decarboxylase (GAD), the GABA-synthesizing enzyme. In this study, combined immunocytochemistry and radioautography were used to demonstrate the co-existence of ^{3}H-GABA uptake and GAD immunoreactivity within the same cortical neurons.

Murine cerebral cortical neurons were studied during development in dissociated cell cultures. At various times after plating, cultures were incubated with 0.1 μM ^{3}H-GABA for 30 min at 37°C, and fixed with 4% paraformaldehyde. Cultures were then reacted with a sheep GAD antiserum, peroxidase-antiperoxidase complexes and 3,3'-diaminobenzidine for the immunocytochemical localization of GAD. Selected areas of the cultures were photographed and neurons scored for GAD immunoreactivity. The cultures were subsequently coated with radioautographic emulsion, exposed for three days, and the same culture areas re-examined for neurons labeled with silver grains as evidence of ^{3}H-GABA uptake. Nearly 5000 neurons were evaluated for GAD immunoreactivity and/or ^{3}H-GABA uptake.

There was a strong correlation between the presence of GAD and ^{3}H-GABA uptake within the same cortical neurons. Of those neurons scored in six cultures from two dissections, GAD immunoreactivity and ^{3}H-GABA accumulation were co-existent in 88% of neurons classified as GABA-ergic by one or the other of these morphologic criteria. In optimal preparations at least three weeks in culture, this correlation approached 95%. 95% of GAD-positive neurons showed ^{3}H-GABA uptake; 98% of ^{3}H-GABA accumulating neurons showed GAD immunoreactivity. In 8 day old cultures, GAD immunostaining was not detectable, and in 14 day old cultures, the anti-GAD reaction product was not as qualitatively distinct as in 22 day old cultures. However, neurons in both 8 and 14 day cultures were well-labeled by ^{3}H-GABA accumulation, suggesting the development of ^{3}H-GABA uptake prior to immunologically detectable GAD.

These studies constitute rigorous experimental evidence that ^{3}H-GABA accumulating cortical neurons contain the GABA-synthesizing enzyme, GAD.

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294.15 IMMUNOCYTOCHEMICAL LOCALIZATION OF ASPARTATE AMINOTRANSFERASE AND ENKEPHALIN IMMUNOREACTIVITIES IN THE GUINEA PIG RETINA. R.A. Altschuler, J.L. Mosinger*, R.J. Wenthold, D.W. Hoffman*, and M. Parakkal*. Lab. Neuro-otolaryngology, N.I.H., Bethesda, MD and Harvard Univ., Cambridge, MA.

Indirect immunofluorescence techniques were used on cryostat sections of parformaldehyde perfused guinea pig retinas to determine the localization of aspartate aminotransferase (AAT) and enkephalin-like immunoreactivities. Previous studies have suggested that AAT is involved in the metabolism of glutamate and aspartate when these amino acids are used as neurotransmitters. We have recently demonstrated AAT-like immunoreactivity in cell bodies, axons and terminals of the auditory nerve. In the present study a band of AAT-like immunofluorescence was seen in the outer plexiform layer (OPL) of the guinea pig retina. In colchicine pre-treated animals AAT immunofluorescence was seen in photoreceptors with cell bodies in the outer portion of the outer nuclear layer giving rise to thin processes ending in large swellings in the OPL. These are likely to be cones and cone pedicles. These results support the hypothesis that aspartate or glutamate may serve as the neurotransmitter of cones. A population of AAT immunoreactive cells was also observed in the position of amacrine cells with fibers in the five distinct sublaminae of the inner plexiform layer (IPL).

Enkephalin-like immunoreactivity in the guinea pig was examined using an antiserum (RA143) raised in rabbit against a *met-enkephalin-bovine thyroglobulin* conjugate. This antiserum has been well defined by RIA and has low cross-reactivities to leucine enkephalin and beta-endorphin. Enkephalin-like immunofluorescence was seen in fibers in the inner plexiform layer. In colchicine pre-treated animals enkephalin-like immunoreactive cell bodies were seen at the inner most margin of the inner nuclear layer giving rise to immunofluorescent processes running in the IPL sublaminae one, three and five. This is the first description of enkephalin-like immunoreactivity in the retina of the mammal and agrees with previous description in several non-mammalian species.

294.16 IMMUNOCYTOCHEMICAL LOCALIZATION OF ASPARTATE AMINOTRANSFERASE AND GLUTAMINASE IN THE AUDITORY NERVE. R.J. Wenthold, W.G. Haser*, R.A. Altschuler, G.G. Harmison*, G.R. Neises*, and J. Fex. LNO, NINCDS, NIH, Bethesda, MD and Dept. Biochem., Univ. Pittsburg, Pittsburg, PA

Several lines of evidence suggest that glutamate or aspartate is the neurotransmitter of the auditory nerve. In studying the synthesis of these amino acids in the auditory nerve, it was found that the two enzymes associated with the metabolism of glutamate and aspartate, aspartate amino transferase (AAT) and glutaminase, appear to be concentrated in terminals and fibers of the auditory nerve. Both enzymes decrease in the cochlear nucleus after auditory nerve lesion and the specific activities are two to five times higher in the auditory nerve than in other nerves tested. To further study the localization of these enzymes, antibodies were made to cytoplasmic AAT from pig heart and phosphate dependent glutaminase from rat kidney. Both preparations were found to cross-react with their respective enzymes from guinea pig brain. Indirect immunofluorescence techniques were then used on cryostat sections for light microscopic visualization and ultrastructural localization was done for AAT using the peroxidase-antiperoxidase technique on vibratome sections.

With immunofluorescence techniques both antibody preparations showed rings of immunoreactivity corresponding to auditory nerve terminals on cells in the ventral cochlear nucleus of the guinea pig. The glutaminase-like immunofluorescence was of a more punctate nature. Both antibody preparations gave intense labeling of spiral ganglion cell bodies. Again the glutaminase immunofluorescence was more punctate producing a web-like appearance over ganglion cell cytoplasm.

AAT-like immunoreactivity in the ventral cochlear nucleus was examined at the ultrastructural level. Immunoreactivity was localized in terminals exhibiting the morphological characteristics of primary afferent terminals. The other classes of terminals showed no immunoreactive labelling.

In recent studies glutaminase and AAT-like immunoreactivities were examined in the retina and hippocampus of the guinea pig. In the retina a similar distribution of immunoreactivity was found for both enzymes in photoreceptor terminals in the outer plexiform layer. In the hippocampus, only glutaminase-like immunofluorescence was observed. This immunoreactivity was found in fibers in the stratum radiatum of the regio inferior.

These studies suggest that one or both of these enzymes may serve as a marker for aspartate/glutamate synapses.

295.1 ADRENERGIC RECEPTORS COUPLED TO ADENYLATE CYCLASE IN RAT PIA-ARACHNOID. G.C. Palmer and S.J. Palmer*, Dept. Pharmacol., Univ. So. Ala. Col. Med., Mobile, Alabama 36688.

Microvasculature consisting of pia-arachnoid were removed from the cerebral cortex of saline-perfused rat brains. A series of adrenergic agonists were added to homogenates of pia-arachnoid in order to evaluate receptor specificity with regard to activation of adenylate cyclase. Norepinephrine and isoproterenol with or without Gpp(NH)p (5'guanylyl imidodiphosphate) were the most potent agents in their ability to elicit adenylate cyclase. The alpha agonists, phenylephrine and methoxamine (preliminary observations), displayed an intermediate potency on enzyme activation. The selective $beta_1$-$beta_2$ agonists (dobutamine, tazolol or salbutamol, metaproterenol) exerted only weak activation of the enzyme. Dopamine was ineffective. The stimulation of adenylate cyclase by norepinephrine was inhibited by the following adrenergic agonists in descending order of potency: propranolol (mixed beta) > butoxamine ($beta_2$) > phentolamine (alpha) > practolol ($beta_1$). In preliminary work the beta adrenergic ligand, dihydroalprenolol, was found to bind to particulate fractions of pia in a stereospecific fashion. An analog of adenosine, 2-Cl-adenosine, in the presence of adenosine deaminase failed to activate adenylate cyclase with any degree of potency. However, 2-Cl-adenosine was active in isolated cerebral capillaries. Chronic treatment of rats with reserpine (one week) produced a heightened sensitivity of pial adenylate cyclase, an action not observed in corresponding capillaries. Thus, the pia-arachnoid possesses a mixed alpha-beta receptor activation of adenylate cyclase. The location of these receptors to pre- or postsynaptic sites is unknown. (Supported by NSF PCM 7911782.)

295.2 BEHAVIORAL EFFECTS OF CYCLIC GMP IN THE CAUDATE NUCLEUS. Irwin N. Lourie*, Michael M. Krieger*, Sarah A. Kilgour* and Nagendran S. Thampi. (Spon. Luis A. Marco) Res. Dept. Norristown State Hospital, Norristown,PA 19401

Previous experiments (Lourie Neuroscience Abstracts,5:564, 1979) indicate a biphasic behavioral response to intraventricular administration of dibutyrl cyclic 3',5' guanosine monophosphate (dbc-GMP) with depression at low doses (5µg) and excitation at higher doses (50ug). As part of a series of experiments to localize this effect bilateral cannula implants were directed at the caudate nucleus of 125 day old male NSH rats (n=8). 21 days following surgery subjects were equilibrated to an open field testing apparatus and handling procedures for 6 days at which time behavior patterns are stabilized. On the subsequent 4 days a randomized latin square design was used to test 4 dbc-GMP levels (0,2,8,32 µg). On day 5 all subjects were given 64 µg. All doses were delivered in 0.5 ul of saline over a period of 31 sec, 30 min prior to assay. Behavioral assay consists of analyzing the first 5 min of a 1 hr exposure to the apparatus. Data was taken in 1 min blocks in order to measure habituation effects. Following each assay the subjects were subjected to a tail pinch measure of nociception.

There was a two-fold increase in motor activity at 32 µg using both time and distance measures with no activation at the lower doses. This effect is unlike that of amphetamine in that the subjects show evidence of habituation on both motor and motivational measures. Grooming shows a biphasic effect with 40.1, 21.2, 39.8 and 12.8 sec of activity for the 0, 2, 8, 32 µg doses respectively. The magnitude of this effect seems independent of exploratory activity measures. The animals also showed a withdrawal effect that was related to cumulative dosing. This was evidenced as an apparent deficit in initial exploratory activity during the first minute. There was no loss of nociceptive responding at any dose. At 64 µg all of the animals showed evidence of convulsive behavior. A unique behavioral feature at this dose was self mutilating behavior in 3 of the eight animals.

The activation data are consistent with the findings of Baker, Psychopharm.Bull. 16:27,1980 who demonstrated the effect of cholinergic stimulation of the caudate with subsequent activation of cortical,hippocampal, and motor systems and those of Greengard Nature, 260:101, 1976 who formulated the c-GMP second messenger concept of the muscarinic action of acetylcholine.

295.3 WITHDRAWN

295.4 CYCLIC NUCLEOTIDE-DEPENDENT PHOSPHORYLATION OF ENDOGENOUS PROTEINS OF BOVINE ROD OUTER SEGMENTS. Rehwa H. Lee*, Bruce M. Brown*, Bernice S. Lieberman* and Richard N. Lolley. Dept. of Anatomy, UCLA School of Medicine, Los Angeles, CA 90024, and V.A. Medical Center, Sepulveda, CA 91343.

A role is proposed for cGMP in the visual process of rod photoreceptors. The mechanism by which cGMP modulates the activities of rod visual cells probably involves protein phosphorylation. We have identified and partially characterized the cyclic nucleotide-dependent protein kinases (CNPK) of bovine rod outer segments (ROS) using exogenous soluble proteins. We have now analyzed the endogenous substrates for CNPK. Soluble proteins that are phosphorylated by CNPK were obtained by centrifugation of ROS homogenates prepared in hypotonic buffer. After labeling with ^{32}P, the phosphoproteins were separated by SDS-PAGE and identified with autoradiography. It was found that a 33K protein (30K, using the Weber & Osborn system, BBRC, 78:572, 1978) and, to a lesser extent, a 45K protein were phosphorylated in a cyclic nucleotide-dependent manner. The concentration of the 33K protein is greater in ROS than in the complete bovine retina, suggesting that this protein is localized in ROS. The 33K protein has an isoelectric point of 5.4, a sedimentation coefficient of 6.0 S and a proposed dimeric configuration in the native state. After partial purification by density gradient centrifugation, the 33K protein was phosphorylated by partially purified CNPK with maximal phosphate incorporation achieved in the presence of either 10^{-4} M cGMP or 10^{-5} M cAMP. Illumination of ROS membranes does not appear to affect the extractability of the 33K protein. These findings support the proposal that cGMP, in regulating the activity of ROS CNPK, controls the rate at which the 33K and, perhaps, the 45K proteins are phosphorylated. These endogenous ROS proteins may be the ultimate effectors of cGMP action in rod visual cell activities. (Supported by NSF grant BNS-79-26806, NIH grant EY00395 and the Medical Research Service of the Veterans Administration.)

295.5 PHOSPHODIESTERASES OF THE CONE-DOMINANT GROUND SQUIRREL RETINA. Debora B. Farber and Dennis W. Souza*. Jules Stein Eye Institute, UCLA School of Medicine, Los Angeles, CA 90024, and V.A. Medical Center, Sepulveda, CA 91343.

There is strong evidence implicating cGMP phosphodiesterase of rod photoreceptors in the visual process. In a light-regulated manner, this enzyme catalyzes the reduction of cGMP levels of rods. Cone- and rod-dominant retinas differ in their cyclic nucleotide concentrations with a cAMP/cGMP ratio of 2.2-8.0, in cone-dominated, vs. 0.2-0.3 for the rod-enriched. In addition, light selectively reduces cAMP levels in the cone-dominant retinas; cGMP content remains unchanged.

We have separated and partially characterized several cAMP and cGMP phosphodiesterases of the ground squirrel retina. Three kinetically distinct cAMP phosphodiesterases (Kms 3.9 x 10^{-5} M, 1.8 x 10^{-4} M and 2.4 x 10^{-3} M) and one cGMP phosphodiesterase (Km 1.6 x 10^{-4} M) were found in crude retinal homogenates. Isoelectric focusing of the post-nuclear supernatant from the homogenate separated two peaks of phosphodiesterase activity at pH 4.1-4.4 and pH 4.8-4.9 which hydrolyzed cAMP; the latter peak also hydrolyzed cGMP. Other peaks of phosphodiesterase activity which used cGMP as substrate were observed at pH 4.3-4.5 and pH 4.9-5.1. Each of the isoelectrically focused fractions was subjected to sucrose gradient centrifugation. Using hemoglobin and alcohol dehydrogenase as internal markers, we estimated the molecular weights of the partially purified enzymes. Two phosphodiesterases which hydrolyzed both cAMP and cGMP had molecular weights of approximately 175,000 and 122,000, and one phosphodiesterase which selectively utilized cAMP as substrate had a molecular weight of 50,000. The enzymes were further purified by HPLC, and SDS-PAGE was used to characterize their subunit composition.

We have used a similar purification scheme for the bovine rod outer segment phosphodiesterase. Our results indicate that this enzyme has a different isoelectric point and different subunit components than the phosphodiesterases of the cone-dominant retina of the ground squirrel.

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295.6 SPECIFIC INHIBITION OF PHOSPHORYLATION OF PROTEIN I, A SYNAPSE-SPECIFIC PROTEIN, BY PURIFIED ANTI-PROTEIN I ANTIBODY. S. Naito and T. Ueda. Mental Health Research Institute, Depts. of Psychiat. & Pharmacol., Univ. of Mich., Ann Arbor, MI 48109.

Protein I is a synaptic protein which serves as an endogenous substrate for cyclic AMP- and calcium-dependent protein kinases. Antibodies raised against purified Protein I have been isolated from rabbit antiserum by affinity chromatography on Protein I-conjugated agarose column. The purified antibodies were identified as immunoglobulin G by SDS-polyacrylamide gel electrophoresis and Ouchterlony double immunodiffusion precipitation test. The purified antibodies (but not immunoglobulin G purified from preimmune serum) showed the ability not only to inhibit the phosphorylation of purified Protein I by exogenous cyclic AMP-dependent protein kinase, but also to inhibit specifically the phosphorylation of Protein I by endogenous cyclic AMP-dependent protein kinase in a homogenate of rat cerebrum or in the synaptic junctional complex and synaptic vesicle fractions of bovine cerebral cortex. The antibodies to Protein I also inhibited with a similar potency calcium-dependent phosphorylation of Protein I without affecting the phosphorylation of other proteins. Moreover, the Fab(t) fragment of anti-Protein I immunoglobulin G, which was produced by tryptic digestion, retained the ability to inhibit specifically the phosphorylation of Protein I. This substrate-directed, specific inhibitor of the phosphorylation of Protein I may provide a unique probe for investigating the function of Protein I phosphorylation.

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295.7 BRAIN CYCLIC NUCLEOTIDE RESPONSE FOLLOWING CENTRAL CHOLINERGIC ACTIVATION IN RATS EXPOSED TO CHRONIC LEAD. R.H. Lenox[1], G.J. Kant[2], J.L. Meyerhoff[2], Z. Annau[3]. Univ. Vermont, Burlington VT 05405[1]; Walter Reed Army Inst. Research, Washington DC 20012[2]; Johns Hopkins University, Baltimore MD 21205[3].

Neurochemical and electrophysiological studies of animals chronically exposed to lead support a role for lead in the inhibition of central and peripheral cholinergic systems (Shih and Hanin, Life Sci., 1978). Chronic lead exposure has also been reported to decrease in vivo turnover of acetylcholine in cortex, hippocampus, midbrain and striatum of the rat. Recent investigations from our laboratory have reported significant changes in both cyclic AMP and cyclic GMP in specific brain regions of rats following central cholinergic stimulation with oxotremorine(Lenox et al. Life Sci., 1980). In order to further define the state of central cholinergic responsivity in animals exposed to chronic low levels of lead,we have examined cyclic nucleotide response to oxotremorine challenge in specific brain regions of the rat.

Long-Evans rats selected for study had been derived from matched mothers exposed to lead acetate (1000 ppm) or distilled water throughout gestation and lactation. Animals with preweaning history of lead exposure were maintained on lead acetate(1000ppm) until sacrifice at 60 days of age. The matched control group of animals were maintained on distilled water until sacrifice. Animals were maintained in light-cycled chambers and all experiments took place at the same time of day. Both chronic lead and control animals received an intraperitoneal injection of oxotremorine (2 mg/kg) or saline and were placed in an activity arena 10 min prior to sacrifice by exposure to high power microwave inactivation system. All animals were pretreated with methylatropine (0.5 mg/kg)40 min prior to sacrifice. Following sacrifice and decapitation, trunk blood was collected for radioimmunoassay of plasma hormones and 21 brain regions were dissected for radioimmunoassay of cyclic AMP and cyclic GMP.

Levels of cyclic AMP in the pituitary increased 15-fold in both control and lead animals confirming our previous findings.Of the remaining regions assayed, no large differences were observed between lead and control animals. However, there was a trend in the lead animals vs. controls toward an increased cyclic AMP response to oxotremorine challenge in the substantia nigra and brainstem. Animals exposed to oxotremorine demonstrated less overall motor activity. There was a trend, however, for higher cyclic GMP levels in the cerebellum of lead animals vs. controls. These data reflect a neurochemical response to central cholinergic stimulation in animals exposed to lead concentrations existing in some environments.

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295.8 MUSCARINIC CHOLINERGIC RECEPTOR REGULATION OF CYCLIC AMP METABOLISM IN CULTURED ASTROCYTOMA CELLS. R.B. Meeker* and T.K. Harden, (Spon: A.T. Dren) Department of Pharmacology, University of North Carolina School of Medicine, Chapel Hill, N.C. 27514

Incubation of 1321N1 human astrocytoma cells with 10 μM (-)-isoproterenol (ISO) results in a rapid increase (>30 x basal) in intracellular cAMP levels. The addition of 100 μM oxotremorine (OXO) concomitantly with ISO reduces the accumulation of cAMP by 50-70% and decreases the $t_{1/2}$ to steady-state from 0.9 min in the presence of ISO alone to 0.2 min in the presence of ISO + OXO. This inhibitory effect of OXO is competitively blocked by 1 μM atropine and noncompetitively prevented by the presence of 0.5 mM isobutylmethylxanthine. The capacity of intact cells to degrade cAMP was measured by incubation for 5 min in ISO or ISO + OXO followed by blockade of the synthetic reaction by the addition of a saturating concentration of propranolol. The rate constant for cAMP degradation (k_{deg}) in the absence of OXO was 0.389 min^{-1}. In the presence of OXO cAMP degradation was biphasic with over 50% of the cAMP being degraded in a rapid phase (k_{deg} = 1.334 min^{-1}; 0-30 sec) followed by a slower rate (k_{deg} = 0.300 min^{-1}) similar to that observed in the presence of ISO alone. These data suggest that activation of muscarinic receptors reduces cAMP levels in 1321N1 cells by increasing phosphodiesterase activity.

Preincubation of 1321N1 cells in 100 μM carbachol results in a rapid loss of the inhibitory effect of OXO. After a 30 min preincubation, the inhibitory activity of OXO is reduced by 50%. The properties of control and desensitized muscarinic receptors were examined using the ligand ^{3}H-quinuclidinyl benzilate (^{3}H-QNB). ^{3}H-QNB binds to a single class of sites with an affinity of 10 pM and Bmax of 50 fmol/mg protein. Rate constants of association and dissociation were 3 x 10^8 $M^{-1}min^{-1}$ and 4 x $10^{-3}min^{-1}$, respectively. A 24 hr incubation with 100 μM carbachol results in a greater than 90% loss of receptors. Receptor loss occurs with a slower time course ($t_{1/2}$ = 4 hr) than does desensitization to the inhibitory effects of OXO. Since greater than 80% of the inhibitory effect of OXO on cAMP accumulation is lost prior to a significant reduction in receptor number, it seems unlikely that receptor loss per se could account for at least the initial stage of desensitization. Supported by NIH Grant GM29536.

295.9 CELLULAR LOCALIZATION OF CYCLIC NUCLEOTIDE CHANGES IN THE RAT SUPERIOR CERVICAL GANGLION. Clark A. Briggs*, Marjorie A. Ariano and Donald A. McAfee, (SPON: Richard E. Wimer). Div.of Neurosciences, City of Hope Research Institute, Duarte, CA 91010 and Dept. Anatomy and Neurobiology, Univ. Vermont College of Medicine, Burlington, VT. 05405.

Preganglionic stimulation produces a two-fold increase in cyclic AMP content and a five-fold increase in cyclic GMP content of the ganglion. Cyclic nucleotide levels increase as a function of the frequency and duration of tetanic preganglionic stimulation, becoming maximal at 10 Hz for 30 sec. Low Ca^{2+} or Co^{2+} (2mM) inhibits cyclic GMP increases and blocks cyclic AMP increases. Also, cyclic nucleotide levels are augmented by bath application of isoproterenol, azide, and KCl (Quenzer et al. (1980) J. Pharmac.Exp.Ther. 215:297). One of our goals was to identify the type of cells showing changes in cyclic nucleotide levels following experimental manipulation of the ganglion.

Pairs of ganglia were isolated and maintained in Locke solution at 23°C; one member of each pair served as a control. At the end of the experiment both ganglia were bisected. One half was homogenized for radioimmunoassay and the other was frozen for immunohistochemistry for cyclic nucleotides. Frozen sections 8 μm thick were treated with either diluted anti-cyclic AMP or anti-cyclic GMP antisera for 1 to 3 hrs. The unbound antisera was washed off and replaced with fluorescein-coupled antirabbit goat antiserum. The fluorescent antisera are assumed to localize the remaining cyclic nucleotide antibody bound to relatively slowly diffusing cyclic nucleotides. Morphological elements were identified through the use of glyoxylic acid-induced catecholamine fluorescence and toluidine blue counterstain.

In non-stimulated ganglia, neurons and satellite cells stained for cyclic AMP. Preganglionic electrical stimulation increased cyclic AMP staining in small neurons and satellite cells while depolarization of the ganglion by KCl increased cyclic AMP staining in all cell types. Sodium azide did not increase cyclic AMP staining. Only large neurons stained for cyclic GMP and this staining increased with preganglionic stimulation, KCl, and sodium azide. These studies demonstrate that 1) cyclic nucleotides can be localized at the cellular level in the rat ganglion by immunohistochemistry; 2) cyclic AMP and cyclic GMP show a differential localization; and 3) changes in cyclic nucleotide levels can be detected by immunohistochemistry in the rat ganglion. (Supported by Grants NS 12116 and BNS 79-12394, and a Biomedical Research Support Grant to the Univ. Vermont College of Medicine).

295.10 DENERVATION SUPERSENSITIVITY IN LUMBAR SPINAL CORD FOLLOWING MID-THORACIC TRANSECTION. D.J.Jones, M.S.Giere* and B.L.Moak*. Departments of Anesthesiology and Pharmacology, The University of Texas Health Science Center, San Antonio, TX 78284

Previous studies from this laboratory have demonstrated agonist specificity for cyclic AMP accumulation in rat spinal cord tissue slices (Jones and McKenna, Neuropharmacol., 19: 669-674, 1980). The presence of descending catecholamine containing neurons in spinal cord and their interruption by transection suggests that adrenergic denervation supersensitivity might be associated with the adenylate cyclase-cyclic AMP response to catecholamines in denervated spinal cord below the transection site.

Transection of the spinal cord was performed at the mid-thoracic level following laminectomy in 125-150 Gm rats. Following various time periods, rats were sacrificed and the cervical (intact) and lumbar (denervated) spinal cord removed and sliced on a McIlwain tissue chopper (300 μm) with subsequent incubation for one hour in oxygenated Krebs-Ringer bicarbonate. Following this period, the slices were placed into incubation flasks for 15 min at which time various agonists were added. At the end of the incubation period, tissue protein was denatured and cyclic AMP measured by radioimmunoassay.

Seven days following transection there was an enhanced accumulation of cyclic AMP in lumbar spinal cord in response to norepinephrine (NE). The enhanced response was concentration dependent with the maximal increase in lumbar cyclic AMP accumulation in the presence of 10^{-5} M NE being 3-4 times greater than the intact cervical cord response. No change in NE EC_{50} was observed in responses of the denervated lumbar vs control cervical spinal cord. Supersensitivity in the lumbar spinal cord cyclic AMP system is present as early as 3 days and lasts at least 30 days (the latest time tested), and occurs in both dorsal and ventral cord sections. Enhanced responsivity is also evident in the presence of isoproterenol, but not K^+ or adenosine. These data suggest that synaptic supersensitivity exists in lumbar spinal cord following transection and that alterations in adrenergic receptor sensitivity may play a role in mediating events following deafferentation.

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295.11 IDENTIFICATION OF AN ESSENTIAL SULFHYDRYL GROUP FOR THE ACTIVATION OF DOPAMINE-SENSITIVE ADENYLATE CYCLASE, E. T. Suen*, P.C.K. Kwan* and Y.C. Clement-Cormier. Department of Pharmacology, The University of Texas Medical School at Houston, Houston, Texas 77025.

A sulfhydryl alkylating agent, N-ethylmaleimide was used to study the role of sulfhydryl groups in the coupling of the dopamine receptor to adenylate cyclase in striatal membranes. N-ethylmaleimide inhibited dopamine-sensitive adenylate cyclase activity in membranes pretreated with the alkylating agent. A fifty percent inhibition of dopamine-sensitive adenylate cyclase activity was observed in the presence of 10μ M N-ethylmaleimide. Similar results were observed with p-chloromercuribenzoate, a heavy metal alkylating reagent. The reducing agents, dithiothreitol, 2-mercaptoethanol, and cysteine had no effect on dopamine-sensitive adenylate cyclase activity. N-ethylmaleimide inhibited GTP and NaF stimulated adenylate cyclase activity but was without effect on adenylate cyclase assayed in the presense of manganese. Pretreatment of membranes with GPP(NH)P or G TP (γ)S prior to exposure to N-ethylmaleimide prevented the inhibitory effect of the sulfhydryl reagent on adenylate cyclase activity. N-ethylmaleimide also produced a ten fold decrease in agonist affinity for the dopamine receptor binding site which was similar to the decrease in agonist affinity observed in the presence of GTP. The effect of GTP on agonist affinity was not additive with N-ethylmaleimide. Overall, the data reveal that a reactive sulfhydryl group is important for the coupling of the components of the cyclase complex and that alkylation of the reactive sulfhydryl group modifies the activity of the guanine nucleotide binding protein. (Supported by U.S.P.H.S. Grants MH035851 and BNS 7816003)

295.12 THE EFFECT OF CHRONIC DOPAMINE AGONIST TREATMENT ON TYROSINE HYDROXYLASE AND ADENYLATE CYCLASE ACTIVITIES. K.D. Wilner*, I.J. Butler, W.E. Seifert and Y. C. Clement-Cormier. Department of Pharmacology, The University of Texas Medical School at Houston, Houston, Texas 77025.

The effect of long-term levodopa, bromocriptine or combination levodopa and bromocriptine therapy was tested on rat striatal adenylate cyclase, dopamine receptor binding and tyrosine hydroxylase activities. An oral dose of sinemet (250 mg levodopa/25 mg carbidopa) and/or intraperitoneal bromocriptine (2 mg/kg) were administered for 21 days. Following chronic sinemet treatment, an increase in the EC_{50} for dopamine stimulation of adenylate cyclase was observed in the drug treated group compared to controls. In addition, a significant increase in the number of specific (^3H) spiroperidol binding sites was observed in the drug treated group. In the chronic bromocriptine study, there was no significant change between control and experimental groups in dopamine-stimulated adenylate cyclase or (^3H) spiroperidol binding. However, the analysis of the CSF neurotransmitter metabolites HVA, 5-HIAA and MHPG indicated a significant increase in HVA following bromocriptine treatment. In addition, there was a significant decrease in tyrosine hydroxylase activity in the bromocriptine treated group as compared to the control group. Combination sinemet and bromocriptine treatment yielded similar results as with bromocriptine alone. These data suggest that the increase in the number of dopamine binding sites following chronic levodopa treatment may correlate with the behavioral supersensitivity seen in Parkinsonian patients on this drug regimen. The results also show that bromocriptine treatment abolishes the levodopa-induced increase in the number of dopamine binding sites. Thus, the data suggest a possible biochemical mechanism for the success of combination levodopa and bromocriptine therapy in the treatment of Parkinson's disease. (Supported by U.S.P.H.S. Grants MH035851 and BNS 7816003)

295.13 CYCLIC GMP METABOLISM: HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC (HPLC) ANALYSIS OF RAT BRAIN SLICES. R. D. Kelson*, M. Cohn, D.J. Wooten* and M.L. Cohn, Lab. Anesthesia Research, Drew Medical School, Los Angeles, CA 90059.

Reports have shown that, following parenteral injection of morphine to rats, cyclic GMP (cGMP) content is differentially altered in various brain areas: e.g. cGMP increases in striatum and nucleus accumbens and decreases in cerebellar cortex. Cholinergic agonists have also been shown to be potent analgetics and to alter brain cGMP content. However, a cause/effect relationship between changes of cGMP concentrations and analgesia regulation has yet to be determined. Like morphine, cGMP administered centrally to rats produces profound analgesia but appears to regulate pain through a different pathway (Cohn et al, 1978). In order to clarify this evidence, we sought additional information on cGMP metabolism in the central nervous system. Since little is known about cGMP metabolic pathways, in the present study, we analyzed the catabolic conversions of the nucleotide using HPLC. Male Sprague-Dawley rats were sacrificed; brains rapidly removed on ice-prechilled plate and sectioned into various parts; sections cut into 260u slices with McIlwain tissue chopper; slices rapidly transferred into a tonometer (Instrumentation Laboratory IL237) containing oxygenated Krebs-Ringer buffer and standard cGMP (.5uM); and incubated at 37°C. Aliquots of incubation mixtures were sequentially withdrawn (0-60min), boiled for 3 min, filtered through Millipore filters (.45u) and analyzed under isocratic conditions with an Altex reversed-phase HPLC system (Cohn, Venkatesan, Cohn, 1981). The analytic profile of rat brain cortex incubated with cGMP shows appearance of guanosine monophosphate (GMP), guanosine and guanine, in this order. The fact that there is no evidence of formation of either inosine monophosphate or xanthosine suggests the absence of both GMP reductase and guanosine aminohydrolase activity under our conditions. Analysis of midbrain slices incubated as described above showed patterns identical to those of cortex slices. With our analytical procedure, we hope to determine whether alterations in cGMP concentration and/or changes in cGMP metabolism induced by morphine are linked to the analgetic properties of the narcotic. Supported by NIH (MBS) Grant RR08140.

295.14 CYCLIC AMP METABOLISM: OPPOSITE EFFECTS OF HALOGENATED HYDROCARBON ANESTHETICS AND INSECTICIDES. Major L. Cohn, Daniel J. Wooten*, Stephan J. Cohn*, Daryl Rayford*. Lab. Anesthesia Research, Drew Medical School., Los Angeles, California 90059.

The Meyer Overton lipid solubility theory is well accepted. Recent reports of strong correlations between oil/water and oil/air partition coefficients and potency of anesthetic agents (AA) support the theory. However, no theory of narcosis is all inclusive. Our recent findings that barbiturates alter cyclic AMP (cAMP) metabolism correlate with our previous behavioral data which suggests that in-vivo cAMP is a key factor in the regulation of narcosis. In the present study we compared the effects of barbiturates on cAMP metabolism to those of halothane, (HA)--a halogenated hydrocarbon anesthetic--and DDT, chlordane, and heptachlor -- three halogenated hydrocarbon insecticides (HHI). The two classes of halogenated hydrocarbons share high lipid solubility but elicit biological activities in opposite directions. Sprague-Dawley rats were sacrificed by decapitation; brains swiftly removed to ice-prechilled plate; cerebral cortex cut into slices; and slices rapidly transferred to ice-cold oxygenated (95% O_2-5% CO_2) Krebs-Ringer buffer for 10 min. preincubation with either DDT, chlordane, or heptachlor (.14-0.6 uM). Next, slices were incubated at 37°C with fresh oxygenated Krebs-Ringer buffer containing standard cAMP (.5 uM) and HHI in concentrations similar to those used in preincubation. Control slices were preincubated and incubated without HHI. Slices treated with HA were prepared as described above, then incubated with cAMP (.5uM) in a tonometer (Instrumentation Laboratory IL 237) to which HA (1%) was delivered for the duration of experiments. Aliquots of incubation mixtures were sequentially withdrawn (0-60min.); filtered through Millipore filters (.45u); and analyzed with an Altex Liquid Chromatographic (HPLC) system. Confirming our previous findings (Cohn and Cohn, 1980), HPLC analysis of control slices showed that adenosine monophosphate, inosine, and hypoxanthine are the major catabolic products of cAMP. Like barbiturates, HA led to increased formation of adenosine monophosphate and adenosine. In contrast, HHI did not shift the metabolic pattern. The fact that HHI do not alter adenylate deaminase (AD) activity supports our argument that inhibition of AD is a specific action of AA on purine metabolism. This analytical data may explain the opposite biological actions of AA and HHI: AA depress CNS while HHI produce excitatory syndrome. Agreeing with our data are close correlations shown between rate of deamination and brain ammonia production; ammonia concentrations and neural activity; and a sharp decrease in brain ammonia levels in anesthetitized rats. Supported by NIH (MBS) Grant RR08140.

295.15

WITHDRAWN

295.16 ACTIVATION OF CORTICAL ADENYLATE CYCLASE BY PROTEIN PHOSPHORYLATION. Scott R. Whittemore*, Robert H. Lenox, Edith D. Hendley, and Yigal H. Ehrlich, Depts. of Physiology & Biophysics, Psychiatry, and Biochemistry, University of Vermont College of Medicine, Burlington, VT 05405

We have reported recently that protein phosphorylation mediates effects of isoproterenol on adenylate cyclase (AC) activity in rat cortical membranes (Whittemore et al, Neurochem. Res. Vol. 8, 1981 in-press). The present studies examined whether the phosphorylation of membrane-bound proteins plays a direct role in the regulation of AC. An assay system has been developed in which pre-incubation of cortical membranes under phosphorylating conditions was followed by extensive washes under conditions which minimize dephosphorylation. Cyclic AMP formation was then measured in resuspended membranes. Preincubation with 2mM ATP + 10mM $MgCl_2$ for 5 min. at 30°C resulted in a 70% increase of AC activity upon reincubation. With longer pre-incubation times, AC activity returned to control levels, suggesting that the activation of AC is reversed by de-phosphorylation. To test this possibility we used ATP-gamma-S in the preincubation buffer. This ATP analog is used by protein kinases to phosphorylate proteins, but the phosphothioester bond formed is known to resist hydrolysis by phosphatases. Preincubation of cortical membranes with ATP-gamma-S for 5 min. resulted in over 300% activation of AC. This activation was irreversible, as compared to ATP, since longer pre incubations further activated AC (740% increase after 40 min). This irreversible activation of AC was found to be dependent on a number of factors: ATP-gamma-S concentration (40μM ATP-gamma-S produced half maximal activation of AC) preincubation time, and most importantly, Mg^{2+} concentration. At 10mM $MgCl_2$ the activation by ATP-gamma-S was five-fold greater than when exogenous Mg^{2+} was not added. Further experiments indicated that this activation cannot be accounted for by changes in ATPase or phosphodiesterase activity. The use of radiolabeled ATP as a substrate for protein kinase, followed by SDS-gel electrophoresis and autoradiography, served to reveal specific phosphoproteins that may be involved in this activation of AC. These data demonstrate a direct role for membrane-bound protein phosphorylation systems in AC regulation. Together with our reports that neurotransmitters regulate protein phosphorylation activity in brain membranes (Ehrlich et al, Life Science, 26:1975, 1980), the results indicate that this process may play a role in the mechanism of receptor-coupled information transfer in the nervous system. (Supported by PHS grants DA 02747 and MH 25811).

295.17 PRELIMINARY STUDIES ON THE EFFECTS OF VARIOUS PEPTIDES ON PHOSPHODIESTERASE ACTIVITY FROM RAT BRAIN. O.Sartor* and M.A.Spirtes
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Peptide hormones are generally thought to control cyclic nucleotide levels by altering the rate of synthesis. However in some peripheral tissues, these hormones have been shown to alter the rate of breakdown as well. At this time there are no reports of transmitter or hormone-induced changes in phosphodiesterase activity from brain. This study sought to explore that potential.

A particulate enzyme fraction was prepared from rat occipital cortex or hypothalamus. The phosphodiesterase activity was assayed by a modification of the Rangel-Aldao et al method using 10^{-6} M H^3 c-AMP as substrate. Results are expressed in terms of percentage of control. Use of boiled enzyme (3.2) or the phosphodiesterase inhibitor IBMX (1.8) confirmed the existence of enzymatic activity. The lack of 100 millimolar KCl (94±7) effect suggests that the activity was primarily due to the low Km phosphodiesterase, however the stimulation by 30 micromolar $CaCl_2$ (128±2 $p<.01$) indicates that high Km enzyme is also present. In the hypothalamus, 10^{-7} M angiotensin II (101±4), 10^{-6} M bradykinin (95±5), 10^{-6} M αMSH (99±2), 10^{-6} M MIF (106±9), 10^{-6} M neurotensin (107±6), and 10^{-5} M substance P (105±5) had no effect. 10^{-6} M substance P had a small but statistically significant effect on the hypothalamic enzyme (112±5 $p<.05$). In the occipital cortex, αMSH at 10^{-6} M (98±3), 10^{-8} M (98±3), or 10^{-10} M (99±2) had no effect on the enzyme.

In whole brain, particulate c-GMP phosphodiesterase activity was assayed with 10^{-5} M H^3 c-GMP serving as substrate. IBMX inhibited activity (11±4) but MIF at 10^{-5} M (102±1) or 10^{-7} M (105±2) had no effect.

As yet there are no peptides which have been shown to effect phosphodiesterase in the brain.

1) Rangel-Aldao, Schwartz, and Rubin (1978) Analytical Biochemistry 87:367-375.

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295.18 CYCLIC NUCLEOTIDE AND CALCIUM LEVELS IN CEREBROSPINAL FLUID (CSF) OF AN INHERITED FELINE TREMOR (IFT). G.A. Hegreberg and M.J. Hamilton*. Dept. Veterinary Microbiology and Pathology, Wash. State Univ., Pullman, WA 99164.

Inherited feline tremors (IFT), an autosomal recessive disorder, is clinically apparent at 2-4 weeks of age and is recognized by fine intentional tremors of the head, trunk, and tail. The disease is progressive and the tremors increase in severity in the young growing kitten. Those kittens surviving beyond 16 weeks of age demonstrate remission of the tremors. During this stage of the disease,convulsive seizures, agressive behavior with unprovoked biting and hissing, diarrhea, and visual impairment have been observed. In adult cases, the clinical manifestations include convulsions, behavioral changes which include catatonia, repetitious activity, unkempt coat and a withdrawn and flattened response to attention.

A close relationship exists between divalent cations, including calcium, and the metabolism of cyclic nucleotides in nervous tissue. Changes in the CSF concentrations of cyclic nucleotides, especially a significant depression of cGMP, have been reported in psychiatric illnesses, including depressed, manic, and schizophrenic groups. A depression in the concentration of CSF calcium has also been reported in association with certain psychiatric illnesses.

Total calcium, cAMP, and cGMP were measured in the CSF of adult affected and nonaffected cats. Cerebrospinal fluid was obtained from atlanto-occipital punctures. cAMP and cGMP were determined using modifications of the radioimmunoassay methods of Steiner, et al. Reagents were obtained from New England Nuclear Co. Calcium was determined using an atomic absorption spectrophotometric method.

Results disclosed that the affected cats have a significant decrease (p 0.005) in CSF levels of cGMP (affected [n=10] [mean ± S.D.] 1.7±0.85 pmoles/ml; nonaffected [n=11] 3.1±1.17). Although cAMP was decreased in the CSF of affected cats, no statistical difference was noted between the affected and nonaffected cats (affected [n=10] 12.9±6.5 pmoles/ml; nonaffected [n=11] 15.0±7.8 pmoles/ml.). Levels of CSF calcium were not significantly different between affected and nonaffected adult cats (affected [n=8] 3.78±0.14 meq/L; nonaffected [n=21] 3.90±0.36 meq/L).

(Supported by NIH grant RR 00515 and Washington State University)

295.19 PROPERTIES OF A GONADOTROPIN-SENSITIVE ADENYLATE CYCLASE IN A LEYDIG TUMOR CELL LINE. R. V. Rebois* and P. H. Fishman*
(SPON: L. Abood). Membrane Biochemistry Section, Developmental and Metabolic Neurology Branch, National Institute of Neurological and Communicative Disorders and Stroke, The National Institutes of Health, Bethesda, Maryland 20205.

The transplantable Leydig tumor M5480P has been adapted to culture and cloned to produce several cell lines that have retained their responiveness to lutropin (LH) and human chorionic gonadotropin (hCG). The cell line most intensively studied has been designated MLTC-1. The cells appear to have a single class of high affinity and specific binding sites for hCG. Concentrations as low as 10 pM hCG are capable of inducing the production of adenosine 3':5'monophosphate (cAMP) in intact cells. Cholera toxin (CT) also stimulated cAMP production in MLTC-1 cells. Levels of cAMP increase over an order of magnitude in the presence of either the hormone or the toxin. Adenylate cyclase activity was directly assayed in membranes prepared from the cells. Activity was stimulated by hCG and LH as well as by a variety of other effectors including NaF, 5'-guanylylimidodiphosphate (GppNHp), and activated CT (plus NAD). NaF activated adenylate cyclase to the greatest extent followed by GppNHp and CT and then the hormones LH and hCG. Thus, MLTC-1 cells possess specific gonadotropin receptors which are functionally coupled to adenylate cyclase through a regulatory component which is also responsive to NaF, guanine nucleotides and CT. Only one other hormone-responsive Leydig tumor cell line has been described (Ascoli, M., Endocrinology 108: 88, 1981); it also was derived from the M5480P transplantable tumor. These unique Leydig cell lines provide ease of experimental manipulation unattainable with material taken from testes and offer an opportunity unattainable with material taken from testes and offer an opportunity to study not only the hormone-responsive adenylate cyclase system but also more characteristic properties of Leydig cells such as steroidogenesis.

295.20 GLUCOCORTICOID INFLUENCE ON A BRAIN PROTEIN KINASE. R. F. Alderson* and P. Y. Sze. Dept. of Biobehavioral Sciences, University of Connecticut, Storrs, CT 06268.

In a previous study (Alderson and Sze, Neurosci. Abst., 6, 536, 1980), we have reported the separation of two fractions of protein kinase from rat brain cytosol. One fraction (peak II), eluted at 0.2 M NaCl on a DEAE-cellulose column, is stimulated by cAMP and appears to be similar to "type II" protein kinase identified in heart muscle. The other fraction (peak I), eluted at 0.1 M NaCl on DEAE-cellulose, contains a protein kinase with a M.W. 90,000 which is not dissociated or stimulated by cAMP.

In this study, we have found that the protein kinase activity in peak I is under the influence of glucocorticoids. Two-day-old male rats were injected with corticosterone (20 µg/g, i.p., twice daily) for a period of 5 days. Littermates were injected with saline as controls. The brain (all tissue anterior to the cerebellum) was homogenized in 50 mM Tris, pH 7.4 - 1 mM DTT - 2 mM EDTA, and the homogenate was centrifuged at 40,000 g. An amount of the supernatant equivalent to exactly 25 mg protein was fractionated on a DEAE-cellulose column (2.5 ml). The elution profiles for protein kinase between the control and steroid-treated animals were compared. Peak II was found to remain unchanged following the corticosterone treatment. In contrast, peak I was substantially increased by the steroid treatment. The increase in the peak size was 40-80% from several chromatograms. To verify the differences, fractions in peak I were pooled and lyophilized, and the specific activity of the protein kinase was further determined.

Regulation of protein kinases by steroid hormones has been described in various tissues by several investigators. For example, a cAMP-dependent protein kinase in the prostate and liver is affected by gonadal steroids (Fuller et al., PNAS, 75, 223, 1978). Glucocorticoids are known to have a variety of effects in the brain. The present data show that in the neonatal brain, corticosterone treatment could modulate the activity of a cytosol protein kinase which appears to be cyclic nucleotide-independent. Although the cellular function of this protein kinase is not clear, the fact that its activity is influenced by glucocorticoids suggests that this protein kinase may be involved in some of the steroid effects in brain.

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295.21 ADENYLATE AND GUANYLATE CYCLASES OF VERTEBRATE RETINAS: BIOCHEMICAL AND HISTOCHEMICAL PROPERTIES. Suhail A. Khoury* and Debora B. Farber (SPON: H. J. Jerison). Jules Stein Eye Institute, UCLA School of Medicine, Los Angeles, CA 90024, and V.A. Medical Center, Sepulveda, CA 91343.

Biochemical measurements and histochemical localization of both adenylate and guanylate cyclase (AC & GC) activities were carried out on retinas of various vertebrates. These included retinas dominated by either cones (13-line ground squirrel) or rods (rat) and duplex retinas, containing both kinds of photoreceptor cells (chicken and teleost). In retinas of ground squirrel, AC has an apparent Km of 5×10^{-4} M (Vmax 3.3 nmol/mg protein/min) when determined in the presence of 10 mM NaF. This enzyme was further activated by NaF, by guanosine and by low concentrations of adenosine. Alloxan, calcium and higher adenosine concentrations inhibited AC in a dose-dependent fashion. Histochemically, AC was localized in the cytoplasmic infoldings of the cone outer segment, in the cilium and along the plasma membrane of the cone inner segment and at the photoreceptor synaptic terminals. AC was found also along the plasma membrane of ganglion cells and on their axons. Similar results were observed in the retinas of chicken and teleost; in the latter, AC activity was found also along the photoreceptor cell nuclear envelopes. GC in the ground squirrel retina shows two apparent Kms: 2.1×10^{-4} M and 3.7×10^{-5} M (Vmax 1.84 and 0.83 nmol/mg protein/min). This enzyme was activated with 0.1 mM ATP and inhibited in a dose-dependent manner by higher concentrations of ATP (1 mM and greater); it was also inhibited by alloxan and calcium. NaF (10 mM) reduced GC activity by 40-50%. No apparent effect of either adenosine or guanosine was detected on GC activity. Similar results were observed with the GC of rat retina and with that of bovine rod outer segments. However, the response of rod photoreceptors to NaF seemed different from that of cones. We observed a 2-fold increase in GC activity of bovine ROS and a 40% increase in the activity of the enzyme from rat retina. Histochemically, GC is localized in the intradiscal spaces of rat outer segments, in the cilium, along the nuclear envelopes and, to a lesser extent, on the photoreceptor synaptic terminals. In ground squirrel, GC is found in the cilium and along the nuclear envelopes of photoreceptor cells. A lower density of reaction product was found on the plasma membranes of ganglion cells and on their axons. Similar localizations were detected in the teleost. Thus, these studies show biochemical and histochemical differences in the synthetic enzymes of cyclic nucleotides from rod- and cone-dominant retinas. (Supported by NIH training grant EY7026 (SK), by NIH grant EY2651 and RCDA EY0144 (DF), and by the Medical Research Service of the V.A.)

296.1 SYSTEMIC ADMINISTRATION OF PUTRESCINE INDUCES GABA-LIKE BEHAVIORS IN RATS. F. D. Feng and B. B. Turner. Depts. of Psych. & Biol., Virginia Polytechnic Institute & State Univ., Blacksburg, VA 24061

Putrescine is a simple polyamine with multiple roles in cell growth and metabolism. It is also a minor precursor of the inhibitory neurotransmitter, GABA. Administration of ^{14}C-putrescine results in slight, but significant conversion to GABA in neural and peripheral tissues. We now present evidence that oral and i.p. administration of pharmacological levels of putrescine induces behaviors that are similar to those induced by GABA.

Male Sprague-Dawley rats (350-425g) were given putrescine or saline. Oral putrescine doses were 50, 250, or 1000 mg/kg; i.p. doses were 25, 50, 100, or 250 mg/kg. Behavioral observations included the time sampling of 15 behaviors over 60 min, followed by 6 additional measures: startle response, open-field ambulation, vertical grid endurance, food and water intake, body temperature, and pain responsivity to electric shock. As an indication of the duration of any analgesic effect, pain responsivity was determined again 21 hr post-treatment.

In the oral putrescine experiment, significant effects were found on all measures except grid endurance. The i.p. putrescine experiment revealed similar findings, though no significant effects were found with respect to food or water intake, or body temperature. Those behaviors scored by time sampling which were affected by putrescine treatment included grooming, sleep, rearing, gnawing, mastication stereotopy, abnormal posture/movement, and at the highest doses, "wet-dog" shakes. A positive, dose-dependent effect was seen for attenuation of initial startle intensity, facilitation of startle habituation, decreased open-field ambulation, and increased pain thresholds in both experiments. Significant hypothermia, polydipsia, and anorexia were seen with 1000 mg/kg putrescine. Additional behaviors associated with high doses included ptosis, piloerection, incomplete grooming sequences, fore-paw flicking, sedation and flacid immobility. Decrements in pain sensitivity remained at 21 hr post-treatment only in animals receiving oral putrescine.

These data provide evidence for the elicitation of a complex of behavioral changes following oral or i.p. administration of putrescine. Many of the observed behavioral effects are similar to those reported following treatment with GABA, GABA agonists, and GABA-transaminase inhibitors. The occurence of "wet-dog" shakes and pronounced analgesia suggest the facilitation of endogenous opiate systems. From the behavioral evidence obtained, we conclude that systemic administration of putrescine may modulate CNS GABA function.

296.2 RAPHE UNIT ACTIVITY IN FREELY MOVING CATS: EFFECTS OF BENZODIAZEPINES. Donald W. Preussler, Gailyn A. Howell, Christopher J. Frederickson and Michael E. Trulson. Dept. Psychol., Univ. of Texas at Dallas, P.O. Box 688, Richardson, TX 75080.

Previous studies have shown that the activity of serotonin-containing raphe neurons in cats is grossly correlated with the level of behavioral arousal or tonic motor activity. These neurons display a progressive decrease in activity with descending levels of arousal, from a maximum of 3-5 spikes/sec during active waking to complete silence during REM sleep. This suppression of activity during REM sleep may be simply due to the fact that tonic EMG activity or motoric output is at a minimum. On the other hand, raphe unit activity may be related to the state (i.e., REM sleep) of the animal. To test these competing hypotheses, in a previous study we compared raphe unit activity in normal cats with that in cats that display REM sleep without atonia (produced by lesions of the pontine tegmentum). Although the activity of raphe neurons in lesioned cats during REM sleep without atonia was significantly below that seen in these cats during waking, the level of activity was often impressive, especially in those cats that displayed the greatest degree of motor activity during REM sleep. These data suggest that the decrease in raphe unit activity during REM sleep is largely a concomitant of the atonia which characterizes that state. In order to further test this hypothesis, in the present study we examined the effects of centrally acting muscle relaxants, the benzodiazepines, on raphe unit activity in freely moving cats. Dorsal raphe unit activity was recorded by means of movable 32 or 62 u dia insulated nichrome wires. Raphe neurons were initially identified on-line on the basis of their characteristic slow and rhythmic discharge rate. This identity was later confirmed by means of histological analysis, i.e., all cells were in the densely serotonergic dorsal raphe nucleus. After obtaining 30 to 60 min of baseline unit activity, the cats were given a single i.p. injection of either saline (0.5 ml/kg), diazepam, or chlordiazepoxide HCl (0.25 to 10.0 mg/kg). These drugs produced dose-dependent decreases in raphe unit activity, from no significant change at 0.25 mg/kg to 80-90 % decreases in activity at the 10 mg/kg dose. The benzodiazepines produced ataxia and decreased EMG activity at the higher dose levels. However, raphe unit activity remained suppressed even when the cat would show high levels of tonic and phasic EMG activity during eating, grooming, or attempting to catch a mouse placed in the recording chamber. These data suggest that the benzodiazepines decrease raphe unit activity by an action on the central motor system, and are consistent with the hypothesis that the depression of raphe unit activity normally observed during REM sleep is largely a result of the centrally induced atonia that characterizes this state.

296.3 EFFECTS OF QUIPAZINE ON RAPHE UNIT ACTIVITY AND BEHAVIOR IN FREELY MOVING CATS Terriann Crisp*, Gailyn A. Howell, Donald W. Preussler and Michael E. Trulson (SPON: A. Rupert). Dept. Psychol., Univ. of Texas at Dallas, P.O. Box 688, Richardson, TX 75080.

Several recent studies have investigated the behavioral and neurochemical effects of quipazine. This drug has been reported to have multiple effects on the central serotonergic system, including a direct stimulation of serotonin receptors, inhibition of serotonin reuptake, and inhibition of monoamine oxidase, as well as decreasing the rate of synthesis and turnover of serotonin. These latter effects are considered to be secondary to a primary action of increasing the functional activity in the central serotonergic system by direct receptor activation. Numerous behavioral studies support the hypothesis that quipazine increases the activity at central serotonergic synapses. While a large number of studies have examined the serotonergic effects of quipazine using behavioral and neurochemical measures, apparently none has investigated the effects of quipazine on the serotonergic system using electrophysiological techniques. Thus, in the present study, we examined the effects of quipazine on the activity of serotonin-containing raphe neurons in freely moving cats. Dorsal raphe unit activity was recorded by means of movable 32 or 62 u dia insulated nichrome wires (see complete methodology in Brain Res. 163: 135-150, 1979). Raphe neurons were initially identified on-line on the basis of their characteristic slow and rhythmic discharge rate. This identity was later confirmed by means of histological analysis i.e., all cells were in the densely serotonergic dorsal raphe nucleus. After obtaining 30 to 60 min of baseline unit activity, the cats were given a single i.p. injection of either saline (0.5 ml/kg) or quipazine (0.5, 1.0, 2.0 or 5.0 mg/kg). Quipazine produced a dose-dependent decrease in raphe unit activity ($P<0.01$, ANOVA). The lowest dose of the drug (0.5 mg/kg) produced no significant change in raphe unit activity from saline baseline, while the highest dose (5.0 mg/kg) produced a nearly complete suppression of unit activity. The duration of suppression of raphe unit activity by quipazine was also dose related; unit activity returned to baseline levels within 1, 3, and 6 hours following doses of 1.0, 2.0 and 5.0 mg/kg of quipazine, respectively. Behavioral observations revealed that quipazine elicited a number of unique behaviors such as limb flicking, abortive grooming, investigatory behavior, and hallucinatory-like behavior, which are also elicited by LSD and related hallucinogens. Furthermore, the behavioral and electrophysiological effects of quipazine were temporally correlated. Similar behavioral and raphe unit changes were observed following administration of certain indole nucleus hallucinogens in our previous studies. However, it should be emphasized that is is unclear whether quipazine possesses hallucinogenic properties.

296.4 AMPHETAMINE POTENTIATES KETAMINE-INDUCED LOSS OF RIGHTING REFLEX AND SLEEP TIME. Marie T. Spoerlein and Christina VanderWende, (SPON: A. Sinha). College of Pharmacy, Rutgers-The State Univ., Box 789, Piscataway, N.J. 08854.

Ketamine is a dissociative anesthetic which produces psychotomimetic effects as post-anesthetic sequelae. These mind altering effects have led to the street abuse of ketamine as well as with the parent compound, phencyclidine. It is possible that a drug abuser will unknowingly use ketamine in combination with other drugs, such as amphetamine, since ketamine is sometimes cut with this drug. Voluntary poly-drug use, however, is also a potential. It would, therefore, appear essential to understand any interactions between these drugs.

Male albino, Swiss Webster mice were used for these studies. Drugs were administered intraperitoneally. Amphetamine was administered 15 min. before the ketamine. Amphetamine (5 mg/kg) increased the number of animals losing the righting reflex induced with ketamine; at 100 mg/kg of ketamine, amphetamine increased the loss of the righting reflex to 90% as compared to 60% in the control group. Amphetamine pretreatment also caused animals to sleep 2.5 times longer than the controls. Thus, amphetamine increased both the number of animals and the length of time they would sleep with ketamine alone.

Since both amphetamine and ketamine have effects on the catecholaminergic (CA) systems, the question was raised whether this potentiation of ketamine related to CA systems. Animals were pretreated with d,l-alpha methyl-p-tyrosine (MPT) (400 mg/kg) to deplete the CA's of the brain. Four hours after MPT pretreatment, the effect of amphetamine on ketamine sleep was reexamined. Ketamine (100 mg/kg) in the MPT pretreated animals now caused 90% of the animals to lose the reflex (as compared to 60% in non- MPT animals) with an average sleep time of 13 min. Amphetamine administered 15 min. before the ketamine in the MPT-treated animals caused 50% loss of righting with the same sleep time as the controls. Thus, the potentiative effect of amphetamine on ketamine was lost with MPT pretreatment. A further attempt was made to determine whether this effect of amphetamine related to the dopaminergic (DA)system. This did not seem to be the case since neither spiroperidol, a DA blocker, nor apomorphine, a DA agonist, altered the amphetamine effect on ketamine.

296.5 SHAKING BEHAVIOR IN RATS INDUCED BY THYROTROPIN-RELEASING HORMONE IS DEPENDENT ON BRAIN DOPAMINE. E.G. Drust* and J.D. Connor (SPON: C.I. Thompson). Dept. of Pharmacology, The Pennsylvania State University College of Medicine, Hershey, PA 17033.

Thyrotropin-releasing hormone (TRH) is a potent inducer of shaking behavior in rats after intracerebroventricular (icv) administration. The mechanism by which TRH produces this response was investigated by treating rats with receptor antagonists or monoamine depletors and then comparing the mean number of shakes (±SEM) in control and treated rats after injection of TRH (100 ng, icv). Pretreatment with p-chlorophenylalanine (pCPA, 400 mg/kg, ip, daily for 3 days) decreased brain 5-hydroxytryptamine (5-HT) concentrations to 15% of control ($p<0.001$) and also reduced brain norepinephrine (NE) and dopamine (DA) to 71% and 80% of control, respectively ($p<0.01$). Pretreatment with pCPA had no significant effect on TRH shakes (saline 20.3±3.6 vs pCPA 15.6±3.6). In rats pretreated with 5,7-dihydroxytryptamine (5,7-DHT, 200 µg, icv, 72h prior), brain 5-HT was reduced to 50% of control ($p<0.001$), brain NE and DA were 104% and 100% of control, respectively. Pretreatment with 5,7-DHT also had no significant effect on TRH shakes (vehicle 19.1±1.5 vs 5,7-DHT 21.3±3.9). In addition, the 5-HT receptor antagonist methysergide (10 mg/kg, ip), injected 5 min before TRH, did not affect TRH shakes (saline 25±2 vs methysergide 24±3). Naloxone (10 mg/kg, ip) injected 5 min before TRH also did not affect TRH shakes (saline 20.4±1.5 vs naloxone 23.8±1.6). Pretreatment with α-methyl-p-tyrosine (α-MpT, 200 mg/kg, ip, 21 and 5h prior) decreased brain DA and NE to 15% and 14% of control ($p<0.001$), respectively; 5-HT was 106% of control. Pretreatment with α-MpT reduced TRH shakes to 21% of control (saline 25.2±3 vs α-MpT 5.2±1; $p<0.001$). After pretreatment with 6-hydroxydopamine (6-OHDA, 2x250 µg, icv, 6 and 7 days prior), brain DA and NE were reduced to 59% and 26% of control ($p<0.001$), respectively; 5-HT was 88% of control ($p<0.05$). 6-OHDA pretreatment reduced TRH shakes to 20% of control (vehicle 28±3.2 vs 6-OHDA 5.7±0.9; $p<0.001$). A combination of 6-OHDA and desipramine (DMI, 30 mg/kg) selectively decreased brain DA (DA 54% of control, NE 82% and 5-HT 100% of control) and also inhibited TRH shakes (DMI + vehicle 26±1.6 vs DMI + 6-OHDA 8±2.8; $p<0.001$). However, after a combination of bupropion (BP, 100 mg/kg) and 6-OHDA which selectively depleted NE (NE 31% of control, DA 93% and 5-HT 90% of control), shakes induced by TRH were not significantly different from control (BP + vehicle 22±2.7 vs BP + 6-OHDA 19±2.1). The results of these studies indicate that TRH-induced shaking behavior is dependent on brain DA mechanisms and is not due to release of endogenous NE or activation of 5-HT or opiate receptors. (Supported by USPHS DA 02007.)

296.6 TOBACCO SMOKING: INFLUENCE ON HUMAN AGGRESSIVE BEHAVIOR D.R. Cherek, J.L. Steinberg, and J.T. Brauchi. Psychiatry Research Unit, Department of Psychiatry, Louisiana State University Medical Center, Shreveport, LA 71130.

Aggressive behavior in human subjects was elicited in a laboratory setting and the effects of not smoking and smoking low (0.42mg) and high (2.19mg) doses of nicotine on that behavior was investigated.

Subjects were given the option of pressing three response devices. Pressing button A was maintained by fixed-ratio 100 schedule of monetary reinforcement, consisting of increments of ten cents indicated on a counter and paid to the subject after the session. We defined as aggressive, pressing button B or switch C which ostensibly delivered aversive stimuli by subtracting ten cents from or delivering a one-second blast of white noise to another "person". Each subject was told that at any time he may have money subtracted from him or presented with blasts of white noise which were attributed to another "person". Research subjects were not actually paired with another "person" and were informed of this after the experiment. The subtractions of money from the research subject took place automatically at random time points throughout the session in order to elicit aggressive responding. Compared to the non-smoking condition, aggressive monetary-subtraction responses (button B) were decreased in all subjects following smoking of either nicotine dose. Smoking the high dose of nicotine resulted in a greater reduction of aggressive monetary subtraction responses than the low dose. In general, smoking resulted in suppression of aggressive noise delivery responses (switch C) and increased the number of non-aggressive monetary reinforced responses (push button A). The increase in non-aggressive responding following smoking, indicates that the suppression of aggressive responding was not due to a non-selective generalized depressant action. For both doses of nicotine cigarettes contained the same amount of tar suggesting that the dose-dependent effects were due to nicotine. The relatively selective suppression of aggressive behavior observed in humans in the present study is consistent with the effects of nicotine observed in a number of infrahuman species.

296.7 COMPARATIVE DOSE EFFECTS OF CAFFEINE, THEOPHYLLINE, AND AMPHETAMINE ON RAT ACTIVITY. F. A. Holloway, H. E. Modrow*, and D. C. Bird*. Dept. Psychiat. & Behav. Sci., Univ. Okla. Health Sci. Ctr., Okla. City, Okla. 73190.

Preparatory to tolerance and dependency studies, stimulatory effects of caffeine on rat activity were assessed under several conditions and in comparison with theophylline and d-amphetamine. Spontaneous activity of rats was monitored over successive 24-hr periods with a vibration-sensitive, phonocartridge microprocessor system. Male albino rats were housed individually on ad lib food and water under 12:12 LD conditions (lights on: 0800-2000).

In Experiment 1, i.p. injections of caffeine (7 doses: 0.1-150 mg/kg), theophylline (10, 32, 56, 100 mg/kg), and d-amphetamine (0.5, 1, 2, 4 mg/kg) were administered 1-2 hrs after lights-on (n=8). All drugs produced a U-shaped dose-effect curve for activity during the 4 hours post-injection. The peak stimulatory effect for caffeine occurred between 10 and 32 mg/kg; for theophylline, between 10 and 20 mg/kg; and for d-amphetamine, between 1 and 2 mg/kg. At peak dosage, caffeine was approximately 10-fold more potent than theophylline and about 2-fold more potent than amphetamine. At the highest dose levels, caffeine but not amphetamine or theophylline still produced a small stimulatory effect.

Experiment 2 examined single vs. dual injections of drugs used in Exp. 1. Half of each dose was injected 20 min prior to and the other half immediately prior to a 1-hr activity sampling period (n=8). Single-injection dose-effect curves were comparable to those of Exp. 1. However, the two-injection procedure appeared to result in a shift to the right in caffeine but not amphetamine or theophylline dose-effect curves.

The final experiment examined caffeine injections 2 hrs after lights-on and 2 hrs after lights out (n=8). At doses of 1, 10, and 20 mg/kg, activity increases were larger for the dark than for the light sample. No light-dark differences were evident at 32 mg/kg, while at 100 mg/kg, dark-activity scores were significantly lower than light period scores.

In summary, for doses examined, caffeine's potency exceeded that for theophylline and amphetamine. Further, there is some indication of tachyphylaxis with caffeine. Finally, the pattern of caffeine-induced increases in activity differed as a function of light-dark conditions. (Supported by NIDA Grant #DA02666-01; FAH, P.I).

296.8 AMPHETAMINE AND MORPHINE: ADDITIVE EFFECTS ON ICSS THRESHOLD. Thomas F. Seeger* and Kristin R. Carlson. (SPON: J. Cooke) Department of Pharmacology, University of Massachusetts Medical School, Worcester, MA 01605.

The complex interactions of dopaminergic (DA) and enkephalinergic systems within the CNS are reflected by the behavioral consequences of amphetamine and morphine treatment. Nigrostriatal DA behaviors induced by amphetamine, such as stereotypy and turning behavior, are inhibited by concurrent morphine treatment. However, a mesolimbic DA behavior, non-specific locomotor activity increase due to amphetamine, can be potentiated by intracerebral injection of enkephalin into the ventral tegmentum.

In order to further characterize the behavioral interactions between DA and enkephalin systems in the mesolimbic system, we have tested the effects of amphetamine and morphine on self-stimulation threshold in rats implanted with electrodes in the ventral tegmental nucleus (A10 DA cell body area). A two-lever, current-decrementing threshold titration paradigm was used in order to partially control for changes in motor activity and arousal level.

Thirteen rats were successfully implanted and trained to stable responding in the titration task. The average threshold current after saline baseline runs was 117 ± 16 µA. Following D-amphetamine treatment (0.5 mg/kg 15 min prior to 15 min test period), average threshold current was decreased by 10% ($p<.01$). After morphine treatment (5 mg/kg 1 hr prior to test), threshold was similarly decreased by 15% ($p < .01$). This decrease was naloxone-reversible. When the morphine and amphetamine pretreatments were combined, an additive decrease of 24% was measured ($p < .05$ vs. morphine or amphetamine alone). These results provide further evidence of a functional interrelationship of opiates and opiate peptide systems with the mesolimbic DA system and further suggest that such interaction is opposite in direction from that seen in nigro-striatally mediated behaviors.

(Supported by USPHS grant DA-02226).

296.9 REGULATION OF MUSCARINIC RECEPTOR SENSITIVITY AND MEMORY PERFORMANCE IN RATS. C.C. Loullis, R.L. Dean, D.I. Benson*, L.R. Meyerson, and R.T. Bartus. Dept. of CNS Research, Medical Res. Div. of American Cyanamid, Lederle Labs, Pearl River, NY 10965.

Data from many laboratories demonstrate that changes in CNS cholinergic neurotransmission, induced through pharmacological and/or precursor manipulation, can have profound effects on performance of memory tasks. In general, administration of anticholinergics impairs retention while that of cholinomimetics enhances it. Recent evidence suggests that cholinergic function may also be modified by drug induced changes in receptor sensitivity. For example, when cholinergic blockers are administered chronically, an increase in muscarinic receptor density has been observed, whereas chronic administration of acetylcholinesterase inhibitors has been reported to induce a decrease in muscarinic receptor density.

In the present study we were interested in examining the relationsip between changes in muscarinic receptor binding induced by chronic administration of cholinergic agents and possible changes in performance on a memory task. Rats were implanted subcutaniously with Alza® pumps and physostigmine or scopolamine were continuously infused for 15 days. Twenty-four hours after removal of the pumps the experimental groups and sham operated control group were trained on a single trial passive avoidance task. Twenty-four hours after training they were tested for retention. Following behavioral testing, animals were sacrificed by decapitation and selected brain regions were dissected, quickly frozen and stored (-20°C) for analysis of muscarinic antagonist binding.

Animals which had previously received chronic infusions of scopolamine performed significantly better than controls, while those which previously received chronic infusions of physostigmine performed significantly worse during the retention test. No differences in training latencies were found between groups, demonstrating that alterations in motor activity cannot easily explain the observed behavioral differences. Determinations of muscarinic receptor binding, using ^{3}[H](-)QNB, revealed regional specific increases in the scopolamine-treated animals and decreases in the physostigmine-treated animals.

Results indicate that: (1) continuous, chronic infusion of drugs known to facilitate or inhibit CNS cholinergic activity can induce reciprocal changes in muscarinic receptor binding, (2) parallel changes in behavioral function, as measured by retention on a memory task, also occur, (3) a relationship between the neurochemical and behavioral changes exists, suggesting that changes in receptor function may be responsible for the behavioral effects.

296.10 SIX-HYDROXYDOPAMINE INDUCED HYPERACTIVITY: NEITHER SEX DIFFERENCES NOR CAFFEINE STIMULATION ARE FOUND. L. Erinoff*, P.H. Kelly and S.R. Snodgrass, Childrens Hospital of Los Angeles, CA 90054, and Depts. of Neurology and Physiology and Biophysics, U. of Southern California, Los Angeles, CA 90033

We investigated possible sex differences in the development of locomotor activity in rats treated neonatally with desmethylimipramine (DMI) followed by intraventricular 6-hydroxydopamine (6HDA) (Concannon, J.T. and Schechter, M.D., Pharmac.Biochem.Behav. 14:5,1981). In addition, the locomotor response to the stimulant drug caffeine was studied in the male rats after they had reached adulthood.

At 3 days of age rats were assigned to one of four litters: control males, treated males, control females, and treated females. At 3 and 6 days of age, all rats were injected with DMI(20mg/kg) followed by intraventricular injection of 6HDA(100 ug in 10 ul) or saline-ascorbate. Beginning on day 10, locomotor activity was measured for 1 hour every other day in photocell cages. Both male and female treated rats exhibited increased activity relative to controls. These differences were maximal during the fourth week of life and were maintained through day 52. No sex differences were seen in either the development or magnitude of this hyperactivity.

Male rats were used to determine the dose effect function for caffeine(0.5, 5, 15, 30 mg/kg) on locomotor activity. Control rats exhibited increased locomotor activity at 5, 15, and 30 mg/kg whereas DMI-6HDA treated rats showed no increases with any dose of caffeine. The lack of stimulation seen with caffeine in 6HDA treated rats is not likely to be due to a ceiling effect since a dose of 2.5 mg/kg of L-DOPA following pre-treatment with the decarboxylase inhibitor RO4-4602 did increase locomotor activity in these animals. We suggest that neonatal 6-HDA treatment changes receptor mechanisms involved in caffeine induced increases in activity.

296.11 MUSCIMOL ENHANCES ACTIVITY LEVEL IN THE RAT: BLOCKADE BY LESIONS OF THE ASCENDING 5-HT SYSTEMS. S.M. Sainati and S.A. Lorens. Pharmacology Dept., Loyola University Strich School of Medicine, Maywood, IL 60153

Acute microinjections of the GABA-agonist, muscimol, into the dorsal raphe (DR) nucleus of ether-anesthetized rats induces hyperactivity (Przewłocka et al, Life Sci. 25: 937, 1979). In our initial work, we replicated these findings. Muscimol (100ng/0.5µl) or vehicle solution (0.5µl) was administered via a 5µl microsyringe into either the DR or the median raphe (MR) nucleus of ether-anesthetized rats. The animals were placed in photocell chambers 15 min. post-op and their activity levels were recorded for 90 min. Injection of muscimol into the MR produced a greater increase (740%) over control than did injection into the DR (270%).

Subsequently, we implanted chronically-indwelling 26-gauge cannulae in either the DR or the MR. Beginning 7 days post-op, the animals were habituated to the testing apparatus. We then established a dose-response curve using muscimol doses of 0, 25, 50, 100, 200 and 400ng/0.5µl. This experiment confirmed that the MR is more sensitive to muscimol than the DR (peak effect of 100ng for the MR vs. 400ng for the DR).

If these muscimol effects are due to activation of GABA receptors, they should be potentiated by chlordiazepoxide (CDP) and blocked by bicuculline. We found that CDP in the subataxic dose 3.8mg/kg i.p. did not itself affect activity level, but enhanced the locomotor responses to low doses (25 and 50ng) of muscimol injected into the MR. Conversely, a subconvulsant dose of bicuculline (1.1mg/kg i.p.) completely blocked the response to 50 and 100ng of muscimol.

Finally, forebrain 5-HT was depleted by injecting the serotonin neurotoxin, 5,7-dihydroxytryptamine bilaterally into either the cerebral ventricles (75µg/5µl) or into the midbrain tegmentum (8µg/2µl) in desipramine (20mg/kg) pretreated rats. These lesions markedly attenuated the locomotor response produced by muscimol injections into the MR.

These data suggest that midbrain GABA neurons modulate activity level in the rat through a direct action on serotonergic neurons.

296.12 REINFORCING PROPERTIES OF PSYCHOSTIMULANTS REVEALED BY A CLASSICAL CONDITIONING PARADIGM. Christina Spyraki and H.C. Fibiger. Div. Neurological Sciences, University of British Columbia, Vancouver, B.C. V6T 1W5, Canada

The intravenous self-administration paradigm has provided strong evidence for the reinforcing effects of a variety of centrally-acting drugs in animals. The so-called place preference procedure is a much more simple procedure that may also be useful in studies on drug reinforcement. In the present experiments we examined the effects of systemically administered cocaine, d-amphetamine and apomorphine on place conditioning in the rat.

Groups of male Wistar rats (n = 10/group) weighing 300±20 gm. were used. The tests were conducted in an aluminum shuttle-box divided into 2 compartments that were distinctive in flooring and wall covering. In the first phase of the study (3 days) each animal was allowed to explore the shuttle-box for 15 min. and the time spent in each compartment provided a measure of preference between the two environments. In the second phase, the animals were injected with the drug on days 1,3,5,7 and confined to the less preferred ("conditioned") side of the chamber, while on days 2,4,6 and 8 the rats received a vehicle injection and were confined to the preferred side. In the third phase (day 12) each animal was placed into the shuttle-box and the amount of time spent in each compartment was recorded over 15 min.

All 3 compounds produced statistically significant conditioned place preference. d-amphetamine produced the most pronounced effect, followed by cocaine and then apomorphine. The dose effect was significant for amphetamine and cocaine ($p<.05$) but not for apomorphine. In another series of experiments, we attempted to block cocaine-induced place preference by pretreatment with neuroleptics. Neither pimozide (0.5 mg/kg) nor haloperidol (1 mg/kg) blocked cocaine-induced place preference. Furthermore, neither neuroleptic alone produced place aversion. Bilateral 6-OHDA lesions of the nucleus accumbens did not affect place conditioning produced by cocaine. Neonatal treatment with 6-OHDA that produced extensive depletions of central norepinephrine also did not affect cocaine-induced place preference.

These results demonstrate reinforcing effects of cocaine, d-amphetamine and apomorphine in the rat using the place conditioning paradigm. However, according to the more extensive studies with cocaine, it appears that the conclusions reached with this procedure may differ substantially from those obtained with the intravenous self-administration paradigm. Specifically, although studies with the latter procedure point to a dopaminergic mechanism of cocaine reinforcement, we were unable to demonstrate this in the place conditioning paradigm.

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296.13 PARADOXICAL ROTATION: LONG-TERM CONSEQUENCE OF A SINGLE APOMORPHINE TREATMENT. P.B. Silverman and B.T. Ho. Neurochem.-Neuropharm. Res. Section, Texas Res. Inst. of Mental Sciences, Houston, Texas 77030.

Rats were lesioned unilaterally in the substantia nigra by stereotaxic microinjection of 6-hydroxydopamine. Lesioned, but otherwise drug-naive, rats showed slight ipsilateral (toward the lesioned side) rotation when placed in a novel environment. Treatment with apomorphine, 0.05 to 1.0 mg/kg, resulted in acute, active contralateral rotation of dose-dependent duration. Upon subsequent exposure to the rotation environment, rats that had received a single apomorphine treatment up to 9 months earlier showed a brief, intense burst of contralateral rotation. Saline-treated controls maintained their initial slight ipsilateral bias. Our data thus suggest that "paradoxical" rotation (Ungerstedt, Acta Physiol Scand. Suppl. 367, 49, 1971) is a long-term consequence of prior drug treatment. Further examination of the phenomenon revealed that paradoxical rotation showed some characteristics of associative learning, e.g., it was environmentally specific and could be extinguished by daily exposure to the rotation environment. Other characteristics were not readily reconciled with an associative learning explanation, e.g., inverse relationship to dose and no recency effect. Preliminary experiments with LSD, which acts like a direct dopamine receptor agonist in the rotation paradigm, showed that it, too, induces paradoxical rotation. Treatment with (+)-amphetamine, an indirect-acting dopamine agonist which induced acute, active ipsilateral rotation, resulted in neither paradoxical rotation nor reverse paradoxical rotation. Paradoxical rotation was abolished by ipsilateral intranigral or intracaudate injection of kainic acid, whether or not such treatment also eliminated the acute response to apomorphine. Some of our data may suggest a long-term physiological or biochemical change resulting from a single small dose of apomorphine or LSD.

296.14 LOCALIZATION OF SULFANILIC ACID, A FOOD DYE METABOLITE, IN DEVELOPING RAT BRAIN FOLLOWING INTRAPERITONEAL INJECTION. J. R. Goldenring* and B. A. Shaywitz. Lab. of Developmental Neurobiology, Pediatric Neurology, Yale University School of Medicine, New Haven, CT 06510.

Artificial food dyes have been implicated in the etiology of behavioral disturbances in children. We have previously shown that chronic ingestion of a mix of artificial food dyes causes significant behavioral changes in developing rat pups (Goldenring, et al, Life Sci., 27:1897-1904, 1980). Furthermore, sulfanilic acid, a metabolite of the azo food dyes Tartrazine (F,D &C Yellow No. 5) and Sunset Yellow (F,D&C Yellow No. 6), induces similar deficits in rat pups during their first postnatal month. In an attempt to define the mode of sulfanilic acid action, we have studied the autoradiographic localization of sulfur label in brain following intraperitoneal administration of (^{35}S)-sulfanilic acid to six-day-old rat pups.

In 24 µm frozen sections, increased uptake was seen in the central regions of the olfactory bulb and in the developing cortical layers of the cerebellum. 8 µm paraffin sections of brain fixed with Bouins fix also showed localization in olfactory bulb and cerebellum. In the cerebellum, the densest grain localization was observed over the external germinal layer. In the olfactory bulb, dense grain concentrations were seen around periglomerular cell bodies and in the deepest regions of the granule cell layer. No significant labelling was seen over the olfactory bulb glomeruli.

These results suggest that sulfanilic acid can cross the blood-brain barrier and produce a localized pattern of uptake in neonatal brain. Whether this uptake is due to neuronal or glial cell elements has not been determined yet. However, at least in the granule cell layer of the olfactory bulb, grains were not localized over cell bodies. The integrity of olfactory function is especially important for the maintenance of normal development in rats (see Teicher, et al., Neurosci. Abs., 6:633, 1980). Therefore, the localization of label in the olfactory bulb may suggest a mechanism for the behavioral effects of sulfanilic acid in developing rat pups.

296.15 ANTICHOLINERGIC-INDUCED FORWARD LOCOMOTION IN RATS TREATED WITH 6-HYDROXYDOPAMINE IN NUCLEUS ACCUMBENS, NIGROSTRIATAL PATHWAY, SPINAL SUBARACHNOID SPACE OR CEREBRAL VENTRICLES. T. Schallert, S. Farrar*, N. Lobaugh*, R. E. Wilcox and D. Vaughn*. Depts. of Psychol. and Pharmacol., Univ. of Texas at Austin, Austin, TX 78712. Intraventricular injections of the irreversible neurotoxin 6-hydroxydopamine (6-OHDA) can produce animals that are chronically akinetic, which has been attributed to the destruction of ascending catecholaminergic pathways. After several weeks, muscarinic anticholinergic drugs produce dose-dependent excessive walking in animals with these lesions (Schallert, Whishaw, Ramirez & Teitelbaum, Science, 199:1461, 1978). The dopamine receptor antagonist, haloperidol, does not block the enhanced locomotion in these otherwise severely akinetic animals. The present study extends these findings with localized microinjections of 6-OHDA into nucleus accumbens, median forebrain bundle (MFB) and spinal cord (SC) in an attempt to localize those regions that may be involved in the enhanced forward locomotion to anticholinergics.

These lesions were performed on male rats via an acutely placed cannula; pargyline, a MAO inhibiter, was administered 30 minutes prior to infusion of either 6-OHDA or vehicle. Intraventricular injections were bilateral (20 mcl; 12 mcg/mcl in artificial CSF). SC injections were made into subarachnoid space (5 mcl; 10 mcg/mcl). Accumbens and MFB injections were bilateral slow infusions (1-2 mcl; 2-4 mcg/mcl).

A battery of neurological tests was performed on all subjects for 30 days following the surgery. Severe deficits in movement initiation and ingestive behavior were found in subjects with intraventricular as well as MFB injections.

Anticholinergic-induced forward locomotion was tested by placing the animals in an activity wheel for 6 hours after injection with atropine sulfate (50 mg/kg) or, on a different day, with saline. Animals were tested every 9-10 days for 35 post-operative days. All lesioned groups showed greatly enhanced locomotion to atropine, which was most pronounced in the intraventricular, MFB and spinal groups. The effect developed and increased dramatically over the course of the five weeks after surgery.

Biochemical analyses included regional (3H)-dopamine uptake, (3H)-spiroperidol binding, (3H)-QNB binding, and (3H)-muscimol binding.

The results suggest that the chronic depletion of striatal, accumbens, and spinal catecholamines are each sufficient but not necessary for atropine-induced excessive forward locomotion. (Supported by N.I.H. grant NSAG-17274 to T.S.)

297.1 MICRODISTRIBUTION OF CATECHOLAMINE SYNTHESIZING ENZYMES IN RAT LOWER BRAIN STEM. G. Chamba*, L. Deneroy*, B. Renaud* and D. Armstrong, (SPON: A. Judd). Laboratoire de Neuropharmacologie and ERA CNRS 894, Faculte de Pharmacie, Universite Claude Bernard, Lyon, France and Laboratory of Neurobiology, Cornell University Medical College, New York, N.Y. 10021.

Since the discovery of the adrenaline (A) containing neurons in the rat lower brain stem, no comparative study of the respective distribution of the A and neighboring noradrenergic (NA) neurons has been performed. In this study we sought: (a) to determine the comparative microdistribution of the A and NA neurons; (b) to develop a microdissection procedure to isolate A or NA neurons. We therefore analyzed the distribution of the enzymes tyrosine hydroxylase (TH), dopamine-B-hydroxylase (DBH) and phenylethanolamine-N-methyltransferase (PNMT) regionally in lower brain stem of rat. Activities of all 3 enzymes were determined simultaneously by sensitive radiometric methods in supernatants obtained by sonication of brain cubes microdissected from 500 um coronal sections of the lower brain stem (5 sections rostral and 4 sections caudal to the obex each containing 8 to 18 cubes depending on the level of the section). No significant enzymatic activity was found in most of the cubes in the dorso-lateral and ventro-medial areas of the lower brain stem. In contrast, significant TH, DBH, or PNMT activities were found in the ventro-lateral and dorso-medial areas of the lower brain stem regions containing, respectively, the A_1-C_1 and A_2-C_2 CA group. In the ventro-lateral area, the distribution exhibited a small rostro-caudal difference between the maximum of TH and DBH activities (respectively 676 $\pm$ 70 and 12285 $\pm$ 720 units/mg protein) and the maximum PNMT activity (161 $\pm$ 37 units/mg protein) located 500 um more rostrally in the section lying from 500 to 1000 um rostral to the obex. In the dorso-medial area, the maximal TH and DBH activities (respectively 330 $\pm$ 30 and 6778 $\pm$ 485 units/mg protein) were present in the section immediately caudal to the obex while the maximal PNMT activity (96 $\pm$ 9 units/mg protein) occured much more rostrally in the section located from 1000 to 1500 um rostral to the obex. The localization of the three enzymes, primarily in neuronal perikarya, was confirmed at several rostro-caudal levels by immunocytochemistry. At the caudal levels, the distribution of the catecholamine containing cell bodies was restricted to relatively small areas while at the rostral levels immunoreactive cells were found scattered between the ventro-lateral and the dorso-medial part of the medulla oblongata as well as near the dorsal midline. The rostro-caudal distribution of catecholamine biosynthetic enzymes suggests a procedure for a preferential microdissection of the A neurons of the C_2 group versus the NA neurons of the A_2 group.

297.2 AN ACTIVATING ANTIBODY TO TYROSINE HYDROXYLASE. *John W. Haycock and Jack C. Waymire*. University of Texas Medical School, Dept. Neurobiology & Anatomy, Houston, TX 77025.

Immunoglobulins from sheep, immunized with rat pheochromocytoma tyrosine hydroxylase (TH), increased the catalytic activity of TH up to 20-fold. The effect occurred within minutes and was maintained for at least 48h. The activation was maximal at the minimal antibody:TH ratio required to remove TH from solution with protein A-bearing *Staphyloccocus aureus* (SAC). Kinetic analysis of the activation revealed a decrease in K_m(app) for pterin cofactor and no change in K_m(app) for tyrosine.

Two immunoglobulin fractions were separated with DEAE Sephacel chromatography. Fall-through protein (IgG2) contained neither anti-TH activity nor activating influence. The other immunoglobulin fraction (IgG1) was eluted at approx. 125mM NaCl. This later fraction contained most of the activating influence as well as most of the anti-TH activity.

The activating influence was also bound by protein-A Sepharose. Both anti-TH activity and activating influence were eluted from protein A-Sepharose at pH 6.5. Further, the active peak from DEAE Sephacel chromatography was bound on protein A-Sepharose and the activating influence was eluted at pH 6.5.

Preadsorption of the active fractions from DEAE Sephacel chromatography with SAC that were precoated with rabbit anti-sheep IgG eliminated the activating influence.

It appears then that all anti-TH antibodies are restricted to the IgG1 subclass and that, within the limited purification steps utilized, all anti-TH antibodies increase the catalytic activity of TH. Although the mechanism of the activation has not yet been determined, preliminary studies indicate that activation of TH by phosphorylation may involve sites on the protein not unrelated to those affected by the antibody. We anticipate that the antibody will prove to be useful in analyzing *in situ* and *in vivo* modifications of tyrosine hydroxylase.

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297.3 REGULATION OF TYROSINE HYDROXYLASE IN RAT RETINA: IN SITU AND IN VITRO STUDIES. T. Lloyd* (SPON: P. Walicke) Depts. of OB/GYN and Pharmacology. College of Medicine, The Pennsylvania State University, Hershey, Pennsylvania 17033.

Dopamine is synthesized by a subpopulation of amacrine cells in the mammalian retina. The dopamine content and the activity of retinal tyrosine hydroxylase, the rate limiting enzyme in the biosynthesis of catecholamines, can be rapidly increased by photic stimulation. Our laboratory has confirmed that tyrosine hydroxylase isolated from light adapted rat retinas is 3-4 times as active as that isolated from dark adapted rat retinas. With the goal of studying intracellular tyrosine hydroxylation, we have developed a rapid and gentle procedure for the dissociation of rat retinas into single cell preparations. After using several more vigorous digestion procedures, we found that brief exposure (7½ min @ 37°C) to a mixture of hyaluronidase and DNase was sufficient to cause complete dissociation of the retina. Microscopic examination indicated that cell features had been preserved and the major cell types could still be identified.

Using dissociated cells and an in situ assay, we found that light stimulation caused only a 20% increase in intracellular tyrosine hydroxylation over the intracellular activity seen in retinal cells from dark adapted animals. Thus, although in vitro assays indicated that light causes a 3-4 fold activation of extractable tyrosine hydroxylase activity, this activation can be suppressed by regulatory mechanisms functioning within the cell. Intracellular tyrosine hydroxylation can be increased by exposure of the dissociated cells to EGTA in the presence of normal levels of medium Ca^{++}. This rapid activation can be blocked by levels of tetracaine or Co^{+++} which did not influence basal in situ tyrosine hydroxylase activity. These results are consistent with our observation that tyrosine hydroxylase isolated from rat retinas can be activated in vitro by exposure to cAMP-independent phosphorylating conditions and suggest that activation of tyrosine hydroxylase occurs by a Ca^{++}-dependent, cAMP-independent protein kinase.

In contrast, exposure of the dissociated retinal cells to agents known to increase intracellular cAMP levels (cholera-toxin, 8-Bromo-cAMP, or 2 chloroadenosine) results in a profound decrease of in situ tyrosine hydroxylation. Correspondingly, we have observed that exposure of the isolated enzyme to cAMP-dependent phosphorylating conditions causes a reduction of in vitro tyrosine hydroxylase activity. These studies indicate that the retina is a useful CNS model for studying regulation of tyrosine hydroxylase activity, in situ and in vitro.

297.4 SUPRANORMAL LEVELS OF RETINAL DA IN YOUNG BW RATS AND SUBNORMAL LEVELS IN OLDER ANIMALS: AN INHERITED DOPAMINERGIC ABNORMALITY. J.P.H. Wyse, Dept. Anat., Faculty of Medicine, Univ. of Calgary, Calgary, Alberta, Canada T2N 1N4.

The Bmn-wys (BW) strain of rat is affected by a number of inherited neurological defects including developmental and degenerative abnormalities of the visual system, a centrally mediated hypogalactia and altered motor activity. Although there are several possible causes for these defects it has been hypothesized that a single inherited dopaminergic abnormality underlies most if not all of the observed neurological changes in these animals (Wyse and Lorscheider, Exp. Eye Res. 32, 1981). Preliminary studies have provided evidence consistent with altered dopamine (DA) neurotransmission in the retina, hypothalamus and nigrostriatal system. For example, catecholamine (CA) histofluorescent techniques together with a sensitive CA radioenzymatic assay (REA) have revealed significantly ($p<0.05$) less retinal DA in one year old BW rats than in age and sex matched controls. The purpose of the present study was to use these same techniques to determine either if retinal DA levels in BW rats are lower throughout life than those in age matched controls or if they change with age relative to those in controls. Whole retinae, one per animal, were obtained by dissecting eyes on iced cooled glass with care being taken to ensure complete removal of the vitreal body. Catecholamine values were determined by REA and expressed as pg CA per ug total retinal protein. Mean retinal DA levels ($\bar{X}\pm$SEM,n) were determined for both BW (b) and control (c) animals at 5 weeks and at 2, 3, 6 and 12 months of age. All DA values and standard errors are normalized with respect to the appropriate control mean. DA levels were significantly higher in BW rats than in controls at 5 weeks ($p<0.001$: b=1.88±0.15, 4; c=1±0.06, 5), 2 months ($p<0.05$: b=1.72±0.28, 4; c=1±0.07, 5) and at 3 months ($p<0.005$: b=1.49±0.10, 5; c=1±0.7, 5) of age. No significant difference was observed at 6 months (b=1.16±0.08, 5; c=1±0.03, 3). In contrast the retinal DA levels were significantly ($p<0.05$) lower in BW rats (0.44±0.08, 6) than in controls (1±0.25, 4) at one year of age. From these data it can be concluded (1) that retinal DA levels in BW rats 5 weeks to 3 months of age are significantly higher than those in age matched controls (2) that there is a continuous age-related drop in retinal DA levels in BW animals relative to those in controls and (3) at some time between six months and one year of age BW retinal DA levels cross over and become lower than those in age matched controls. Results of this study provide further support of the hypothesis that BW rats are affected by an inherited dopaminergic abnormality and demonstrate that the abnormality is expressed as early as 5 weeks of age. (Supported by MRC and Natl. Ret. Pig. Found. Can.)

297.5 HPLC METHOD FOR ANALYSIS OF DOPAMINE SULFATE ISOMERS. Mary Ann Elchisak and Joanne H. Carlson*. Purdue University, School of Veterinary Medicine, West Lafayette, IN 47907.

Conjugated dopamine (DA) occurs in the tissues and fluids of many species, and much of this is thought to occur as dopamine sulfate ($DASO_4$). Since $DASO_4$ may not simply be a biologically inert metabolic waste product, and since it can occur as either of two isomers, DA-3-SO_4 or DA-4-SO_4, we have developed a method utilizing reverse-phase paired-ion high performance liquid chromatography (HPLC) to separate and quantitate each $DASO_4$ isomer.

Separations were performed on a 25 cm X 4 mm stainless steel column prepacked with 5 µm octadecyl silica. The mobile phase was 0.025 M monochloroacetic acid, pH 2.8, containing 1 mM EDTA and 4 mM n-octylamine. The flow rate was 0.5 to 0.9 ml/min. $DASO_4$ isomers were quantitated by ultraviolet detection at 280 nm or electrochemical detection utilizing a thin-layer carbon paste working electrode with the potential maintained at +0.90 or 0.95 volts vs an Ag/AgCl reference electrode. The k' values for DA-3-SO_4 and DA-4-SO_4 were 4.8 and 5.3, respectively. The minimum detectable amounts (producing a peak height twice baseline noise) of DA-3-SO_4 and DA-4-SO_4 by ultraviolet detection were 15 and 20 picomoles, respectively, and 7 picomoles for each isomer by electrochemical detection.

This method was used to determine the occurrence of DA-3-SO_4 and DA-4-SO_4 in normal human urine and rat adrenal glands. Cation exchange chromatography was used as a preliminary purification step for the urine. Excretion of DA-3-SO_4 ranged from 0.44 to 3.4 µ mol/day in six normal human volunteers. DA-4-SO_4 was not detected. DA-3-SO_4 accounted for 34 to 96 percent of the conjugated DA excreted in the urine of these six subjects as determined by an acid hydrolysis method (Carlson and Elchisak, abstract this meeting). Our previous work utilizing thin-layer chromatographic methods also detected only DA-3-SO_4 in urine from normal volunteers (Carlson and Elchisak, unpublished). In rat adrenal glands, neither DA-3-SO_4 nor DA-4-SO_4 was detected by the HPLC method described above. This negative finding was verified by the absence of any conjugated DA released upon acid hydrolysis of the same samples, even though free DA was released during the hydrolysis procedure after addition of authentic $DASO_4$ standards to the same samples.

The method described here is rapid, simple, and direct, and it should prove useful in investigations concerning the physiological role of dopamine sulfate. (Partially supported by Pharmaceutical Manufacturer's Association grant to MAE.)

297.6 HPLC METHOD FOR ANALYSIS OF FREE AND CONJUGATED CATECHOLAMINES J.H. Carlson* and M.A.Elchisak (SPON: C. Henrikson). Purdue Univ, School of Veterinary Medicine, W.Lafayette, IN 47907.

In the course of developing methods to analyze the physiological role of dopamine sulfate ($DASO_4$), we found it necessary to have a simple assay available for the analysis of free and conjugated dopamine (DA) in various body tissues and fluids. We report this procedure here, as modified to measure free and total norepinephrine (NE), epinephrine (EPI), and DA. An aliquot of body fluid or tissue supernatant containing EDTA and sodium metabisulfite was hydrolyzed in 0.4 N perchloric acid at 100°C for 120 min to convert conjugated catecholamines (CA) to free CA. The hydrolysis step was omitted for assay of free CA. The free NE, EPI, and DA were then isolated by standard alumina procedures. Conjugated NE, EPI, or DA was the difference between total and free amounts in duplicate aliquots. Dihydroxybenzylamine (DHBA) and/or deoxyepinephrine (DOE) were used as internal standards.

HPLC with electrochemical detection was utilized; conditions were similar to those employed by Davis and Kissinger (Analyt Chem, in press). The stationary phase was octadecyl silica; the mobile phase was .075 M phosphate buffer, pH 2.8, containing EDTA (1 mM), sodium octyl sulfate (30 mg/l), and methanol (5%). Flow rate was 1.2 to 2.4 ml/min. A gradient of increasing flow was used for most analyses. A thin-layer carbon paste working electrode, with the potential maintained at +0.65 or 0.70 volts vs an Ag/AgCl reference electrode, was used for detection. The k' values varied depending on the exact flow gradient conditions for each experiment, but were as follows for an 18 min flow gradient from 1.2 to 2.2 ml/min: NE, 2.2; EPI, 3.2; DHBA, 4.1; DA, 5.9; DOE, 7.1.

This method was used to determine free and conjugated NE, E, and DA in normal human urine and in rat adrenal glands. CA in urine were isolated by alumina as described above, and adrenal gland homogenate supernatants were injected directly onto the HPLC column before and after hydrolysis. The percent of each CA found to be conjugated was as follows:

	NE (SD)	EPI (SD)	DA (SD)
RAT ADRENAL (n=8)	3.2%	2.3%	.98%
HUMAN URINE (n=6)	51% (7)	57% (13)	67% (7)

DA-3-SO_4 excretion accounted for 34 to 96 % of the conjugated DA excreted in the human urine, but no $DASO_4$ was detected in rat adrenals (Elchisak and Carlson, abstract this meeting).

This method should prove useful in future investigations concerning the physiological functions of conjugated CA. (Partially supported by a PMA Research Starter Grant to MAE.)

297.7 THE SELECTIVE PHARMACOLOGICAL EFFECTS OF SOME NEW ACETYLENIC INHIBITORS ON BRAIN MONOAMINE OXIDASE. Rosa H. Huang* (SPON: G.L. Longenecker). Department of Biochemistry, University of South Alabama College of Medicine, Mobile, AL 36688.

We have synthesized three new acetylenic inhibitors, p-hydroxypargyline (HO-P), O-[3(2-acetamido)-2,2,5,5-tetramethyl-1-pyrrolidinyloxyl]-p-hydroxypargyline (SLPS) and O-[3-[[2-[2-(2-acetamido)ethoxyl]ethyl]carbamoyl]-2,2,5,5-tetramethyl-1-pyrrolidinyloxyl]-p-hydroxypargyline (SLPL). Their irreversible inhibition of the rat brain MAO using 5HT or PEA as the substrate was studied. The dose response relationship indicated the ratio of PI_{50} for the MAO-A to that for the MAO-B was 0.62, 2.02, 0.397 and 22.99 for HO-P, SLPS, SLPL and pargyline, respectively. The preincubation time required to block 50% of the MAO-A was 101 min for pargyline (1.8×10^{-7} M, 37°), 48 min for OH-P (5.6×10^{-7} M, 37°), 42 min for SLPS (3.2×10^{-6} M, 37°) and 28 min for SLPL (3.2×10^{-5} M, 20°). Under the same experimental conditions the preincubation time required to achieve 50% inhibition of the MAO-B was found to be 8 min, 67 min, 20 min and 75 min for pargyline, OH-P, SLPS and SLPL, respectively. The results suggested highly selective pharmacological effect on the multiple MAO forms of these acetylenic inhibitors which varied in size, polarity and hydrophobicity. (Supported by NIH grant #NS14434.)

297.8 TURNOVER OF CATECHOLAMINES (CA) IN DISCRETE HYPOTHALAMIC AREAS AND THE EFFECTS OF CHLORPROMAZINE (CPZ). Sarah F. Leibowitz, Meena Jhanwar-Uniyal* and Barry E. Levin. Rockefeller Univ., New York 10021; Dept. Neurosci., N.J. Medical School, Newark 07103.

A sensitive radioenzymatic assay was used to measure the three CA, norepinephrine (NE), epinephrine (EPI), and dopamine (DA), in five microdissected areas of the rat hypothalamus. These areas were the paraventricular nucleus (PVN), dorsomedial nucleus (DMN), lateral hypothalamic medial forebrain bundle (MFB), and two perifornical hypothalamic areas just dorsolateral (dPFH) and ventrolateral (vPFH) to the fornix. To determine the rate of CA turnover in these areas, synthesis of the CA was inhibited by the tyrosine hydroxylase inhibitor α-methyl-p-tyrosine methyl ester (α-MpT, 300 mg/kg, i.p.), and the animals (adult, male, albino Sprague-Dawley rats) were sacrificed at variable times (15-180 min) after α-MpT injection. The results of this experiment revealed a linear decline of both NE and DA levels after synthesis inhibition. The turnover rate of these two CA varied as a function of hypothalamic area, with the highest rate of NE turnover, for example, occurring in the DMN and lowest rate in the dPFH. For EPI, however, no appreciable decline in levels was detected in any site up to 3 hr after α-MpT, indicating that there may be little or no turnover of EPI following inhibition of tyrosine hydroxylase activity.

To evaluate the impact of CPZ on CA levels in the different hypothalamic areas, a separate group of rats was injected with saline or CPZ (2 mg/kg, i.p.) and sacrificed 30 min later. Only two significant changes in CA levels were detected in the CPZ-treated animals: DA levels were decreased (by 50%) in the PVN, and EPI levels were increased (by 100%) in the MFB. No significant changes in NE levels were observed. To assess the effect of CPZ on the turnover of CA, a similar experiment was conducted in α-MpT-pretreated rats that were sacrificed 3 hr after α-MpT and 30 min after CPZ or saline. In these rats, CPZ affected the disappearance or turnover of NE and DA in only one of the five hypothalamic areas analyzed. This was the dPFH area (dorsolateral to the fornix) where the turnover of these two CA was significantly potentiated.

This finding is consistent with other evidence from this laboratory suggesting that the perifornical hypothalamus may be involved in mediating the overeating caused by antipsychotics in humans as well as in rats. Perifornical hypothalamic injection of CPZ potentiates eating behavior in the rat, and overeating induced by peripheral CPZ injection is attenuated by perifornical hypothalamic lesions.

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

297.9 EFFECT OF PROTEIN DIET ON HYPOTHALAMIC BIOGENIC AMINE ACTIVITY AND HORMONE REGULATION. E.J. Hawrylewicz, James Q. Kissane*, Elizabeth A. Drab*, and Henry H. Huang*. Dept. of Research, Mercy Hospital & Medical Center, Chicago, IL. 60616.

Protein diet quality influences brain development and behavior. The biologic mechanisms involved have not been defined. The hypothalamic biogenic amines; norepinephrine (NE), epinephrine (Epi), dopamine (DA) and serotonin (5-HT) have a major regulatory role controlling the function of the anterior pituitary gland. Changes in this neuroendocrine relationship due to altered protein diets may in part explain the observed biologic effects.

Virgin Sprague-Dawley rats were fed, ad libitum, isoenergetic diets consisting of 8%, 19.5% and 31% casein during conditioning, pregnancy and lactation periods. All litters were reduced to 8 pups and after weaning, the female pups were continued on their respective diet. Animals were sacrificed 3 hrs. before proestrous surge at 3 wks. after sexual maturity and 8 mos. of age. Six hypothalamic nuclei were removed by punch technique and analyzed by radioenzyme procedure for DA, NE, Epi and 5-HT.

At the age of 3 wks. after sexual maturity, significant decrease in DA conc. (ng/mg protein ± s.d.) was evident in the median eminence (16.7±19.8 vs. 43.5±23.1 and 49.2±26.5) and in the paraventricular nucleus (3.3±0.6 vs. 5.3±2.2 and 4.4±2.5) in the low protein group (8% casein) compared to the normal protein group (19.5% casein) and high protein group (31% casein). No significant decrease in DA was evident in the periventricular, arcuate, suprachiasmatic and supraoptic nuclei. NE was also significantly reduced in the periventricular nucleus, 9.4±4.1 (8%) vs. 29.3±13.4 (19.5%). In contrast, NE was significantly increased in the suprachiasmatic nucleus in the low protein group. Epi was not altered in the low protein group.

At 8 mos. of age the decreased DA concentration persisted in the paraventricular nucleus (5.2±4.3 (8%) vs. 12.0±5.0 (31%)) and median eminence (17.1±13.3 (8%) vs. 43.0±17.1 (31%)). NE also remained depressed in the paraventricular nucleus (13.5±10.6 (8%) vs. 30.6±23.7 (31%)). Epi remained unaltered. Serotonin was reduced in the median eminence; 3.2±4.7 (8%) vs. 7.2±12.7 (31%) and the supraoptic nucleus; 1.1±1.9 (8%) vs. 4.7±3.8 (31%). Serum prolactin concentration was determined in the same animals. At the age of 3 wks. after sexual maturity, low protein animals had a decreased serum prolactin concentration. DA and 5-HT data were examined in regard to the serum prolactin change. Dietary protein does affect neurohormonal regulation. (Grant No. CA-26547, National Cancer Institute).

297.10 SEQUENTIAL CHANGES OF BIOGENIC AMINE LEVELS IN NEOCORTEX, SEPTUM AND MEDIOBASAL HYPOTHALAMUS AFTER 6-HYDROXYDOPAMINE LESIONING OF THE ASCENDING NORADRENERGIC BUNDLES. T.A. Reader, O. Bosler*, C. Mercure* and L. Descarries. Centre de recherche en sciences neurologiques, Département de physiologie, Université de Montréal, Montréal, Qué., Canada H3C 3T8.

The noradrenaline (NA), dopamine (DA) and serotonin (5-HT) contents of adult rat neocortex (frontal including gyrus cinguli, between A: 7020-8920 μ in König and Klippel's atlas), septum (from A: 6670-8920 μ) and mediobasal hypothalamus (from A: 3750-4890 μ , including median eminence) were measured radioenzymatically 2, 15 and 90-120 days after bilateral microinjection of 6-hydroxydopamine (2 x 2 μg/μl) on either side of the decussation of the superior cerebellar peduncles (A: 0.16, L: 1.75 and H: -1.60 mm). By comparison with controls, NA levels were significantly ($p < 0.01$) lowered in all 3 regions and at every survival time examined (-60 to -80% in cortex, -66 to -77% in septum and -22 to -48% in hypothalamus). DA levels were also changed but followed a differential sequence in each of the regions. In cortex, DA content was normal at 2 and 15 days but considerably increased (+95%) after 90-120 days. In septum, there was an initial reduction of DA at 2 days (-43%), an augmentation at 15 days (+134%) and a return to the normal range after 90-120 days. In hypothalamus, DA was diminished by 35-52% from 2 to 90-120 days. The 5-HT levels also showed marked changes in cortex and septum but were not significantly altered in hypothalamus. Cortical 5-HT was lowered after 2 and 15 days (-35 and -61%), and increased (+57%) after 90-120 days. In septum, unchanged levels of 5-HT were found at 2 and 15 days but a large augmentation (+139%) was again measured after 90-120 days. These results indicate that, in frontal cortex and septum, the NA depletion secondary to 6-hydroxydopamine lesioning of the noradrenergic ascending bundles may be associated with marked increases in DA (also see Tassin et al., Neurosci., 4: 1569, 1979) and 5-HT levels. Moreover, the augmentation of the DA content in septum appears to be reversible within a period of 90-120 days. The interpretation of such changes will require some assessment of local turnover of the biogenic amines as well as of the sprouting capacity of DA and 5-HT terminals arising from nerve cell bodies located at various distances from their territories of innervation (Supported by MRC Grants MA-6967 and MT-3544).

297.11 EVIDENCE FOR A RELATIONSHIP BETWEEN MEDIAN EMINENCE NORADRENALINE AND PROLACTIN IN RATS. William W. Morgan and Edward G. Rennels*. Dept. Anat., Univ. Texas Health Sci. Ctr., San Antonio, TX 78284.

The involvement of median eminence (ME) noradrenaline (NA) in the regulation of prolactin (PRL) secretion is controversial. In order to investigate this relationship in vivo, 32 adult female Sprague-Dawley rats (125-140 grams) were ovariectomized and 2 weeks later were either hypophysectomized (HYPEX) or were treated by sham surgery (CONTROL). One week later one half of the HYPEX and one half of the CONTROL rats were ether anesthetized and given 2 anterior pituitary homografts under the right kidney capsule (GRAFT). The homografts were from female donors. The remaining rats were treated with the same surgical perturbations but no tissue was implanted (SHAM). Eight days later the rats were sacrificed and the ME, the remaining hypothalamus and the telencephalon were collected from each animal and frozen. The levels of NA in each tissue were subsequently determined by a radioisotope-enzyme technique and protein levels were quantified by an automated Lowry procedure. Statistical analysis of the data was performed by the analysis of variance and subsequently by the Student-Newman-Keuls test. The concentration of NA (ng/mg protein) was significantly elevated ($p<0.01$) in the ME of the HYPEX-SHAM rats (41 ± 3) (mean ± standard error of mean) as compared to the CONTROL-SHAM rats (25 ± 1). The levels of NA in the ME of the CONTROL-GRAFT (12 ± 2) and the HYPEX-GRAFT rats (12 ± 1) were not significantly different from one another but were significantly lower ($p<0.01$) than those observed in either the HYPEX-SHAM or the CONTROL-SHAM rats. The levels of NA in the other brain regions were not significantly different among the experimental groups. The results show that hypophysectomy elevates NA in the ME and that this effect is reversed by anterior pituitary homografts. Anterior pituitary homografts alone significantly decrease NA in the ME. Collectively, these data suggest an inverse relationship between NA levels in the ME and circulating PRL levels. It is hypothesized that median eminence NA may stimulate PRL secretion indirectly perhaps by effecting the release of a PRL releasing factor. Supported by DA 00755 and NS 14855 to WWM.

297.12 EFFECTS OF CENTRAL INJECTION OF 6-HYDROXYDOPAMINE ON REGIONAL CEREBRAL BLOOD FLOW, SMALL VESSEL BLOOD CONTENT, AND CEREBRAL OXYGEN CONSUMPTION IN RABBITS. E. Buchweitz, and H. R. Weiss* Dept. Physiology and Biophysics, College of Medicine and Dentistry of New Jersey, Rutgers Medical School, Piscataway, NJ 08854

Evidence has suggested that the catecholaminergic neurotransmitter systems play a role in the regulation of cerebral O_2 metabolism. Our investigation focused on the effects which long-term interruption of these systems had on the distribution of cerebral blood flow (rCBF), cerebral O_2 consumption ($C\dot{V}O_2$) and cerebral small vessel blood content (rSVBC, an index of open capillary density). Eight rabbits received bilateral intracerebroventricular injections of 100 μg 6-hydroxydopamine (6OHDA), while a control group received an equal volume of .1% ascorbic acid in 0.9% saline. 6OHDA has been shown to produce a relatively specific destruction of brain catecholaminergic neurons. The efficacy of the lesion was monitored by DA and NE determination in various brain regions. Three weeks later the animals were anesthetized with pentobarbital, artifically respired, and femoral and superior saggital sinus catheters inserted. rCBF was monitored by the injection of 141-Ce labeled microspheres (15 ± 3 μ in diameter) into the left atrium. rSVBC was monitored by determination of 59 Fe-labeled plasma siderophilin. After sacrifice 17 brain regions were isolated and radioactivity counted. Blood pressure, heart rate, and arterial and venous blood gas values were within normal ranges in both groups and were similar in the two groups. Total CBF averaged 43.6 ± 16.4 (mean ± S.D.) ml/min/100 g in control and 42.1 ± 39.4 ml/min/100 g in the experimental group. No significant changes in rCBF were noted between the groups or among the various regions of either group. O_2 extraction averaged 4.05 ± 1.86 ml O_2/100 ml blood in the control and 3.83 ± 2.31 ml O_2/100 ml blood in the experimental group. These values were not significantly different. $C\dot{V}O_2$ was not altered by treatment. rSVBC was significantly increased by 95% in the lesioned group. There were no regional differences in rSVBC in either group. This significant increase in SVBC, reflecting an increase in the number of open capillaries in the brain, may be due to the secretion of a vasodilator substance, or a decrease in the secretion of a vasoconstrictor substance. Long term interruption of catecholaminergic neurotransmission did not affect rCBF, $C\dot{V}O_2$ or O_2 extraction probably due to an adaptive alteration in the activity of other neuronal elements which maintained cerebral metabolic activity.

297.13 A SINGLE DOSE OF d-AMPHETAMINE PRODUCES AN ENDURING RECOVERY OF LOCOMOTION AFTER MOTOR CORTEX INJURY IN THE CAT. D. A. Hovda* and D. M. Feeney (SPON: D. Trevino). Dept. of Psychology, University of New Mexico, Albuquerque, NM 87131.

We have previously reported (Feeney et al., Neurosci. Abst., 6:802, 1980) that a single dose of d-amphetamine (d-AMP) given at 24 hrs. following unilateral ablation of the motor cortex produces an enduring recovery of locomotor ability in the rat. We now report an extension of these findings to the cat. Like the rat, cats with unilateral ablation of motor cortex are able to walk and run normally on a flat surface but show a serious contralateral deficit when placed on a narrow beam. The rat spontaneously recovers within 14 to 21 days, however, the cat displays a deficit for more than 60 days on this task. In this study cats were trained to walk the length of a 3.8 m x 5.1 cm beam and then a unilateral suction ablation of motor cortex (Primarily areas 4 and 6) was performed using standard procedures. These cats were given 4 mg/kg of d-AMP at 24 hrs. postsurgery. Two of the cats died and the neurological status of the other cat deteriorated. Clearly in the cat, 24 hrs. post injury is too early for d-AMP treatment, therefore, in 11 cats, drug administration was delayed until 10 days post injury, with 5 cats receiving saline as a control and 6 cats receiving 5 mg/kg d-AMP. The cats' locomotor performance on the beam was rated by two observers, one uninformed regarding drug or saline assignment. By 6 hrs. post drug treatment, there was a clear improvement in the locomotor performance of the d-AMP animals compared to the saline control group's performance. An even further improvement of the d-AMP group's locomotion compared to the saline control's agility on the beam was observed at 24 hrs. post drug. This significantly improved performance of the d-AMP group compared to the saline control level has endured for 35 days following d-AMP administration. These results in the cat support our hypothesis, that much of the locomotor deficit following motor cortex injury is attributable to a transient depression of catecholamine function in intact areas of the brain which is reversible by d-AMP.

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297.14 HALOPERIDOL PRODUCES AN ENDURING BLOCKADE OF RECOVERY OF LOCOMOTION FOLLOWING MOTOR CORTEX INJURY IN THE RAT. D. M. Feeney, A. Gonzales* and Wendy A. Law*Department of Psychology, University of New Mexico, Albuquerque, NM 87131.

We previously reported (Feeney, D. M., et al. Neurosci. Abst., 6:802, 1980) that the contralateral hemiparesis produced by a unilateral motor cortex ablation in the rat is dramatically reversed by a single dose of d-amphetamine (d-AMP). After this lesion rats can locomote on a flat surface but are unable to walk or run on a narrow beam. This deficit gradually recovers over a 2 to 3 week period. However if a single dose of d-AMP (2-4 mg/kg) is given 24 hours postinjury, these motor cortex injured animals show a dramatic improvement of locomotor performance within 3-6 hrs and this improvement is maintained over the subsequent weeks of testing. This d-AMP facilitated recovery of function is blocked by administration of haloperidol (HAL) suggesting a dopaminergic (DA) involvement. We proposed that after motor cortex injury in the rat, much of the locomotor deficit is due to a transient DA depression which is reversible by d-AMP. To further test this hypothesis that recovery of DA function is important for the recovery of locomotion after motor cortex injury, we examined the effect of HAL, a DA antagonist, on recovery of this behavior. Rats were trained to run a narrow beam as previously described and the entire motor cortex was then unilaterally removed by suction ablation. All animals were retested at 24 hr postinjury and most could not maintain their balance or run on the beam. Locomotor performance was independently rated by two observers, one uniformed of subsequent drug treatment. Five of the animals were then given a single dose of saline and 6 were given 0.4 mg/kg of HAL (i.p.) immediately following the 24 hr postsurgery testing. The animals were retested every hour for 6 hours, once at 12 hours and once every other day for 30 days postdrug. The animals receiving a single dose of HAL showed a severely retarded recovery of locomotion compared to saline control animals. In addition, their performance did not reach the control group's level until 3 weeks postdrug. At 38 days postinjury when the saline control group's performance had completely recovered these animals were also given 0.4 mg/kg HAL and were tested hourly for the first 6 hrs postdrug and again at 24 hrs. This dose of HAL had no effect on locomotion in saline recovered animals. These data compliment our previous d-AMP results and indicate that DA plays an important role in the process of recovery of function after motor cortex injury.

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298.1 PROCONVULSANT EFFECTS IN BABOONS OF β-CARBOLINE, A PUTATIVE ENDOGENEOUS LIGAND FOR BENZODIAZEPINE RECEPTORS. J. Rossier, C. Cepeda, T. Tanaka, R. Besselièvre, P. Potier and R. Naquet. Laboratoire de Physiologie Nerveuse and Institut de Chimie des Substances Naturelles, C.N.R.S., 91190 Gif-sur-Yvette, France.

β-Carboline-3-carboxylic acid ethyl ester (B-CCE) has been recently isolated from human urine and brain tissues. B-CCE has the property of displacing ^{3}H-diazepam from specific cerebral binding sites. It was proposed that B-CCE was a putative endogenous ligand for benzodiazepine receptors. We have studied the effects of B-CCE on two models of epilepsy in papio papio baboons: 1° kainic acid induced limbic status epilepticus and 2° natural photosensitive epilepsy.

Limbic status epilepticus was produced by a microinjection of kainic acid (10 µg) in the amygdala. Twenty days after cessation of the limbic status epilepticus, B-CCE was tested. Intravenous injection of 1 mg/Kg of B-CCE induced repetitive secondary generalized seizures. Diazepam (10 mg i.v.) prevented further possible seizures. At lower doses B-CCE induced a reappearance of interictal spikes restricted to limbic structures (0.04 mg/kg) or a localized limbic seizure (0.5 mg/kg).

The action of B-CCE on light-indiced epilepsy was also studied. Two types of baboons have been utilized : 1° naturally non-photosensitive baboons and 2° naturally photosensitive baboons. Non-photosensitive animals did not display any clinical symptom to intermittent light stimulation (ILS). However, when a single dose, ranging from 40 to 100 µg/kg was injected i.v. the animals became photosensitive and exhibited myoclonic jerks in response to ILS. Generalized seizures were induced with ILS when doses of B-CCE were slightly increased (50 to 100 µg/kg). In naturally photosensitive animals 8 µg/kg of B-CCE i.v. produced a latency shortening of the photomyoclonic response. At a dose of 20 µg/kg generalized seizures were obtained in the seconds following the exposure to ILS. The effects of B-CCE were of very short duration lasting no longer than 5 min, indicating a short half-life.

The most common proconvulsant drugs are usually active at doses over 1 mg/kg. In this study B-CCE was 50 to 100 times more active than pentylenetetrazole. The high potency of B-CCE may reflect its great affinity for benzodiazepine receptors. However B-CCE and benzodiazepine have opposite effects. Indeed the proconvulsant effect of B-CCE was antagonized by an injection of benzodiazepine. B-CCE should now be considered as an antagonist of the benzodiazepines. We are now examining if the other recently described benzodiazepine antagonists (Hunkeler et al, Nature, 290, 514,1981) are also proconvulsant in the baboons.

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298.2 CHOLINERGIC NATURE OF SEPTO-HIPPOCAMPAL MODULATION OF PYRAMIDAL CELL FIRING. K. Krnjević and N. Ropert, Anaesthesia Research & Physiology Depts, McGill University, Montréal, PQ, Canada

Acetylcholine (ACh) strongly facilitates discharges of population spikes in CA1 in response to fimbria-commissural stimulation, probably mainly by reducing the effectiveness of inhibitory pathways that normally tend to suppress pyramidal firing (Krnjević, K. et al., J. Physiol., 308:73P, 1980). A comparable effect can be elicited by stimulation of the medial septum (Ropert, N. et al., Canada Physiology, 11:118, 1980). We now report that the facilitatory action of septal stimulation is susceptible to blockade by the muscarine antagonists atropine and especially scopolamine.

In these experiments on rats under urethane, stereotaxic stimulation of the hippocampal commissure at a moderate intensity (2-3 times threshold) and low frequency (<1 Hz) evoked prominent positive field responses in the CA1 pyramidal layer. When a brief tetanus (100 Hz for 100 ms) was applied in the medial septum, it produced (for a period of 20-50 ms) a striking facilitation of negative population spikes in response to the same commissural stimulus. A significant feature is that single or multiple stimulation at the critical site in the medial septum by itself evoked minimal field responses in the hippocampus. The similar modulatory effects of ACh and septal stimulation both were rapidly reversible and they were both markedly diminished after intravenous injections of atropine or scopolamine, though rather high doses of these antagonists were required to produce a major change (typically 10 mg of scopolamine per kg). This is in accordance with the fact that the most potent ACh-agonist, muscarine, was antagonized only by such high doses of scopolamine. In addition, there was evidence that nicotine receptors may also be involved in the action of ACh.

These observations confirm previous suggestions that the septo-hippocampal pathway is cholinergic. Its mechanism of operation is mainly through muscarine receptors, but there may be a significant component of nicotinic transmission; therefore full blockade of septo-hippocampal cholinergic activation may require the administration of centrally-acting nicotinic as well as muscarinic antagonists.

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298.3 **MODULATION OF ACETYLCHOLINE BY THYROTROPIN-RELEASING HORMONE IS RECEPTOR-MEDIATED.** **D. J. Braitman, C. R. Auker and S. L. Hargett*. Physiology Dept, Armed Forces Radiobiology Research Institute, Bethesda MD 20014.**

We reported earlier (Braitman et al., Brain Res. 194: 244, 1980) that thyrotropin-releasing hormone (TRH) could cause long-term potentiation of slow excitatory responses to acetylcholine (ACh) on pyramidal tract (PT) neurons in sensorimotor cortex of cat. Since then we have conducted additional experiments indicating that the potentiation of ACh by TRH is a receptor-mediated phenomenon.

A multi-barrelled micropipette was used to record extracellular action potentials from neurons in sensorimotor cortex of chloralose-anesthetized cats. Ionophoretic aplication of TRH and ACh was closely paired (<1 sec separation) in 59 of 227 characterized neurons. TRH potentiated the slow excitatory response to ACh in 14 of the 59 tested cells. In each case, TRH had no excitatory action when applied alone. After paired administration of TRH and ACh, the response to subsequent applications of ACh alone was typically potentiated. This effect often lasted for several minutes before the excitatory ACh response returned to pre-TRH levels. Potentiation could be repeated on the same cell by again pairing TRH and ACh.

Eleven neurons were identified as PT cells by antidromically stimulating the medullary pyramids with a bipolar electrode. Since this potentiation is found almost exclusively on PT neurons, it supports the hypothesis that the modulatory effects of TRH on ACh responses is a receptor-mediated phenomenon found on a particular subpopulation of cells.

In order to further test the specific action of TRH on ACh, the ionophoretic applications of TRH and ACh were separated temporally by 10 to 30 sec. When separated temporally, potentiation of the ACh response did not occur. However, when TRH and ACh were paired closely on these same neurons, the ACh response was potentiated. Thus modulation of ACh by TRH does not occur unless both transmitters are present at the same time.

To demonstrate that TRH was not nonspecifically potentiating the action of other transmitters, TRH was paired with glutamate. The fast excitatory action of glutamate was never potentiated by TRH even when the ACh response was potentiated. This demonstrates that the specific modulatory action of TRH on ACh responses is not a function of a general long-lasting subthreshold effect.

These data suggest that the specific long-lasting modulation of ACh responses by TRH is receptor-mediated. This may occur by (1) TRH acting directly at the ACh receptor or at a linked receptor or (2) receptor-mediated stimulation of a second messenger that specifically acts on slow excitatory responses.

298.4 PARTIAL PURIFICATION OF A DENERVATION-DEPENDENT PHOSPHOPOLYPEPTIDE FROM RAT SKELETAL MUSCLE. S.P. Squinto*, J.A. McLane and I.R. Held. Loyola Univ. Med. Ctr. and VA Hosp., Hines, IL 60141.

Denervation of skeletal muscle results in early changes in both membrane-associated and cytosolic events which may be related to some neuroregulatory influence. We have reported that denervation of both the soleus and extensor digitorum longus (EDL) muscles of the rat result in the stimulation of adenylate cyclase and cyclic AMP phosphodiesterase (J. Neurosci. Res., in press, 1981). We have also shown that within hours after denervation there is an increase in the in vitro phosphorylation of an endogenous cytosolic polypeptide(s) of approximately 40K daltons (Neurosci. Lett. 20:295, 1980; Neurochem. Res. 6:203, 1981) which is temporarily correlated with the length of the distal nerve stump remaining attached to the muscle (Trans. Am. Soc. Neurochem. 12:230, 1981). The cytosolic 40K polypeptide has been resolved by SDS polyacrylamide gel electrophoresis from both the soleus and EDL. Samples of ^{32}P-labeled cytosolic protein in 50mM Tris:HCl, 2mM $MgCl_2$ and 0.1M NaCl were passed over Sephadex G-150 and a peak of radioactivity comparable in molecular weight to the polypeptide resolved on SDS polyacrylamide gels obtained. A radiolabeled peak of similar molecular weight was also obtained when ^{32}P-labeled cytosol was fractionated by exclusion chromatography with a mobile phase of 0.05 M sodium phosphate pH 7.4 on a Waters I-125 HPLC column. Since peaks of similar apparent molecular weight were obtained under denaturing (SDS) and non-denaturing conditions, apparently the radiolabeled polypeptide is not a subunit of a larger protein complex. Also, the denervation-dependent increase in protein kinase activity is apparently due to alterations in endogenous modulators. Combined aliquots from the cytosolic fractions of denervated and contralateral, sham-operated muscles were assayed and the results evaluated with respect to those predicted from assays with each sample separately. While the specific phosphorylating activity of the denervated sample was twofold greater than that from the sham-operated muscle, this activity with the "mixed" samples was less than the predicted value. When either sample is pre-incubated at 37°C before the initiation of the phosphotransferase reaction with α-^{32}P [ATP], then the phosphorylation of the endogenous substrate is decreased. Pre-incubation of "mixed" samples, however, produces an even greater decrease in the formation of phosphorylated product. This suggests that denervation of skeletal muscle may have an effect on the suppression of an endogenous soluble inhibitor of protein kinase. Supported by NINCDS Grant NS-11755, the Medical Reserach Service of the Veterans Administration and BRSG funds from Loyola University.

298.5 SEROTONIN EFFECTS ON PROTEIN PHOSPHORYLATION WITHIN A SINGLE LIVING NERVE CELL. José Lemos*, Ilse Novak-Hofer* and Irwin B. Levitan. Friedrich Miescher-Institut, P.O.Box 273, CH-4002 Basel, Switzerland.

Serotonin causes a cyclic AMP-mediated increase in potassium conductance in the identified Aplysia neuron R15 (Drummond, Benson and Levitan, PNAS 77(1980)5013-5017). This response can be inhibited by intracellular injection of the 10,000 dalton protein kinase inhibitor from rabbit skeletal muscle, indicating that protein phosphorylation may play a role in regulation of this potassium channel (Levitan and Adams, Adv.Cyc. Nuc.Res. 14(1981)647-653). To identify any phosphoproteins which might be involved in such regulation, we have injected γ-(^{32}P)ATP into R15, and have measured protein phosphorylation within this single living neuron. In addition, the cell's electrical properties were monitored throughout the experiment using voltage clamp techniques. Within 30 min after intracellular injection of 10^5-10^6 cpm of γ-(^{32}P)ATP into R15, 1000-20,000 cpm of ^{32}P were incorporated into phosphoproteins which could then be separated according to their molecular weights on SDS-polyacrylamide gels. At least 12 protein bands, ranging in molecular weight from 22,000 to 230,000 daltons, were phosphorylated within neuron R15. The extent of phosphorylation of several of these bands was altered in cells in which potassium conductance was increased by treatment with serotonin. The most consistent changes were seen in bands of molecular weight 26,000 and 135,000 daltons. The possible role of these phosphoproteins in regulation of the activity of potassium channels is under investigation.

298.6 NET OUTWARD CURRENTS OF BAG CELL NEURONS ARE DIMINISHED BY A cAMP ANALOGUE. L. K. Kaczmarek and F. Strumwasser. Division of Biology, California Institute of Technology, Pasadena, CA 91125

Both chemical and electrophysiological evidence indicate that the onset of afterdischarge and the subsequent profound enhancement of spike broadening that occur in the bag cell neurons of Aplysia are related to an increase in intracellular cAMP concentration. We have now used a two electrode voltage clamp to study the properties of isolated bag cell neurons in cell culture and their response to 8 benzylthio-cAMP (8BTcAMP). This membrane permeant and phosphodiesterase resistant cAMP analogue induces spontaneous discharge and spike broadening in both the intact bag cell cluster and isolated bag cell neurons in cell culture.

Cells were chosen, within two days of seeding, that had not yet extended elaborate neuritic networks, and were penetrated with two microelectrodes (resistances 35-50 MΩ). A delayed outward current, sometimes preceded by a transient inward current, was observed with command steps (80 msec) to between -10 and +10 mV from a holding potential of -40 mV, in Eagle's MEM made up in artificial seawater. On repetition at a frequency of 1 Hz these outward currents inactivate, enhancing the early inward currents. The inward currents, in 3 of 4 cases, were abolished by the calcium antagonist Co^{2+} (20 mM). Both the magnitude and the degree of inactivation with repetition of the outward currents were also diminished by Co^{2+} (20 mM) and by Ni^{2+}(5 mM) for these command pulses although larger commands (>+10 mV) still produced substantial inactivation with repetition.

8BTcAMP produced a decrease (31-51%) in the net outward currents between -10 and +10 mV and in their degree of inactivation with repetition. The early inward currents were however unchanged or enhanced by 8BTcAMP. We have attempted to isolate the inward currents pharmacologically in media containing TEA (50-460 mM) with either Na^+ and Ca^{2+} or with Ba^{2+} alone as carriers of inward current. Under these conditions we have not seen an effect of 8BTcAMP on the inward currents, suggesting that 8BTcAMP exerts its effect on the electrical properties of bag cell neurons by depressing an outward potassium current. We have further examined both the transient potassium current, I_A (Connor and Stevens, 1971), by command steps to -40 mV from holding potentials between -80 and -100 mV, and the voltage activated late potassium current I_K, by depolarizing commands from a holding potential of -40 mV in the presence of 20 mM Co^{2+}. Neither of these currents was affected by 8BTcAMP. These results suggest, therefore, that 8BTcAMP may diminish a calcium dependent potassium current. It remains to be determined, however, whether the pharmacological manipulations used to separate the inward and outward currents affect the ability of the cells to respond to cyclic AMP analogues or themselves effect biochemical changes such as protein phosphorylation.

298.7 CALMODULIN MEASUREMENT IN BIOLOGICAL TISSUE BY HPLC-UV DETECTION. Marty Javors*, D.H. Ross and Charles L. Bowden. Depts. Pharmacol. & Psychiatry, Univ. Tex. Hlth. Sci. Ctr., San Antonio, TX 78284.

Calmodulin is a protein which mediates many of the cellular functions triggered by intracellular calcium concentration changes. In neuronal tissue, a calcium-calmodulin complex modulates the activation of cyclic nucleotide phosphodiesterase, adenyl cyclase, Ca-Mg-ATPase activity, plasma membrane calcium transport and neurotransmitter release. To date, the measurement of calmodulin in biological tissues has required utilization of a bioassay in which the activation of phosphodiesterase activity or Ca-Mg-ATPase activity by calmodulin is observed. In this paper, we describe an HPLC method of detection for calmodulin, using UV detection at 214 nm. This assay is sensitive to 0.5 micrograms and linear to at least 20 micrograms, using purified calmodulin from three different sources. Studies with a rat brain striatal P_2 preparation indicate that 30% of the calmodulin was membrane-bound and the membrane contained approximately 2 micrograms calmodulin per mg membrane protein. Determinations of calmodulin content in cytosol and membranes of RBC's and platelets have also been made. This method provides a useful tool for the quantification of calmodulin in biological tissues.

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298.8 DISTRIBUTION OF CALCIUM SENSITIVE PHOSPHODIESTERASE (PDE) IN DIFFERENT RAT BRAIN REGIONS. M. S. Yang* (SPON: Mary J. Kallman). Div. of Neurosurgery, Med. Coll. VA, Richmond, VA 23298.

Using cGMP as substrate, the catabolic enzyme PDE was shown to distribute unevenly in mouse brain. The highest activities in the presence of calcium were found in the substantia nigra pars reticulata and the basal ganglia (Yang et al., J. Neurochem. 36:1272, 1981). It was also shown that several PDE isoenzymes exist in brain. One is specific for cAMP. At least one other, which can hydrolyze both cAMP and cGMP, is located in the cytosol, sensitive to calcium and inhibitable by antipsychotic drugs (Wolff and Brostrom, Adv. Cyclic Nucleotide Res. 11:41, 1979).

Quantitative histochemical analysis demonstrated that PDE distribution in rat brain is similar to that in the mouse. Furthermore, the following study sought to characterize the calcium contribution to the high PDE activity in different rat brain regions.

After homogenization in different buffers, 100,000 g ultracentrifugation and enzymatic assay (Carter et al., J. Histochem. Cytochem. 17:505, 1979), part of the total PDE activity in rat brain cortex was recovered from the supernatant fraction and the other part from the particulate. Recovery was close to 100%. PDE in the supernatant fraction was sensitive to calcium, while that in the particulate was not. The calcium-sensitive fraction of the cortex was also more sensitive to trifluoperazine and thioridazine as compared to the particulate calcium-independent fraction which was more sensitive to papavarine and IBMX when those drugs were presented at low concentrations. In the presence of EGTA, trifluoperazine failed to inhibit the supernatant enzyme. Bromocryptin and Hydergine were less effective in inhibiting both fractions.

Seven rat brain regions were studied. In the presence of 1 mM EGTA, the supernatant PDE activity showed highest activity in the cortex, followed by the striatum, the hippocampus, the thalamus, the pons, the medulla and the cerebellum. In the presence of 0.2 mM calcium chloride, activity increased in all regions, but the increase was not uniform. The enzyme activity increased by 600% in the striatum, 400% in the pons, 300% in the cortex, thalamus and medulla and only 60% in the cerebellum. The large increase in calcium-dependent activity in the striatum resulted in the striatum showing the highest activity of all regions tested. These data demonstrate that the high PDE activity in the striatum is related to a calcium-sensitive component. (This study was supported by Sandoz AG, Basel, Switzerland).

298.9 SEROTONIN-INDUCED PHOSPHORYLATION OF A 28,000 DALTON PROTEIN IN A LOBSTER NERVE-MUSCLE PREPARATION. M. F. Goy*, T. L. Schwarz* and E. A. Kravitz. Dept. of Neurobiology, Harvard Med. Sch., Boston, MA 02115.

The biogenic amine serotonin is part of a complex hormonal control system that influences the dactyl opener muscle of the walking legs of the lobster Homarus americanus. In this preparation serotonin causes an increase in transmitter release from both the excitatory and inhibitory axons that innervate the muscle. In addition, the amine acts directly on the muscle to cause a long-lasting contracture and increase the magnitude of a voltage-dependent Ca^{++} current.

To look for biochemical events that might underlie these physiological processes, intact opener muscles with their attached nerves were incubated in $^{32}PO_4^{2-}$. Phosphorylated proteins were separated by SDS polyacrylamide gel electrophoresis and detected by autoradiography. Adding serotonin to the incubation medium leads to phosphorylation of a 28,000 dalton protein. The time course of this phosphorylation is slow, like the physiological actions of serotonin; it rises over several minutes and is sustained for at least an hour. The phosphorylation can be induced by physiologically reasonable concentrations of the amine (10^{-7}M).

We have begun to investigate the significance of this phosphorylation in the physiological actions of serotonin. A variety of agents that cause contractions (high K^+, caffeine, and glutamate - the presumed transmitter of the excitatory axon) do not cause significant increases in the phosphorylation of the 28,000 dalton protein. The phosphorylation is not, therefore a consequence of contraction or its associated Ca^{++} movements.

Two other neurohormones, octopamine and proctolin, cause long-lasting contractures of the opener muscle similar to those of serotonin. Octopamine causes at most a very slight phosphorylation of this protein and proctolin has no observable effect. Thus the reaction is probably not involved in a common pathway for the three hormone-induced contractures.

The phosphorylation may be mediated by changes in cyclic nucleotide metabolism. In a homogenized preparation, cGMP and (to a lesser degree) cAMP stimulate the phosphorylation of a 28,000 dalton protein which may be identical to the protein phosphorylated in vivo. Experiments to clarify the relationship of the phosphorylation to cyclic nucleotides and the physiology of serotonin action are in progress.

Supported by NIH and the Muscular Dystrophy Association.

298.10 MODULATION OF THE HIPPOCAMPAL α-ADRENERGIC RECEPTOR POPULATION BY LESION OF THE SEROTONERGIC RAPHE-HIPPOCAMPAL PATHWAY IN RATS. H. Ladinsky*, S. Consolo*, G. L. Forloni* and P. Grombi* (SPON: L. VALZELLI). Lab. of Cholinergic Neuropharmacology, Istituto di Ricerche Farmacologiche "Mario Negri", Milan, Italy 20157.

Electrolytic lesion of the ascending serotonergic fibers in the median raphe nucleus caused more than 90% depletion of 5-HT in the hippocampus and cortex (18 days) without affecting NE and ACh contents. α-Adrenoceptor binding of (^{3}H)WB-4101 was increased in the hippocampus but not in the frontal cortex. Scatchard analysis revealed that the increase in (^{3}H)WB-4101 binding in the lesioned hippocampus was the result of an elevated density of α-adrenergic receptors of about 30%. This phenomenon began 8 days postlesion and persisted for at least 80 days postlesion. Similar qualitative results were obtained following lesion of the median raphe nucleus with 5,7-DHT. Selectivity of the phenomenon was demonstrated as β-adrenoceptor binding with (^{3}H)dihydroalprenolol and cholinergic muscarinic receptor binding with (^{3}H)dexetimide were not significantly affected in the hippocampus. By contrast, when NE in the hippocampus was depleted by more than 90% by bilateral lesion of the ascending noradrenergic fibers with 6-OHDA (18 days), α-adrenoceptor number was not significantly changed while β-adrenoceptor number was enhanced. It is currently being investigated whether the increase in α-adrenoceptor number in the presence of unchanged NE content and in the absence of 5-HT signifies heterologous modulation of the NE receptor population by 5-HT.

298.11 EFFECT OF 6-HYDROXYDOPAMINE ON RELEASE OF ATP FROM MYENTERIC SYNAPTOSOMES PREPARED FROM GUINEA-PIG ILEUM. M. Alhumayyd* and T.D. White. Department of Pharmacology, Dalhousie University, Halifax, Nova Scotia, Canada B3H 4H7.

It has been suggested that ATP may be an inhibitory neurotransmitter in the myenteric plexus of vertebrate intestinal smooth muscle. There is also evidence that ATP may be stored and co-released with biogenic amines and acetylcholine to modulate synaptic function. Recently, we have studied the depolarization-induced release of ATP from synaptosomes prepared from the myenteric plexus of guinea-pig ileum by detecting the light produced when the released ATP reacted with firefly luciferin-luciferase present in the incubation medium. Depolarization with elevated extracellular K^+ or veratridine released ATP by a mechanism which required extracellular Ca^{2+}. In the present study we chemically lesioned the noradrenergic nerves of the myenteric plexus by administering two-doses of 6-hydroxydopamine (250 mg/kg i.p.) to guinea-pigs, the second dose being administered 3 days after the first dose. The guinea-pigs were killed 7 days after the first dose and myenteric synaptosomes prepared from the longitudinal muscle of the ileum. Control synaptosomes contained 61.7 ± 5.0 ng noradrenaline/mg protein as measured by HPLC. This was reduced to 29.2 ± 5.5 ng/mg protein by 6-hydroxydopamine pretreatment (6 experiments). 6-Hydroxydopamine pretreatment reduced K^+-induced release of ATP from synaptosomes by 25% and veratridine-induced release by 36% (6 experiments). Preliminary studies indicate that in vitro pretreatment of myenteric synaptosomes with 6-hydroxydopamine abolishes both K^+- and veratridine-induced release of ATP. These findings suggest that at least a portion of the ATP was released from noradrenergic nerve terminals and support a possible cotransmitter function for ATP and noradrenaline in this tissue. (Supported by the MRC of Canada).

298.12 VASOPRESSIN POTENTIATES NOREPINEPHRINE-STIMULATED CYCLIC AMP ACCUMULATION. A. C. Church. Dept. of Anatomy, Univ. of Pennsylvania, School of Medicine, Phila., Pa. 19104

Vasopressin (also known as antidiuretic hormone) is a nonapeptide which has been shown to strengthen memory by a centrally mediated action. In addition to its effets on memory (deWied, D., Nature 232:137, 1973), vasopressin has also been shown to prolong functional tolerance to both ethanol (Hoffman, P.L., et al., Nature 276:614, 1798) and morphine (van Ree, J.M., et al., Biochem. Pharmacol. 27:1973, 1978). Since lesions of brain norepinephrine (NE) systems resulted in a loss of vasopressin efficacy (Hoffman, P.L. and Tabakoff, B., Drug Alc. Depend. 4:249, 1979), a study was undertaken to determine whether vasopressin might not interact with NE stimulated cyclic AMP accumulation in brain slices.

Slices of mouse hippocampus were prepared according to Quik et al. (Biochem. Pharmacol. 27:2209, 1978) and were incubated at 37°C in Krebs-Ringer bicarbonate containing 1 mM theophylline for 60 mins. Aliquots of tissue were then removed and added to buffer containing either NE (10 uM), lysine vasopression (1 uM), both or neither. After incubating 10 mins at 37°C, the reaction was stopped by boiling. Cyclic AMP concentrations were measured by the method of Brown et al. (Biochem. J. 121:561, 1971).

In the presence of NE, cyclic AMP values were increased by about 4-5-fold over basal values, while lysine vasopressin had no significant effect. However, the mixture of lysine vasopressin and NE produced cyclic AMP concentrations which were 9-10-fold higher than basal values. Thus, vasopressin substantially potentiated the stimulative effect of NE, while producing little effect by itself. These results suggest that vasopressin is capable of neurochemically modulating the responsiveness of the hippocampal NE-cyclic AMP system. Further characterization of the vasporessin-NE interaction is currently being investigated.

298.13 CHARACTERIZATION OF ADENOSINE RECEPTORS IN RAT BRAIN. J. Patel* and P. J. Marangos. Clinical Psychobiology Branch, NIMH, Bethesda, MD 20205.

Adenosine has multiple effects in the nervous system, the most notable of which are modulation of neurotransmitter release and adenylate cyclase activity, depression of the spontaneous firing rate of cortical neurons and potent behavioral effects which include sedation. The neuromodulatory effects of adenosine are thought to be mediated by specific adenosine receptors but these receptor sites have been difficult to characterize using ^{3}H adenosine since adenosine is apparently generated in synaptosomal membrane preparations.

Using ^{3}H N^6 cyclohexyladenosine (CHA) and the enzyme adenosine deaminase it is possible to specifically label high affinity binding sites for adenosine that satisfy the criteria for specific receptors. Two populations of adenosine receptors exist in rat brain with respective K_D values of 0.7 nM and 2.4 nM. The respective B_{max} values are 230 and 120 fmoles/mg protein in rat forebrain. Adenosine deaminase treatment of the membranes is essential for maximal binding since less than 30% binding is observed in the absence of this enzyme. Regional distribution studies indicate high densities of adenosine receptors in hippocampus with lower levels observed in cerebral cortex, cerebellum and striatum and still lower levels in the spinal cord. Subcellular distribution studies are consistent with the adenosine receptor being localized in synaptosomes.

Extensive analysis of various anions and cations for their effect on ^{3}H CHA binding reveals that both copper and zinc are potent non-competitive inhibitors of binding with respective IC_{50} values of 30 μM and 150 μM. Calcium and magnesium had weak stimulatory effects on binding with EC_{50} values greater than 100 μM. Sulfhydryl reducing and alkylating agents inhibit ^{3}H CHA binding indicating that the adenosine receptor is a sulfhydryl dependent protein.

The results obtained indicate that ^{3}H CHA is an advantageous ligand to use for adenosine receptor binding studies.

298.14 ADENOSINE ANALOGS INCREASE ELECTRICAL POTENTIAL IN BRAIN SYNAPTIC MEMBRANE VESICLES. M.L. Michaelis and E.K. Michaelis. Neurobiology Section, Dept. of Human Development, Univ. of Kansas, Lawrence, KS. 66045

The purine nucleoside adenosine has been shown to modulate the release of neurotransmitters in both the peripheral and central nervous systems and to influence postsynaptic responses to released transmitter substances. The mechanism(s) by which adenosine acts to modulate neurotransmission is as yet unknown. It has been suggested that presynaptic modulators may decrease transmitter release by either decreasing the influx of Ca^{2+} upon depolarization or by hyperpolarizing the presynaptic membranes by an alteration in the membrane conductance for either Cl^- or K^+. We have used isolated, resealed synaptic plasma membrane vesicles (Life Sciences, 28:37) to study the influence of the adenosine analog 2-chloroadenosine (2-ClAdo) on synaptic membrane potentials in an effort to identify which ionic conductance is affected by this purine. The lipophilic cation [^{3}H] methyl triphenylphosphonium ($TPMP^+$) has been used to measure the membrane potential $\Delta\Psi$ which developed when resealed synaptic membrane vesicles were loaded internally with K_2-succinate and incubated in a Na_2-succinate or NaCl medium with [^{3}H] $TPMP^+$. The membranes have been found to develop a $\Delta\Psi$ of approximately -7 mV in Na_2-succinate, -28 mV in NaCl, and -2 mV in KCl, the latter condition representing the absence of a K^+ diffusion potential.

Preincubation of the K_2-succinate-loaded vesicles with 2-ClAdo led to a substantial increase in membrane $\Delta\Psi$ in the presence of a K^+ diffusion potential. The EC_{50} for the effect of 2-ClAdo was determined by log probit analysis to be 55 nM. 2-Chloroadenosine did not influence $TPMP^+$ uptake when vesicles were loaded internally with Na_2-succinate instead of K_2-succinate. Blockade of anion channels with SITS did not block the 2-ClAdo-induced increase in $\Delta\Psi$, suggesting that Cl^- fluxes are not responsible for the effects of 2-ClAdo on membrane polarity. However, adenosine receptor antagonists such as the methylxanthines did inhibit the effects of 2-ClAdo. In addition, adenosine itself (50 nM concentration) as well as several adenosine derivatives such as phenylisopropyl adenosine, ADP, and α,β-CH_2-ADP also produced rapid increases in membrane $\Delta\Psi$. These preliminary observations suggest that the adenosine-induced hyperpolarization observed in electrophysiological studies may be due to enhancement of K^+ efflux from neurons. Exactly how this event leads to a decrease in the release of neurotransmitters from presynaptic nerve terminals remains to be determined. (Supported by NIH grant NS 16364 and by 79-KS-529 from the AHA, Kansas Affiliate.)

298.15 MODULATION OF MONOAMINE OXIDASE (MAO) BY SMALL MOLECULAR PROTEINS IN PLASMA, C.T. Giambalvo and R. Becker. Rhode Island Psychiatric Research and Training Center and Dept. of Pharmacology, Univ. of Rhode Island, Kingston, RI 02881.

Human plasma contained modulators that affected platelet and striatal MAO activity when added *in vitro*. It was found that addition of plasma inhibited tryptamine deamination by platelet MAO. The inhibition was uncompetitive in nature, reversible by prior precipitation of plasma proteins by perchloric acid, but was not reversed by prior ultrafiltration of plasma through PM30 Amicon membranes that remove substances > 30,000 MW. These results suggested that the inhibitor is a small molecular weight protein. Addition of plasma also inhibited tryptamine deamination by bovine striatal MAO. The inhibition was noncompetitive, reversible by perchloric acid pretreatment as well as by prior ultrafiltration of plasma. Plasma also inhibited the deamination of serotonin by striatal MAO-A. The inhibition was non-competitive in nature, reversible by perchloric acid pretreatment but not by PM30 ultrafiltration. These results suggested that this inhibitor is a small molecular weight protein. The inhibition of platelet or striatal MAO by plasma is unlikely to be mediated by serum albumin because ultrafiltration would have removed serum albumin. Moreover, addition of bovine serum albumin *in vitro* affected the kinetic properties of MAO differently than the ultrafiltered plasma.

298.16 DIFFERENCES IN THE BRAIN RECOGNITION SITES FOR TYPICAL AND ATYPICAL ANTIDEPRESSANTS. N. Brunello*, D.M. Chuang* and E. Costa. (SPON: S. H. Koslow). Lab. Preclin. Pharmacol., NIMH, St. Elizabeths Hosp., Washington, D.C. 20032

Repeated administrations of typical or atypical antidepressants down regulate the norepinephrine (NE) activation of adenylate cyclase in brain slices. This action requires the presence of brain adrenergic axon terminals but not that of brain serotonergic axon terminals. The synaptic membranes prepared from various areas of a mamalian brain contain high affinity binding sites for typical antidepressants (Nature 281:148, 1979). These sites are saturable, stereospecific, unevenly distributed in brain and appear to be located presynaptically on serotonergic axons (Science 210:1133, 1980). Also [^{3}H]mianserin binds to crude synaptic membranes (Eur. J. Pharmacol. 68:395, 1980); these sites appear to be associated with serotonergic synapses (J. Pharmacol. Exp. Ther. 216:142, 1981). We have investigated whether the recognition sites for both classes of drugs are exclusively located in association with serotonergic synapses and whether both drugs bind to the same type of synapses. With these studies we hoped to define more precisely the site of action of antidepressants and, indirectly, to obtain information on biochemical markers specific for affective disorders. Since serotonergic synapses have been singled out as a possible site where the recognition site of both classes of compounds is located, we began our studies by perturbing serotonergic neuronal function with intracerebral injections of 5,7 dihydroxytryptamine, with intraperitoneal injections of p-Cl-phynylalanine and have lesioned the fimbria fornix. The Bmax of [^{3}H]imipramine was reduced by the toxin injections but the extent of the reduction differed in various areas though the extent of 5HT depletion was maximal in all areas. In contrast, in the same rats the Bmax of [^{3}H]mianserin binding was increased. Chronic injections of p-Cl-phenylalanine which deplete the 5HT content of various areas maximally and uniformly caused a variable degree of increase in the Bmax of [^{3}H] mianserin binding while the binding of [^{3}H]imipramine remained unchanged. Lesions of the fimbria fornix decreased the [^{3}H] imipramine binding in the hippocampus but failed to change that of [^{3}H]mianserin. Chronic treatment with imipramine down regulated its high affinity binding; in contrast, chronic treatment with mianserin failed to down regulate its high affinity binding. From these and other results that will be presented it is concluded that the two classes of compounds bind to different sites. In both instances the sites are associated with various types of synapses suggesting as a working hypothesis that the endogenous ligand that binds physiologically to the sites where antidepressants bind, could be a cotransmitter that tunes up the sensitivity of the detection sites for a given group of postsynaptic receptors.

299.1 METABOLIC COMPARTMENTATION OF GLUTAMATE: CELLULAR LOCALIZATION OF CERTAIN RELEVANT ENZYMES AND THE INDUCTION OF GLUTAMINE SYNTHETASE IN THE BRAIN IN VIVO. A.J. Patel, A. Hunt‡ C.S.M. Tahourdin‡ R.D. Gordon‡ and S. Bunn*. MRC Developmental Neurobiology Unit, Institute of Neurology, 33 John's Mews, London WC1N 2NS, UK.

We have previously developed a model to account for kinetic observations indicating that glutamate and the associated tricarboxylic acid cycles ('cycle') are compartmented in the brain, and have assigned the functional compartments to morphological structures. It was proposed that transmitter amino acids released from nerve terminals are taken up, in part, by glial cells, where they are converted into glutamine. Glutamine in turn is taken up, after diffusion into the extracellular space, by neuronal structures and hydrolysed to glutamate, thus balancing the loss of carbon units in the 'cycle'. The availability of relatively pure separated and cultured neurones and glia in our Unit has now permitted the investigation of their biochemical properties and testing of this proposal. The results were consistent with the hypothesis in showing that the astrocyte to neurone specific activity ratio were 3.6-8.4 for glutamine synthetase (GS), 1.5-5.3 for glutamate dehydrogenase and 0.1-0.4 for glutaminase, depending upon the class of neurone and the type of preparation used for comparison. On the other hand, the specific activity of succinate dehydrogenase was more even in the different cell types, indicating that the differences in the distribution of mitochondrial enzymes are not simply related to variations in the concentration of mitochondria in the two classes of cells. The possible involvement of GS in processes associated with amino acid neurotransmission was also suggested by the observation that the activity of this enzyme was unevenly distributed throughout the CNS and in different regions of the brain the activity of GS was significantly correlated with that of glutamate decarboxylase but not with that of choline acetyltransferase. Furthermore, in agreement with previous studies on GS in chick retina in vivo and various avian and mammalian cells in vitro, we observed that the activity of this enzyme in the mammalian brain in vivo is increased by glucocorticoids. The increase was more marked in young than in adult rats and comparison of the effect on various brain areas suggested that elevation of GS was dependent on the maturational state of the region at the time of the hormone treatment. The effect of glucocorticoids on GS activity was due to induction, since the immunochemically detectable GS in cerebellar homogenates fractionated by SDS-PAGE and transferred to DBM paper showed an increase in the amount of enzyme.

299.2 RELEASE AND METABOLISM OF NEWLY SYNTHESIZED DOPAMINE IN RAT STRIATAL SLICES: EFFECT OF SELECTIVE MONOAMINE OXIDASE INHIBITION, D.D. Schoepp* and A.J. Azzaro, Departments of Neurology and Pharmacology-Toxicology, West Virginia University Medical Center, Morgantown, W.V. 26506

Studies were designed to determine the relative roles of the type A and B forms of monoamine oxidase (MAO) in the deamination of released rat striatal dopamine (DA). In vitro release and subsequent metabolism of ^{3}H-DA was examined using rat brain slices. Striatal slices were pre-incubated in the presence of ^{3}H-L-tyrosine (20 µM) followed by a short incubation period in the presence of the irreversible MAO inhibitor agents described below. Tissue pretreated in this manner was then subjected to a release incubation using varying concentrations of potassium chloride (KCl).

In the absence of MAO inhibition, KCl produced a concentration-dependent release of newly formed ^{3}H-DA as well as an increased formation of ^{3}H-deaminated metabolites; dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA). Formation of *^{3}H-DOPAC and ^{3}H-HVA were maximal at 30 mM KCl with* DOPAC representing the major deaminated metabolite. Incubation in the presence of the DA uptake inhibitor nomifensine (10^{-5}M) further enhanced the KCl-induced increase of released ^{3}H-DA as well as the formation of both ^{3}H-DOPAC and ^{3}H-HVA. Pretreatment of striatal slices with a selective concentration of the type A MAO inhibitor clorgyline (10^{-7}M) significantly decreased K^+-induced (30 mM KCl) formation of both ^{3}H-deaminated metabolites (DOPAC 11% of control, HVA 37% of control) while enhancing ^{3}H-DA present in the medium (250% of control). The type B MAO inhibitor deprenyl (10^{-7}M) did not significantly affect these parameters. However, pretreatment with both clorgyline and deprenyl (10^{-7}M) resulted in further reduction of ^{3}H-deaminated metabolites (DOPAC 2% of control, HVA 17% of control).

These results suggest that under the conditions employed, released DA is deaminated primarily by a type A form of MAO that is extraneuronal to the DA neuron. Furthermore, in the absence of the type A enzyme minor but significant metabolism also occurs via the type B form of MAO.

Supported by the West Virginia University Medical Corporation. D.D. Schoepp is a West Virginia University Foundation fellow.

299.3 CALCIUM-STIMULATED GLUTAMATE DECARBOXYLASE ACTIVITY IN SLICES AND IN BROKEN CELL PREPARATIONS OF RAT BRAIN. Barry I. Gold and Francis P. Huger. Dept. of Pharmacol., Uniformed Services Univ., Sch. of Med., Bethesda, MD. 20014.

Glutamate decarboxylase (GAD) increases activity in response to *in vitro* models of increased neuronal activity. Ca^{2+} plays an established role in excitation-secretion coupling in neurons and in many other cells. These studies were undertaken to define the role of Ca^{2+} in the regulation of GAD activity.

Slices of corpus striatum, prepared from freshly dissected brain, were incubated in an oxygenated Krebs-Ringer-bicarbonate (KRB) medium which contained varying concns of $CaCl_2$. [^{3}H]GABA synthesis was initiated by adding L-[2,3-^{3}H]glu and the reactions were terminated with 15% trichloroacetic acid (TCA). Samples were homogenized with a Polytron; centrifuged, and the supernatants were applied to a cation exchange column of AG 50W X-8(Na^+ form). [^{3}H]GABA, eluted at pH 5.5, was estimated by liquid scintillation chromatography (LSC). GABA synthesis without exogenous Ca^{2+} was 86.6 pmoles/mg wet wt/15 min at 0.5 mM [^{3}H]glu. A concn-related increase in GABA synthesis was seen in the presence of Ca^{2+}; maximum increase was 33% at 2.6 mM Ca^{2+}. GABA synthesis in the presence of 1.3 mM Ca^{2+} was completely inhibited by 1 mM La^{3+} while 1 mM La^{3+} in the absence of Ca^{2+} had no effect. The divalent cationic ionophore, A23187 at concns up to 6µM, failed to affect the response to 1.3 mM Ca^{2+}. Co^{2+} (1 mM) did not reverse the response to 1.3 mM Ca^{2+} and had no effect on synthesis in the absence of Ca^{2+}.

GAD activity was also studied in synaptic membranes derived from a crude mitochondrial pellet (P_2). Pellets were lysed in imidazole acetic acid (25 mM, pH 7.4 containing aminoethylisothiouronium bromide (AET). Membranes were washed twice by sedimentation and rehomogenization in buffer with AET and they were dialyzed for 20 h against 50 vol of buffer which contained 0.5 mM EGTA. Membranes were washed again and resuspended for assay. GAD activity was estimated in aliquots of the membrane suspensions in a reaction mixture consisting of buffer with AET, 3.0 mM [^{3}H]glu, 100 µM pyridoxal phosphate, and 100 µM Ca^{2+} or other divalent cation. The mixture was incubated for 5 min at 30° and it was terminated wth TCA. [^{3}H]GABA was estimated as above. Control GAD activity under these conditions was 705 nmoles/mg protein/5 min and activity was increased 22% in the presence of Ca^{2+}, 32% with Co^{2+}, and <10% with Mg^{2+} GAD activity was inhibited 79% by La^{3+}, 97% by Cd^{2+}, and <10% by Mg^{2+} and Sr^{2+}.

These results are suggestive that neuronal membrane Ca^{2+} recognition sites, perhaps the same as voltage-sensitive Ca^{2+} channels, are in proximity to membrane-bound GAD, and that these Ca^{2+} sites are functionally related to the regulation of GAD.

299.4 INDICATIONS OF INCREASED DOPAMINE TURNOVER IN THE NEOSTRIATUM OF RATS TREATED THREE WEEKS PREVIOUSLY WITH TOXIC DOSES OF METHYLAMPHETAMINE. G.A. Ricaurte*, C.R. Schuster* and L.S. Seiden. (SPON: R. Lim). Dept. of Pharmacol. & Physiol. Sci., University of Chicago, Chicago, IL 60637.

Following partial 6-hydroxydopamine (6-HDA) lesions of the nigrostriatal DA projection, there is an increase in neostriatal DA turnover, as evidenced by enhanced conversion of 3H-tyrosine into 3H-dihydroxyphenylalanine (DOPA) and 3H-DA (Agid et al. Nature New Biol. 245:150,1973). It has been postulated that this acceleration of DA turnover is an adaptive response to diminished DA neurotransmission resulting from partial DA neuronal loss (ibid,1973), and the increase in DA turnover at least partly underlies the recovery of function observed in 6-HDA treated animals (Zigmond and Stricker, In: Chemical Tools for Catecholamine Res. 1:319,1975). In this study, the metabolism of neostriatal DA was examined in rats previously treated with high doses of methylamphetamine (MA) since MA, like 6-HDA destroys DA terminals (Wagner et al. Brain Res. 181:151,1980; Ricaurte et al., submitted for publication). The purpose of the study was to determine whether after MA, like after 6-HDA, there is an increase in neostriatal DA turnover. DA turnover was estimated with the DOPA accumulation (Carlsson et al. Naunyn-Schmiedeberg's Arch. Pharm. 275:153,1972) and DOPAC (Roth et al., Eur. J. Pharmacol.) methods. Separate but identically treated groups of rats were assayed with each method 3 weeks after MA treatment (50.0 mg/kg x 3,8 hours apart). With the DOPA accumulation method, the DA synthesis rate constant was found to be increased in MA treated rats. However, this increase was dependent on neostriatal DA depletion size. In rats with 0-35% (n=6) DA depletions, the rate constant was not significantly different from that in controls (0.20 ± 0.01 h^{-1}), whereas in rats with 36-70% (n=9) and >71% (n=6) DA depletions, it was increased by 25 and 150%, respectively. With the DOPAC method, the DOPAC/DA ratio (an index of DA release per neuron) was also found to be increased in MA treated rats (by 66%), but only in those with >71% (n=4) DA depletions. No increase in DA turnover was detected with the DOPAC method in rats with 36-70% (n=8) DA depletions, whereas a small (25%) but significant increase was found with the DOPA accumulation method. Although the reason for this is unclear, it may be that the latter method is more sensitive than the former in detecting DA turnover changes. These results suggest that after MA, like after 6-HDA, there is an increase in neostriatal DA turnover, and that a large number of DA terminals must be destroyed before an increase in DA turnover occurs. (Supported by IMSS Fund, Home Life Ins. Co. New York; PHS-NIDA #DA-00250; NIDA #DA-00085; RSA MH-10562).

299.5 EVIDENCE THAT PYRUVATE CARBOXYLASE IS AN ASTROCYTE SPECIFIC ENZYME IN CNS TISSUES. R.P. Shank, G.LeM. Campbell*, S.O. Freyteg* and M.F. Utter*. Department of Physical and Life Sciences, The Franklin Institute, Philadelphia, PA 19103 and Department of Biochemistry, Case Western Reserve, Cleveland, Ohio 44106.

It has been established that pyruvate carboxylase is the most active CO_2-fixing enzyme in CNS tissues (M.S. Patel, J. Neurochem, 22, 717, 1974). Metabolic studies by Berl, Cheng and their colleagues have demonstrated that CO_2-fixation occurs predominantly (if not exclusively) in a small "synthetic" metabolic compartment that rapidly synthesizes glutamine (S. Berl et al, J. Biol. Chem. 237, 2570, 1962), and that CO_2-fixation accounts for at least 10 percent of the pyruvate carbon entering the citric acid cycle (S.C. Cheng et al, Nature 216, 928, 1967). Because glutamine synthesis is now known to occur almost exclusively in astrocytes (M.D. Norenberg, J. Histochem. and Cytochem. 27, 756, 1979) these metabolic data suggest that pyruvate carboxylase activity may occur predominantly or exclusively in astrocytes. In our experiments we have examined the cellular localization of pyruvate carboxylase using immunocytochemical techniques and assay of pyruvate carboxylation activity. The rat and mouse cerebellum was the tissue examined in most experiments. Antisera against purified rat liver pyruvate carboxylase was elicited in goats as described previously and partially purified by isolating the IgG fraction. Reaction product to pyruvate carboxylase was visualized using rhodamine conjugated rabbit anti-goat antisera and the peroxidase-antiperoxidase procedure of Sternberger. In the cerebellum specific reaction product appeared to be restricted to Bergmann glia cells and other types of astrocytes. In astrocyte-like cells obtained from the cerebral hemispheres of 3 day old rats and grown in vitro for 3 weeks there was considerable reaction product which appeared to reside in discrete organelles (presumably mitochondria). In isolated populations of cell bodies obtained from the cerebellum of 10 to 14 day old mice the activity of pyruvate carboxylase was several times greater in a population enriched in astrocytes as compared to a population enriched in granule cell bodies. Based upon these and other data we postulate that through the anaplerotic function of pyruvate carboxylase, astrocytes generate a net excess of citric acid cycle intermediates which in the form of α-ketoglutarate is released into the interstitial fluid, taken up by nerve terminals (Shank and Campbell, Life Sciences 28, 843, 1981) and used to replenish the neurotransmitter pools of glutamate and GABA. An advantage of the astrocytic localization of pyruvate carboxylase is that is allows neuronal ATP to be conserved for other energy requiring processes. (Supported in part by NIH grant NS16004).

299.6 RAT BRAIN NE METABOLISM: SUBSTANTIAL CLEARANCE THROUGH 3,4-DIHYDROXYPHENYLGLYCOL (DHPG) FORMATION. P.P. Li*, J.J. Warsh, D.D. Godse*. Section of Biochemical Psychiatry, Clarke Institute of Psychiatry, Toronto, Canada.

We have recently shown that rat brain regional DHPG concentrations are either equivalent to or greater than respective 3-methoxy-4-hydroxyphenylglycol (MHPG) levels (Warsh et al., J. Neurochem. 36: 893-901, 1981). These observations suggest that metabolic clearance of brain NE through DHPG formation may be quantitatively greater than through MHPG production in this species. As the steady state concentrations of these metabolites may not reflect their respective turnover rates, we have examined the rate of formation of these metabolites in rat brain regions using the probenecid technique.

Rat brain regional free and total DHPG and MHPG were simultaneously quantitated by selected ion monitoring mass fragmentography. Conjugated DHPG and MHPG concentrations were calculated as the difference between the free and total glycol levels. Administration of increasing doses of probenecid (100-500 mg/kg, i.p.) 90 min before sacrifice produced a dose-dependent increase of conjugated DHPG and MHPG concentrations. The maximum increment of these conjugated metabolites occurred at a dose of 300 mg/kg or higher. During the first hour following probenecid (300 mg/kg, i.p.), rat brain conjugated DHPG and MHPG levels accumulated linearly at a rate of 646 and 319 pmol/g/h, respectively. Using the probenecid technique, the estimated brain regional formation rates of conjugated DHPG and MHPG were as follows:

	Conjugated DHPG (pmol/g/h)	Conjugated MHPG (pmol/g/h)	Formation Rates Ratio(DHPG/MHPG)
Hypothalamus	2800 ± 82*	951 ± 62	3.0
Midbrain	734 ± 60*	297 ± 47	2.5
Brainstem	650 ± 67*	393 ± 58	1.6
Cerebral Cortex	372 ± 25*	281 ± 20	1.3
Hippocampus	325 ± 40*	222 ± 28	1.5
Striatum	284 ± 43	212 ± 29	1.3
Cerebellum	104 ± 19	53 ± 23	1.9

*$p<0.05$ vs conjugated MHPG formation rates

In whole rat brain and most brain regions studied, the estimated formation rate of conjugated DHPG significantly exceeded that of conjugated MHPG. These results lend additional support to the notion that formation and efflux of DHPG is the major route of metabolic clearance of rat brain NE.

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299.7 TIME COURSE AND DISTRIBUTION PATTERNS IN RAT TISSUES AFTER L-DOPA ADMINISTRATION. Jackson V, Reches A*, Prasad ALN*, Fahn S. Dept. Neurol., Columbia Univ., College of P & S, New York, NY 10032.

L-DOPA is widely used therapeutically in the treatment of Parkinson's disease. While it is true that L-DOPA is converted to DA, a major part of the drug given to patients is O-methylated by COMT to 3-O-methyldopa which is therapeutically inactive. Due to the prolonged half-life of OMD, which is 12 hours, this metabolite accumulates in the blood and CSF of L-DOPA treated patients. Clinical evidence indicates that OMD interferes with the beneficial effects of DOPA, thus parkinsonian patients treated with L-DOPA deteriorate when OMD is added to their regimen. Recently, we were able to demonstrate that OMD interferes with striatal uptake utilization of L-DOPA. In the experiments reported here we have studied peripheral metabolism of DOPA in the rat over a 24 hour period to determine the distribution of DOPA and its metabolites.

Rats were injected with DOPA (250 mg/kg i.p.) and sacrificed at 1,6 and 24 h after L-DOPA treatment. Brain, liver, kidney, spleen, muscle and RBC were assayed fluorimetrically (Prasad & Fahn, 1973) for DOPA, 3-O-methyldopa(OMD), dopamine,(DA), and HVA. DOPA levels peaked at 1 h, concentrations obtained in decreasing order were: RBC, 17.61±3.82; spleen 13.47±2.33; muscle 3.89±2.42; kidney 1.91±1.80; liver 1.651±1.347 and brain 1.392±0.661 (all results are expressed as mean nmoles/g tissue ±SEM of n=5). Dopamine formation was highest at 1 h. The concentrations obtained in the kidney (104.92±11.59) were significantly higher than the levels obtained in the liver (6.77±0.982, $p<.0005$) or the brain (6.746± 0.221, $p<.0005$). The concentration of dopamine in RBC, muscle and spleen were smaller than 2 nmoles/g tissue. HVA formation as highest at 1 h, levels obtained in the kidney (119.20±11.7) were significantly higher than those of the liver (11.308±1.148, $p<.0005$ or brain (11.274±0.916, $p<.0005$) levels obtained in RBC, muscle and spleen were 3.473±0.771, 2.22±0.33, and 1.144±.33, respectively. OMD formation was highest at 6 h in muscle, 45.26±12.9; brain 24.795±4.517; RBC 20.52±2.03; and spleen 14.70±3.92. In the liver and kidney, OMD peaks at 24 h and 6 h, 5.195±.763 and 5.921±1.63, respectively. A detailed time course of OMD levels in plasma and brain in rats given DOPA 250 mg kg^{-1}, p.o. revealed a linear rise in OMD concentration with a peak at 6 hrs(brain 39.76±7.1;plasma 25.02±1.08), followed by a slow decline. It is concluded that the pattern of metabolism is different aong the tissues studied.

299.8 SEPARATION OF THREE CYSTEINE SULFINATE DECARBOXYLASE ACTIVITIES FROM HOG BRAIN. Ronald M.Spears* and David L. Martin (SPON: M. G. Pierson). Division of Laboratories and Research, New York State Department of Health, Albany, NY 12201

Cysteine sulfinate decarboxylase catalyzes one step in the biosynthesis of taurine. In an earlier study (Spears and Martin (1980) Soc. Neurosci. Abst. 151.11) hog-brain cysteine sulfinate decarboxylase (CSD) activity was resolved into two components using hydroxylapatite chromatography. Improvements in our chromatographic procedures have since enabled us to resolve three distinct components of CSD activity also using hydroxylapatite chromatography. The first two components (termed peaks I and II) did not decarboxylate and were not inhibited by glutamate whereas the third component (peak III) co-chromatographed with glutamate decarboxylase (GAD) activity. The likelihood that GAD is responsible for both CSD and GAD activities in peak III is supported by the observation of mutual competetive inhibition between cysteine sulfinate (Ki=8.5mM) and glutamate (Ki=1.6mM)for this enzyme and the finding that ATP enhanced the time-dependent, substrate-promoted inactivation of the enzyme when either glutamate or cysteine sulfinate was used as substrate. The Km values of peaks I and II CSD (0.55mM and 0.13mM for peaks I and II respectively) were much lower than the Km of peak III CSD (4.5mM). This fact, together with the strong inhibition of peak III CSD by glutamate, suggests that peaks I and II CSD are the isoenzymes involved in taurine biosynthesis.

Inhibition of peak I CSD by a variety of cysteine sulfinate substrate analogs was measured using 0.25mM cysteine sulfinate and 10 mM inhibitor. The most potent inhibitors were 3-mercaptopropionate (97% inhibition), 3-sulfonopropionate (94%) and DL-penicillamine (88%). Four closer structural analogs of cysteine sulfinate were weaker inhibitors; L-aspartate inhibited by 36%, L-cysteate by 60%, 2-amino-3-phosphonopropionate by 54%, and DL-homocysteate by 30%. In view of the large excess of inhibitor these results together with the failure of glutamate to serve as a substrate indicate that CSD has a high degree of structural specificity. The product, hypotaurine, inhibited by 38%. L-cysteine and α-ketoglutatate inhibited 53 and 69% respectively. Comparable effects were observed with peak II CSD. A high degree of purity of peaks I and II CSD has been achieved by further chromatography of each enzyme on QAE-sephadex, followed by phenyl Sepharose and finally by Sephadex G-100.

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299.9 EFFECTS OF ATP, 4-DEOXYPYRIDOXINE PHOSPHATE AND INOSITOL HEXASULFATE ON BRAIN GLUTAMATE DECARBOXYLASE ACTIVITY. Mary P. Meeley*, Sandra Bloom Martin* and David L. Martin. Div. Labs. and Research, New York State Dept. Health, Albany, NY 12201 and Dept. Chemistry, Univ. of Maryland, College Park, MD 20742

A partially purified preparation from hog brain was used to study the effects of ATP, 4-deoxypyridoxine phosphate (DPX-P) and inositol hexasulfate (IHS) on glutamate decarboxylase (GAD) activity. These compounds were selected on the basis of their structural diversity. ATP is a nucleoside triphosphate known to be a potent inhibitor of GAD; DPX-P is a close structural analog of pyridoxal-5'-phosphate (pyridoxal-P), the cofactor for the enzyme; IHS is a polysulfate anion with no obvious structural relationship to either ATP or DPX-P. The substrate, glutamate, promotes a time-dependent inactivation of holoGAD, probably by means of decarboxylative transamination, thereby generating apoGAD. ATP (50 μM) has been shown previously to accelerate the glutamate-promoted inactivation in the presence or absence of added pyridoxal-P (Meeley and Martin (1980) Soc. Neurosci. Abst. 23.3). DPX-P and IHS also accelerated inactivation in the absence of added pyridoxal-P. These results indicate that inhibition by these three compounds cannot be attributed solely to competition with pyridoxal-P, although some contribution by this mechanism in the presence of pyridoxal-P cannot be ruled out by current data. IHS (IC50<1μM) was a more potent inhibitor than either ATP (IC50<50μM) or DPX-P (IC50<50μM). Reactivation of glutamate-inactivated GAD by pyridoxal-P was also examined. Previous studies have shown that inorganic phosphate (Pi) stimulates activation of apoGAD by pyridoxal-P. Following partial inactivation of holoGAD with 10 mM glutamate, a slow and incomplete recovery of activity (requiring > 30 min to reach 50% of initial activity) was observed when 20 μM pyridoxal-P and 10 mM Pi were added to the reaction mixture. However, complete and rapid (< 5 min) reactivation with pyridoxal-P and Pi occurred when GAD was inactivated with both glutamate and low concentrations of ATP (10 - 50 μM). Increasing the ATP concentration above 100 μM resulted in only partial reactivation. Also, reactivation was incomplete if 10 μM ATP was added at the same time as pyridoxal-P and Pi to GAD which had been inactivated with glutamate alone. Similar results were observed with DPX-P. The results available to date show that the effects of these compounds are remarkably similar despite their apparent structural differences.

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299.10 LOCALIZATION OF RENIN ACTIVITY IN RAT BRAIN SYNAPTOSOMES. A. Husain*, R.R. Smeby*, R.C. Speth, Research Division, Cleveland Clinic Foundation, Cleveland, OH 44106.

In addition to its well known peripheral actions, angiotensin II has a variety of actions in the central nervous system, for example, induction of drinking behavior and elevation of blood pressure. However, the role of an endogenous renin-angiotensin system in brain remains controversial. The recent demonstration in this and in other laboratories of a neutral protease in brain, capable of forming angiotensin I (brain renin) did not determine its origin. To determine whether brain renin activity was localized in neuronal cells, an investigation of brain renin in subcellular fractions was carried out. Using the techniques established by DeRobertis et al, (J. Neurochem. 9: 23-35, 1962), rat brains were fractionated by differential and discontinuous sucrose density gradient centrifugation. Simultaneous measurements of the activities of brain renin and a synaptosomal marker, choline acetyltransferase (CAT), were made on each fraction obtained by differential centrifugation and on further subfractions of the crude mitochondrial pellet (P_2, 11,500 g x 20 min) obtained by discontinuous sucrose density gradient centrifugation. Five subfractions of the crude mitochondrial pellet were obtained by the following sucrose concentrations: Fraction A (<0.8M, Myelin); Fraction B (0.8-1.0M, Synaptic debris); Fraction C (1.0-1.2M, Synaptosomes); Fraction D (1.2-1.4M, Synaptosomes); Fraction E (>1.4M pellet, Mitochondria). Upon differential centrifugation, 65% of brain renin activity was recovered from the crude mitochondrial (P_2) fraction, while less than 10% of the brain renin activity was recovered from the nuclear (P_1) fraction. Brain renin activity does not appear to be enriched in the cytosol as less than 10% of the brain renin activity was recovered in the 100,000 x g x 60 min supernatant (S_3). Recovery of brain renin activity was between 90 and 117%. Within the subfractions of the crude mitochondrial pellet, the highest amount of CAT activity was observed in Fraction C, the lighter of the two major synaptosomal fractions, confirming earlier observations of DeRobertis et al. By comparison, the largest amounts of brain renin activity were observed in both of the major synaptosomal bands, Fractions C and D. The high amount of brain renin activity in the synaptosomal fractions relative to the other fractions suggests that brain renin is localized in a neuronal compartment and further supports a possible functional role of an endogenous brain renin-angiotensin system.

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299.11 AMINO ACID METABOLISM IN HUMAN PLATELETS: OBSERVATIONS IN HUNTINGTON'S DISEASE. Richard M. Mangano and Robert Schwarcz. Maryland Psychiatric Research Center, Baltimore, MD 21228.

Blood platelets have been frequently employed as a peripheral model for many aspects of central nervous system function. Following an earlier report on the biosynthesis of glutamate and aspartate from glucose and acetate in human platelets (Puszkin et al., J. Lab. Clin. Med. 75, 234, 1970) we have now re-examined human platelet metabolism of both amino acids in view of their hypothetical involvement in the etiology of the heritable neurodegenerative disorder, Huntington's disease (HD; Coyle et al., Prog. Neuropsychopharmacol. 1, 13, 1977).

Routine assays were conducted by incubating fresh platelet suspensions (containing 1.5-2 mg platelet protein) for 10 minutes at 37°C with 50 μCi ^{3}H-acetate and 20 μCi ^{14}C-glucose. Subsequently, glutamate, aspartate and their amides glutamine and asparagine were separated according to the method of Berl et al. (J. Neurochem. 15, 131, 1968) and incorporated radioactivity and levels of all amino acids were determined. The specific activities of the compounds derived from both precursors constitute markers of the efficiency of the enzymatic cascades originating from acetate and glucose, respectively. Metabolism was determined simultaneously in control (N=9)- and HD (N=7)-platelets.

After the incubation, platelet levels of glutamate, aspartate, glutamine and asparagine in control donors were 34.8 ±14.6, 8.5 ± 2.3, 6.6 ± 1.3 and 9.7 ± 3.3 nmol/mg protein, respectively. In HD-patients, the equivalent values were 26.3 ± 3.4, 10.2 ± 2.3, 6.9 ± 1.4 and 13.0 ± 4.3, thus showing no significant differences as compared to controls. Radioactivity from ^{3}H-acetate was incorporated in all four amino acids with the specific activities of glutamine and asparagine being 25 and 100 fold lower than those of their immediate precursors glutamate and aspartate. ^{14}C-label derived from glucose could be demonstrated in glutamate and aspartate only. Platelets incubated at 4°C in all cases resulted in less than 10% of the incorporation observed at 37°C, indicating that the precursors were converted by active temperature-dependent processes. No differences between normal and HD-tissue were found in any one of the specific activities investigated.

The present findings suggest that there is no significant dysfunction in glutamate or aspartate metabolism in HD. While noting two important caveats- the possible existence of isozymes or different regulatory mechanisms for identical proteins in brain and peripheral tissue- we conclude that a genetic defect in glycolytic and tricarboxylic acid cycle enzymes is not likely to underlie HD-neuropathology.

This work was supported by a grant from the Wills Foundation.

299.12 SOLUBILIZATION OF HUMAN PLATELET MAO B WITH β-OCTYL GLUCOSIDE. M.Baron, A.S.Perumal and G.Cannova*. New York State Psychiatric Institute and College of Physicians and Surgeons, Columbia University, 722 West 168th Street, New York, N.Y. 10032

Biogenic amines are known to exert numerous physiologic effects and are involved in behavior modification. Monoamine oxidase (MAO)(E.C. 1.4.3.4.) is primarily responsible for the degradation of biogenic amines and thus determines the level of neurotransmitters in various tissues. Platelet MAO is implicated in the etiology of several psychiatric disorders, including schizophrenia. Since MAO is a membrane-bound enzyme, our immediate goal was to solubilize the enzyme. Current methods use Triton X-100 to solubilize liver MAO. Since Triton X-100 has low critical micellar concentration (0.2-0.3mM) it is difficult to remove this detergent completely even after extensive dialysis. Preliminary experiments, using a variety of detergents to extract the ^{3}H-pargyline bound MAO, showed that β-octyl glucoside was more potent in solubilizing human platelet MAO whereas Triton X-100 was most active against hamster liver MAO.

In order to solubilize and study human platelet MAO experiments were conducted to determine the influence of β-octyl glucoside on enzyme activity. Platelet MAO was assayed at the optimal concentration of 10 nmoles of phenylethylamine as substrate and inhibition was observed beyond this concentration. β-Octyl glucoside exhibited a concentration dependent inhibition of platelet MAO above 0.1%. However, this detergent is easily dialysable due to its high critical micellar concentration (25mM). More than 90% activity was recovered by dialysing overnight. In order to check whether the solubilized enzyme behaves similar to the membrane bound enzyme the inhibitory effect of increasing concentrations of deprenyl (MAO B inhibitor) and clorgyline (MAO A inhibitor) was tested with membrane bound enzyme as well as with dialysed preparations of Triton X-100 and β-octyl glucoside solubilized enzymes. The solubilized enzyme retained the characteristics of MAO B. Response to deprenyl was similar with the two preparations. However, the I_{50} values of β-octyl glucoside solubilized enzyme behaved closer to membrane bound enzyme with clorgyline whereas Triton solubilized enzyme showed less sensitivity. These results indicate that β-octyl glucoside can be used to better advantage in the solubilization and hence in the purification of human platelet MAO B.

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299.13 BIOGENIC AMINE CONTENT AND TURNOVER RATES DETERMINED BY LCEC WITH THE TL-9A CELL. C. Co*, J.E. Smith and J.D. Lane (SPON: P.C. Jobe). Psychiatry Research Unit, Department of Psychiatry, Louisiana State University Medical Center, Shreveport, LA 71130.

The content and utilization of the biogenic amines and their metabolites were determined simultaneously by injecting a crude tissue extract into a high pressure liquid chromatography system. The HPLC system utilized a reverse-phase μBondapak C18 column coupled with a TL-9A detector cell. This cell contained the working glassy carbon, auxiliary, and reference electrodes in the same compartment, which reduced the cell dead space to approximately 5 μl. The mobile phase was made up of 0.1M citrate-phosphate buffer, pH 3.5, containing 0.01% sodium octyl sulfate, which was filtered, degassed under vacuum, and methanol added to a final concentration of approximately 9%. Known amounts of standards [norepinephrine (NE), dopamine (DA), serotonin (5-HT), 4-hydroxy-3-methoxyphenylethyleneglycol (MHPG), 5-hydroxyindoleacetic acid (5-HIAA), homovanillic acid (HVA), dihydroxyphenylacetic acid (DOPAC), normetanephrine (NMe), tyrosine (Tyr), tryptophan (Trp), and 3,4-dihydroxybenzylamine (DHBA)] were injected into the system and the peak heights measured. The biogenic amines and their metabolites were identified by the retention time of the standards, and the peak heights determined the content. The utilization of the biogenic amines was determined by injecting radioactive precursors, 1.0 mCi [^{3}H]-tyrosine and 0.5 mCi [^{3}H]-tryptophan, through a jugular catheter which had been previously implanted. Sixty or 90 minutes prior to sacrifice, the animals were injected with the precursors, and the animals were immersed in liquid N_2. The brains were removed at -20°C, sectioned, and dissected into discrete areas. The tissue was pulverized in liquid N_2, extracted in 1N formic acid/1N acetone (v/v:15/85), delipidated with heptane/$CHCl_3$ (v/v:8/1) and concentrated by drying under dry N_2. The dried tissue extract was reconstituted with the HPLC mobile phase, 20 μl injected into the HPLC system, and the individual peaks were collected directly into individual counting vials for specific radioactivity determination. This method yielded 90% recoveries and utilizes a tissue sample as small as 8 mg wet weight. The content and turnover values are comparable to those previously reported. (Supported in part by NIH Grant MH-31835 and USPHS Research Grant DA-01999-04).

299.14 EFFECTS OF PATERNAL EXPOSURE TO CYCLOPHOSPHAMIDE ON THE F_1 PROGENY BRAIN CHEMISTRY. L. L. Hsu, P. M. Adams, J. Fabricant*, and M. S. Legator* Departments of Psychiatry and Behavioral Sciences and Pharmacology and Toxicology, The University of Texas Medical Branch, Galveston, Texas 77550.

The effect of exposing male rats (Fischer 344) to either acute (10 mg/kg) treatment with the alkylating agent cyclophosphamide (CP) or chronic (10 mg/kg 1 day X 5 days X 5 weeks) treatment prior to breeding with normal female animals was studied in the F_1 generation progeny. The present results focus on the changes produced in the brain enzymes of the F_1 progeny. Previous reports (Adams, et al., 1981) described behavioral effects produced in the F_1 progeny following comparable paternal CP exposure.

The F_1 progeny at 90 days of age were decapitated and brains were rapidly removed. Selected brain regions including cerebellum, corpus striatum, hippocampus, hypothalamus were dissected on ice and stored at -20°C until analyses. Glutamic acid decarboxylase (GAD), Choline acetyltransferase (CAT) and acetylcholinesterase (AChE) were assayed in the tissue homogenate of each brain region. Results showed that chronic paternal treatment of CP produced the following biochemical changes in the F_1 brain: (1) a marked decrease (30%, $P<0.03$) in cerebellar GAD activity but a significant increase (13%, $P<0.05$) in the striatal GAD activity of the F_1 females; (2) a marked decrease (34%, $P<0.03$) in cerebellar AChE but a significant increase (22%, $P<0.03$) in the hippocampal AChE activity of F_1 females and a 50% increase in hypothalamus AChE in the F_1 male rats.

Acute paternal treatment of CP affected brain enzyme activities of the F_1 offspring as follows: (1) It caused a 30% ($P<0.02$) decrease in hippocampal GAD activity in the F_1 males only. (2) It decreased cerebellar CAT activity significantly in both F_1 male and F_1 female offspring (26%, $P<0.009$; 26%, $P<0.03$ respectively); it caused a decrease in striatal and hypothalamus CAT activity only in the F_1 females (20%, $P<0.01$; 18% $P<0.03$) but an increase in hippocampal CAT activity in the F_1 females (14%, $P<0.05$); (3) The acute paternal treatment resulted in a 47% decrease in the cerebellar AChE activity in the male F_1 rats only whereas a 26% decrease in the hippocampal AChE activity in only the F_1 rats was observed.

These results indicate the ability of paternal exposure to a mutagenic chemical to induce brain enzymatic changes in their F_1 progeny. The extent of the induced changes in brain enzymes appears to be sensitive to the gender of the F_1 progeny.

299.15 PLASMA AMINO ACID LEVELS, BRAIN TRYPTOPHAN UPTAKE AND BRAIN SEROTONIN SYNTHESIS IN DIABETIC RATS. R.G. MacKenzie, R. Luboshitzky*, J.K. Goldman*, L.L. Bernardis, M.E. Trulson and J.H. Jacoby. V.A. Med. Center, SUNY, Buffalo, New York, 14215 and Department of Psychology, Princeton University, Princeton, New Jersey, 08540.

To determine the effects of diabetes on brain tryptophan (TRP) uptake and serotonin (5-HT) synthesis, male Sprague-Dawley rats received i.p. injections of either streptozotocin (SZ) 75mg/kg in citrate buffer pH 4.2 or buffer alone. The SZ-injected rats became diabetic as evidenced by polyphagia, polydipsia, polyuria and glucosuria. At 1 mo. post-treatment, diabetics (Ds) and controls (Cs) were injected i.v. with 100 μCi [^{3}H] TRP in 0.9% NaCl and groups of rats were killed 1, 2 and 3 hs post-injection. Plasma analysis showed that the Ds were hyperglycemic (D=466±12; C=105±3 mg%) and had vastly elevated branched-chain amino acid levels (VAL: D=706±74, C=132±11; ILEU: D=292±34, C=65±5; LEU: D=527±61, C=120±8 nmol/ml). Total TRP (bound + free) levels were low in Ds (D=41±2; C=81±3 nmol/ml) but free TRP levels did not differ (D=17±1; C=15±2 nmol/ml). In the brain, TRP was reduced by 38% in Ds (D=20±2; C=32±1 nmol/g) but 5-HT levels did not differ (D=2.71±0.04; C=2.81±0.06 nmol/g). As a relative measure of brain TRP uptake, the relative specific activity (RSA) (see Johnson and Dewey, JPET, 207, 140, 1978) of brain TRP SA/plasma TRP SA was determined for 1, 2 and 3 hs following injection of the [^{3}H] TRP tracer. Relative estimates of brain 5-HT synthesis were found by determining the RSA of brain 5-HT SA/brain TRP SA. These data are presented below:

	RELATIVE BRAIN UPTAKE BRAIN TRP SA/ PLAS TRP SA			RELATIVE 5-HT SYNTHESIS BRAIN 5-HT SA/BRAIN TRP SA		
t	1	2	3	1	2	3
D	.21±.02	.20±.02	.20±.03	6.4±0.3	7.7±0.6	7.5±0.3
C	.39±.03	.35±.02	.35±.03	3.4±0.2	5.9±0.3	5.4±0.3

We conclude that brain TRP levels are low in diabetic rats due to the high plasma levels of other neutral amino acids which can compete with TRP for transport across the blood brain barrier and that 5-HT synthesis is increased in diabetic brains to normalize 5-HT levels in the face of reduced substrate.

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299.16 GLUTAMIC ACID DECARBOXYLASE (GAD) ACTIVITY IN CEREBRAL BLOOD VESSELS: CHARACTERISTICS AND KINETICS. Edith Hamel*, Diana N. Krause and Eugene Roberts, Division of Neurosciences, City of Hope Research Institute, Duarte, CA 91010.

γ-Aminobutyric acid (GABA), an inhibitory neurotransmitter in the mammalian CNS, appears to be involved in cerebrovascular function, and its synthesis has been demonstrated in cerebral blood vessels. This study was undertaken to characterize vascular GAD activity. Cerebral blood vessels from fresh bovine brains were cleanly dissected from the pia-arachnoid membranes. Microartery and capillary fractions were prepared from brain parenchyma according to Reinhard et al. (Science 206: 85-87, 1979). GAD activity in the above preparations was measured in the 27,750g supernatant of crude homogenates by a radiometric assay using ^{14}C-glutamic acid as the substrate. The ^{14}C-GABA formed was separated from unreacted ^{14}C-glutamic acid by chelation of the latter substance with copper and passage through an anion exchange column. GAD activity of the pial vessel fraction was found to be B_6-dependent and showed pH optima at 6.5, 7.2 and 8.0 with apparent Km values for glutamate of 7.59, 4.04 and 4.61 mM, respectively. The Km value for brain GAD was found to be 4.60 mM at pH 7.2. GAD activity in both pial vessels and brain parenchyma was inhibited totally by 10 mM aminooxyacetic acid, while 10 mM 3-mercaptopropionic acid (3-MCPA) inhibited 100% of the brain activity but only 58% of the vascular activity. In contrast, cysteine sulfinic acid (CSA) at 10 mM inhibited the vascular activity (49%) significantly more than the cerebral one (22%). GAD activity from rat brain and rat pial vessels was precipitated by specific anti-GAD (mouse brain) antiserum. For comparison, bovine mesenteric arteries also were assayed, and the GAD activity and pharmacology in these peripheral vessels were similar to that of the pial vessels. GAD activity was enriched in cerebral microvessels, the highest value being found in the capillary fraction, which suggests an endothelial localization. These data show that both cerebral and peripheral blood vessels can synthesize GABA. The vascular enzyme appears kinetically and immunologically similar to brain GAD, although it is affected differently by 3-MCPA and CSA. Studies are in progress to elucidate the significance of the findings. Supported in part by USPHS grant NS 12116 from the NINCDS. E. Hamel was supported by a fellowship from the Medical Research Council of Canada.

299.17 MONOCLONAL ANTIBODIES TO DROSOPHILA MELANOGASTER CHOLINE ACETYLTRANSFERASE REACT AT THE ENZYME CATALYTIC SITE. G.D. Crawford*, P.M. Salvaterra, and J.R. Slemmon*. Division of Neurosciences, City of Hope Research Institute, Duarte, CA 91010.

We have recently reported the production of two monoclonal antibodies selective for Drosophila melanogaster choline acetyltransferase (ChAT; E.C. 2.3.1.6, Abstr. 81, Ann. Mtg. Am. Neurochem. Soc., 1981). These two antibodies were shown to directly inhibit the enzyme activity. The two cell lines were grown as ascites tumors in BALB/c mice and the IgG purified from fluids by adsorption and pH elution from a protein A Sepharose column. Scatchard analysis of enzyme inhibition curves using the purified antibodies revealed a single class of binding sites with respect to ChAT for each antibody. Affinities of 22.7 x 10^7 M^{-1} and 2.7 x 10^7 M^{-1} were determined for antibodies 1C8 and 1G4 respectively. We have used substrates and inhibitors of the ChAT enzyme to further elucidate the antigenic determinants involved in the immunological reaction. Choline does not protect the enzyme from inhibition by either antibody. Acetyl CoA, coenzyme A and adenosine diphosphate do, however, protect the enzyme from antibody inhibition. Under standard conditions protection from antibody inhibition is less for 1C8 antibody than for 1G4 consistent with the different antibody affinities for ChAT. Both antibodies thus appear to interact at or near the acetyl CoA binding site (ADP subsite) of the enzyme and not at the choline site. The observed interference of ADP for ChAT-antibody interaction has allowed the 1G4 (lower affinity) antibody to be used as an affinity resin for ChAT purification followed by elution with high concentrations of ADP. Quantitative aspects of the domain mapping and the use of 1G4 affinity resin for production of ChAT will be presented. (Supported by NS-12116).

299.18 MOLECULAR CHARACTERIZATION OF CHOLINE ACETYLTRANSFERASE FROM DROSOPHILA MELANOGASTER. J.R. Slemmon*, P.M. Salvaterra, and E. Roberts. Division of Neurosciences, City of Hope Research Institute, Duarte, CA 91010. (SPON: J. Vaughn)

Choline acetyltransferase (EC 2.3.1.6), the enzyme that catalyzes the formation of the important neurotransmitter acetylcholine, was previously purified from Drosophila melanogaster in this laboratory. Subsequent studies have centered on the enzyme's molecular composition and evidence has been obtained that a major portion of it is made up of two polypeptide chains.

The polypeptide composition of the denatured enzyme, determined by polyacrylamide gel electrophoresis in SDS (PAGE-SDS, 10% acrylamide and 0.07% bis-acrylamide), showed a major protein band at 54K and a lighter band at 67K daltons. The identification of both of these bands as ChAT was accomplished in two ways. First, two-dimensional analysis of the iodinated tryptic peptides from each band indicated that they had very similar primary structures and therefore were related proteins. Secondly, immunoprecipitation of purified ChAT, with two different inactivating monoclonal antibodies to Drosophila ChAT, yielded both the 67K and 54K dalton PAGE-SDS protein bands. Both protein bands were associated with the enzyme and the molecular weight of the major component, under denaturing conditions, was 54K daltons.

The non-denatured enzyme showed only one peak of activity at 67K daltons on Sephacryl S-200 chromatography (at either pH 5.9 or 7.2). Velocity sedimentation of the native enzyme in a 5 to 20% sucrose gradient also showed a single activity peak at 5.2s consistent with a molecular weight of 69K daltons. PAGE-SDS analysis of the 67K dalton Sephacryl peak showed both a 67K dalton minor polypeptide and a 54K major polypeptide.

This discrepancy in the molecular weight of the native enzyme compared to the major polypeptides of the denatured enzyme was further investigated using PAGE-SDS at a higher acrylamide concentration (15% acrylamide, 0.1% bis-acrylamide). Under these conditions, two previously overlooked protein bands could be resolved at 13K and 14K daltons. Two-dimensional analysis of the iodinated tryptic peptides from these bands showed that the 13K dalton band had three peptides that were found in the 67K but not in the 54K dalton band. This result indicates that the 13K dalton band may have originated from the 67K dalton band. The loss of the smaller (13K dalton) polypeptide from the 67K dalton protein under denaturing conditions would be expected to generate the 54K dalton protein. Supported by NS12116.

299.19 SYNAPTOSOMAL TRYPTOPHAN UPTAKE MECHANISMS AND DEVELOPMENTAL-GENETIC VARIATION. James A. Diez. School of Biology, Georgia Institute of Technology, Atlanta, GA 30332.

Intraneuronal tryptophan (trp) concentrations appear to be significant in regulating serotonin synthesis, but is transport of trp across the neuronal membrane a regulated step? This work aims to clarify one issue about the mechanism of trp transport, and to demonstrate two different conditions where transport kinetics vary physiologically.

Synaptosomes (P_2) were prepared from whole mouse brain. The initial rate of trp accumulation was assayed by incubating P_2 with varying concentrations of ^{3}H-trp in 400 uL of HBSS for 60sec at 37°. Uptake was stopped by centrifugation after cold dilution.

When a wide range of trp concentrations (e.g., 10 uM to 5 mM) is used, a biphasic Lineweaver-Burk plot is obtained; this is often interpreted as demonstrating two uptake systems with high and low affinity (typical Km's of about 25 uM and 1 mM). Although the Km of the "low affinity system" is far above physiological levels, attempts to eliminate it by iteratively increasing the passive diffusion term do not really straighten the plot to a single high affinity uptake system. Experimental evidence for exchange diffusion was obtained by incubating pre-labelled P_2 with non-radioactive trp, but kinetic models combining exchange with one saturable uptake system did not explain the experimental data. However, an accumulation of trp greater than predicted by diffusion and a single high affinity uptake system could occur if trp is bound intracellularly. Evidence to support this hypothesis was obtained in washout experiments with trp-loaded P_2, and more directly by Sephadex G25 chromatography of pre-labelled/disrupted P_2: a significant peak of radioactive trp eluted with the void volume, clearly separated from the peak of free trp.

Physiological variation in high affinity trp uptake was found during postnatal development and in adults from several strains of mice. First, a method for storing frozen P_2 for several weeks before assaying trp uptake was developed and validated. Freezing did not affect Km; although it lowered Vmax by about 50%, differences between preparations were neither created nor obscured. For example, fresh P_2 from adult C57BL/6 and Swiss albino mice had Km's of 23 uM and Vmax= 1.87 and 1.43 nmol/hr/mg prot.; after storage, the Km's were 21 uM, and Vmax= 1.02 and 0.68. Other mouse strains showing significant differences in uptake kinetics were: C57BL/10, BALB, and C3H. In adult mice, the major strain differences were found in Vmax. The only large differences in the Km for trp uptake were found during the first 3 weeks of postnatal development; the affinity of the uptake system for trp was higher during the period of rapid brain maturation. Genetic differences in maximum uptake rates developed more slowly. (BNS 7905601)

299.20 THE MINIMUM MOLECULAR SIZE OF THE ACETYLCHOLINESTERASE FROM MANDUCA SEXTA. L.H. Silver and D.J. Prescott*. Dept. of Biology, Bryn Mawr College, Bryn Mawr, PA 19010.

Previous work has demonstrated that the central nervous system of the hawk moth Manduca sexta provides a rich source of "true" acetylcholinesterase (AchE)(EC 3.1.1.7). Optimal extraction of the enzyme was obtained with a non-ionic detergent (1% Triton X-100, 0.5MNaCl) when compared with other extraction procedures. Density gradient centrifugation of detergent extracted enzyme on 5-20% linear sucrose density gradients demonstrated that the enzyme sedimented as a single peak whose sedimentation coefficient was dependent upon the amount of enzyme layered on top of the gradient. When greater than 0.02 I.U. were applied to the gradient, a sedimentation coefficient of 8.6S (205,000 D) was obtained and extrapolation to zero units produced a minimum value of 5.7S (110,000 D). Enzyme extracted in either sodium phosphate buffer (10mM, pH 7.4) or buffer plus NaCl (1.0M) followed by velocity sedimentation in the absence of detergent demonstrated a significant amount of pelleted activity and a species with a mean value of 17.5S (600,000 D).

Detergent extracted enzyme also demonstrated a concentration dependent size in gel filtration experiments on Bio-Gel A-5m. When less than 0.3 I.U. in 0.5 ml was applied to a column of Bio-Gel A-5m (0.9 x 83 cm) a single peak of enzymatic activity eluted with a calculated molecular radius of 41.8 ± 2.7Å (113,000 D). When the sample contained 0.3 to 1.3 I.U., two peaks of enzymatic activity were observed with molecular radii of 41.8 ± 2.7Å and 79.3 ± 3.5Å (322,000 D). These values were unaffected by gel filtration in the presence of 1% alpha-methyl-mannoside suggesting that the enzyme did not interact with the gel matrix. Finally, a single enzymatic peak, which eluted in the void volume, was observed when extracts prepared in 1.0MNaCl were subjected to gel filtration.

These results suggest that the sedimentation and gel filtration pattern of the AchE isolated from Manduca sexta exhibit a marked dependence on the concentration of the enzyme. In the presence of Triton X-100, 0.5MNaCl, the enzyme may exist as a soluble aggregate, which may dissociate to an active form with a minimum molecular weight of approximately 110,000 D. This minimum molecular weight has been verified using both sucrose density gradient centrifugation and gel filtration.

299.21 THE INTERACTION OF DESIPRAMINE WITH NORADRENERGIC SYSTEMS IN RAT CEREBRAL CORTEX. M.W. Dudley*, B.W. Siegel*, R.C. Ursillo* and N.L. Wiech, Department of Pharmacology, Merrell Dow Pharmaceuticals Inc., Cincinnati, Ohio 45215.

Studies have demonstrated that chronic administration of low doses of desipramine (DMI) leads to adaptive processes which result in increased norepinephrine turnover and a down-regulation of beta receptors in the brain. Other studies have shown that DMI (10 mg/kg, b.i.d.) for 4 days also produces a marked reduction in beta receptors, whereas, norepinephrine turnover, as measured by whole brain levels of MHPG-SO_4, is decreased. Since it has been suggested that down-regulation of beta receptors is a compensatory response to increased synaptic levels of norepinephrine, it is difficult to reconcile these latter findings. Therefore, we sought to determine whether levels of DMI in the cortex bore any relationship to beta receptor down-regulation and whether norepinephrine turnover in the cortex might correlate with receptor down-regulation more than turnover as measured in the whole brain.

DMI was administered at concentrations previously shown to reduce beta receptors. The levels of DMI in the cortex were determined 24 hr after the last dose. There was no detectable DMI in cortex isolated from rats treated for 28 days with 5 mg/kg, DMI or 4 days with DMI 5 mg/kg plus yohimbine 2 mg/kg b.i.d. After treatment for four days with DMI 10 mg/kg b.i.d., the tissue level was 4.14 $\pm$ 0.1 μg/g, 24 hr after the last dose. Following a single 10 mg/kg dose, the maximum tissue level of DMI (7.28 $\pm$ 1.14 μg/g) was reached 1 hr later, but at this time there was no reduction in the number of beta receptors. Thus, there was no relationship between down-regulation and DMI levels in the cortex.

In contrast to the reported effect on whole brain MHPG-SO_4, we observed that a single 10 mg/kg dose of DMI produced a transient decrease of MHPG-SO_4 (at 1 hr) followed by an increase at 4 and 7 hours. On the fifth day of treatment with DMI 5 mg/kg, there was a similar initial decrease of MHPG-SO_4 levels with an increase at later hours.

Thus our studies show that acute DMI treatment does increase NE turnover in the cortex and that these changes might be responsible for the subsequent down-regulation of the beta receptors in this brain region.

299.22 THE ROLE OF CITRATE AND ATP-CITRATE LYASE IN SYNTHESIS OF CYTOSOLIC ACETYL-CoA IN SYNAPTOSOMES OF MATURING BRAIN. A. Szutowicz*, J. Kabata* and H. Bielarczyk*. (SPON: I. Crawford). Dept. of Biochem., Univ. of Texas Health Sci. Ctr., and VA Medical Center, 4500 S. Lancaster Rd., Dallas, TX 75235 and Dept. of Clin. Biochem., Med. Acad., 80-211 Gdansk, Poland.

Citrate is known to be an important source of acetyl-CoA for lipid and acetylcholine synthesis in rat brain. However, the mechanisms regulating the supply of acetyl units through this pathway remain obscure. Therefore, the aim of these studies was to investigate the changes in endogenous citrate metabolism during brain development when marked changes in both lipid and acetylcholine synthesis take place.

The activities of pyruvate dehydrogenase and citrate synthase in rat brain synaptosomes increased about three times until the fourth month of life. However, pyruvate utilization increased only by 60% from 4.8 to 7.8 nmol/min/mg of protein. Activity of ATP-citrate lyase decreased from 9.2 to 6.9 nmol/min/mg of protein. Also the rate of citrate accumulation in synaptosomes of adult rat brain was less than half of that found in 1 or 10 day old pups (1.15 and 2.90 nmol/min/mg of protein, respectively). 5 mM ATP-Mg decreased (50%) and 1 mM (-)hydroxycitrate increased (two times) citrate accumulation in synaptosomes at any age. However, absolute values of metabolic flux of citrate to acetyl-CoA through ATP-citrate lyase step decreased from 1.40 to 0.49 nmol/min/mg of protein during brain maturation. The addition of 0.25 mM 3-bromopyruvate decreased pyruvate consumption and citrate accumulation by 70% but completely blocked citrate utilization by ATP-citrate lyase in every age group. These data indicate that production of acetyl-CoA in synaptosol may be regulated either by adaptative age dependent changes in the permeability of mitochondrial membrane to citrate or by the inhibition of intramitochondrial synthesis of acetyl-CoA.

Metabolic parameters of cerebellar synaptosomes were the same as cerebral ones. Therefore, the rates of metabolic fluxes of pyruvate to cytosolic citrate and acetyl-CoA are presumably the same in both cholinergic and noncholinergic nerve endings. On the other hand, ATP-citrate lyase activity in whole synaptosomal fractions was about 10 times and that in cholinergic ones over 100 times higher than the flux of citrate to acetyl-CoA in the synaptosomal cytosol. Assuming that the substrates for ATP-citrate layse (CoA, ATP, citrate) are not limiting then it is possible that citrate transport may be a regulatory step in acetyl-CoA formation in synaptosol. (This work supported by the Polish Academy of Sciences Project No. 10.4.2).

300.1 CO_2 SENSITIVE NEURONS IN THE CAT PONTINE RETICULAR FORMATION. M. E. Levine,* S. M. Carlton,* D. P. Becker, J. D. Miller* and R. L. Hayes* (SPON: L. Malis). Div. of Neurosurgery, Med. Coll. of Virginia, Richmond, VA 23298.

Previous studies utilizing stimulation and ablation techniques suggest a role for the region of the locus coeruleus (LC) in the modulation of cerebrovascular responses to alterations in arterial CO_2 tensions. The present study examined whether or not neurons in the region of the LC could encode changes in $PaCO_2$.

Subjects were 17 mechanically ventilated cats, maintained under N_2O anesthesia. $PaCO_2$ was manipulated through a range of normocapnea, hypocapnea, and hypercapnea by varying the CO_2 content of the inspired gas mixture. Spontaneous single unit firing rates were monitored prior to, during, and after $PaCO_2$ alterations. An additional 5 cats underwent bilateral extracranial high cervical vagotomies prior to recording brain stem activity.

Three types of neuronal responses were seen: 1) increase in firing rate to hypercapnea and/or decrease in firing rate to hypocapnea, n=16; 2) decrease in firing rate to hypercapnea and/or increase in firing rate to hypocapnea, n=14; 3) no change in firing rate, n=15. For neurons (n=15) recorded from vagotomized animals, the distribution of response types was similar. Neuronal responses were unrelated to systemic cardiovascular changes. Cells which responded to manipulations of $PaCO_2$ clustered anatomically at the level of the principal nucleus of LC. Type 1 responses were seen in the more medial portion of the LC region. Type 2 responses occurred more laterally in the parabrachial region.

These data are consistent with the hypothesis that neurons in the region of the LC may be involved in cerebrovascular responses to changes in $PaCO_2$. Also, it appears that information from peripheral chemoreceptor afferents is not necessary for the encoding of arterial CO_2 tensions by neurons in the vicinity of LC in the cat. Supported by NIH Grant # NS 12587.

300.2 DYNAMIC RESPONSES OF CAT PULMONARY STRETCH RECEPTORS TO PRESSURE CHANGES DURING SPONTANEOUS BREATHING. C.K. Knox and M. Passatore*, Lab. of Neurophysiol., Univ. of Minn., Minneapolis, MN 55455.

The discharges of single slowly adapting pulmonary stretch receptors were recorded from small filaments of the cervical vagus nerves of 24 anesthetized, tracheotomized cats while the animals breathed spontaneously on a servo-controlled respirator. Data collected included conduction velocity and volume threshold of each unit, and the changes in firing rate to step increases in tracheal pressure superimposed on normal breathing movements. Pleural pressure and lung volume were also recorded to measure total dynamic compliance of the lungs and chest wall during the inflations.

Conduction velocities ranged from 14 to 70 m/s (average for 180 units = 35 m/s) and were uncorrelated with either volume threshold or dynamic characteristics of the unit. Thus, fiber size is not a determinant of discharge pattern. Dynamic response data were obtained from 29 receptors which had a spontaneous discharge at resting lung volume and which could be recorded long enough to complete the analysis. A series of step inflation pressures of increasing size were applied during either inspiration or expiration, and the response of the unit was computed as the change in discharge rate from that of a spontaneous control breath. Both the unit response and input pressure change were Fourier transformed to obtain the frequency response characteristic of the receptor (Bode plot). The dynamic characteristics of the stretch receptor were found to differ substantially during the two phases of the respiratory cycle: During expiration, static sensitivity is proportional to transmural pressure, while velocity sensitivity increases nonlinearly with pressure step size. During inspiration, static sensitivity decreases with time into inspiration, some units accomodating completely to the added inflation. Velocity sensitivity is, again, nonlinearly related to step size.

The results demonstrate that both the static sensitivity and adaptation properties of pulmonary stretch receptors are highly dependent on the size and time of application of lung inflation during the respiratory cycle. The difference in dynamic characteristics between the inspiratory and expiratory phases suggests that the dynamic compliance of the airways containing the receptors becomes progressively less with time during inspiration.

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300.3 INTERACTIONS AMONG MEDULLARY INSPIRATORY(I) NEURONS. Jack L. Feldman and Dexter F. Speck*, Departments of Physiology and Anesthesia, Northwestern University, Chicago, Il 60611

Interactions among medullary I neurons were studied by correlating the activity of simultaneously recorded pairs of neurons in chloralose-urethane, gallamine paralyzed, thoracotomized cats artificially ventilated by a cycle-triggered pump. Two microelectrodes, mounted on a custom fabricated arc that allowed independent placement of each electrode along with precise determination of interelectrode distance (±50 um), were inserted into the regions of I modulated unit activity in the ventrolateral nucleus of tractus solitarius(NTS) and/or nucleus retroambigualis(NRA). Contralateral phrenic and ipsilateral recurrent laryngeal nerve activities were also recorded. The results of our preliminary analyses are indicated in the TABLE below [Peaks(troughs) represent the presence of bins with significantly increased(decreased) counts within 5 msec of Ø lag]. It is important to note that: 1) Over 25% (63/239) of the unit pairs showed some evidence of short-latency interaction; since extracellular spike-spike crosscorrelations tend to indicate the presence of those interactions with the largest and fastest rising PSPs, but not those with small or slowly rising PSPs, these results suggest extensive interactions among medullary I neurons. 2) All troughs occurred on one side of Ø lag only, which suggests an inhibitory projection from one neuron to the other in a given pair; this is the first evidence of inhibition between identified pairs of I neurons. (Supported by NIH Grants HL-00554, HL-23820 and NIMH Grant T32-MH16097.)

TABLE OF UNIT-UNIT CORRELATION ANALYSES

	NTS-->NTS PAIRS	NTS-->NRA PAIRS	NRA-->NRA PAIRS	TOTALS
FLAT	24	42	110	176
PEAK	9	6	37	52
TROUGH	Ø	2	9	11
TOTALS	33	50	156	239

300.4 RESPONSES OF PHRENIC (Phr) AND RECURRENT LARYNGEAL (RL) NERVES TO MICROSTIMULATION AND MULTIPLE MICROLESIONS IN THE VENTRAL RESPIRATORY GROUP (VRG). Dexter F. Speck* and Jack L. Feldman (Spon. R.C. Rogers). Departments of Physiology and Anesthesia, Northwestern University, Chicago, Il 60611.

The role of VRG neurons in the region of nucleus retroambigualis in the medulla in the generation of respiratory pattern was investigated by examining the effects of microstimulation and multiple microlesions on ipsilateral RL and ipsi- and contra-lateral Phr nerve activities. These experiments were conducted in chloralose-urethane anesthetized, thoractomized, gallamine paralyzed and artificially ventilated cats. In almost all areas of the VRG where concentrated respiratory neuronal activity was recorded from tungsten microelectrodes, single shocks (10-25 uA, 80 uS) produced short-latency (4-8 mS) ipsi- and contra-lateral Phr inhibition lasting 5-25 mS followed by a brief post-inhibitory excitation. A similar pattern of response with a shorter onset latency was also seen in the discharge of inspiratory neurons in the region of the ventrolateral nucleus of tractus solitarius (vlNTS). Single shocks also elicited a short latency (3-8 mS) RL excitation lasting 4-12 mS which was followed by a period of inhibition. At some VRG sites located primarily within 0-2 mm rostral to the obex, low frequency (10-30 Hz) respiratory cycle triggered stimulation produced premature inspiratory termination and/or expiratory prolongation. Subsequent to recording spontaneous activity and the response to microstimulation at each VRG site, a 300-700 um microlesion was made by passing 10-20 uA for 30-60 seconds. After making a lesion, the electrode was removed from the brainstem and reinserted at another site within 500 um. We rarely noticed any transient or long-term changes in rhythm of Phr discharge subsequent to lesion, even after numerous previous lesions at adjacent sites; this is in contrast to the marked transient and sometimes long-term changes in Phr activity that follow small lesions in the nucleus parabrachialis medialis. Multiple microlesions in the rostal most part of the VRG near the retrofacial nucleus ("Botzinger Complex") did not alter Phr discharge [2 cats]; neither did extensive lesioning of the ipsilateral VRG [5 cats] nor extensive bilateral lesioning [2 cats]. In one cat, extensive bilateral lesions in both the VRG and vlNTS failed to significantly alter Phr respiratory patterns. These results suggest that most of the respiratory neurons within the VRG do not play a significant role, if at all, in the generation of respiratory rhythm; rather that their major function is to influence to development of activity in the inspiratory or expiratory phase of a given respiratory cycle. (Supported by NIH Grants HL-00554, HL-23820 and NIMH Grant TG-MH16097.)

300.5 EFFECT OF 5,7-DIHYDROXYTRYPTAMINE (5,7-7HT) ON BRAIN THYROTROPIN RELEASING HORMONE (TRH) AND THE RESPIRATORY RESPONSE TO TRH.
G. R. Breese, C. D. Kilts, K. Beaumont and R. A. Mueller.
Neuropharmacology Laboratory, BSRC, University of North Carolina, Chapel Hill, NC 27514

Previously we have shown that treatment of 3 day old rats with 5,7-DHT plus desmethylimipramine or pargyline severely reduces CNS serotonin without altering dopamine or norepinephrine content. The present experiments were designed to explore the effect of similar treatment on brain TRH and the control of respiration.

In 3 day old rats administration of 50 µg 5,7-DHT intracisternally produced a significant decrease in extrahypothalamic immunoreactive TRH, while not changing hypothalamic peptide content. Respiratory frequency was significantly decreased in treated animals when measured at 3-5 days intervals after treatment.

When lightly anesthetized with halothane in oxygen and placed in a plethysmograph as adults 5,7-DHT treated rats still maintained a reduced minute ventilation as a result of a reduction in respiratory frequency. Moreover, the mechanical response to CO_2 exposure indicated a decreased sensitivity to CO_2. Vagotomy in control rats did not alter basal $PaCO_2$, whereas in 5,7-DHT treated CO_2 sensitivity appeared reduced further. Animals given TRH 1-30 µg i.c.v. evidenced an increase in minute ventilation. The relative increase in mechanical response to TRH in 5,7-DHT treated rats was greater than that of control animals.

The decreased ventilation of 5,7-DHT treated rats, and the enhanced responsiveness of these rats to TRH-induced respiratory stimulation suggest that TRH related peptides may play a role in the central integration of respiration.

Supported by USPHS grant AA 02334; and HD 03110.

300.6 CENTRAL PROJECTION OF SINGLE SLOWLY ADAPTING PULMONARY STRETCH RECEPTORS (PSRs) TO THE REGION OF THE TRACTUS SOLITARIUS (TS) AS DETERMINED BY SPIKE TRIGGERED AVERAGING (STA).
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The central projection of single PSRs to the region of the TS is not known. When HRP was injected into the right main bronchus, HRP reaction product was distributed primarily lateral and to a lesser extent dorsomedial to the TS (Kalia and Mesulam, J. Comp. Neurol. 193:467, 1980). We wished to determine which regions received terminations of single PSRs.

The central terminations of ten PSRs were mapped utilizing STA of extracellular fields (Munson and Sypert, J. Physiol. 296:315, 1979). The extracellular activity of single PSRs was recorded from the nodose ganglion in 10 anesthetized, paralyzed, artificially ventilated cats. The receptor endings were located in either the right lung or its main airways. PSRs were classified based upon axonal conduction velocities and adaptation indices. Medullary extracellular potentials were recorded with a low impedance tungsten electrode. The occurrence of a spike generated in the nodose ganglion triggered an averaging computer. The resultant average of the medullary field potentials was attributable to the terminations of the single PSR. Multiple tracts in a mediolateral plane were made at 0, +1, and +2 mm rostral to obex. Small lesions were placed in the medulla to locate recording sites; subsequently, medullary tissue was excised for histology and electrode track reconstruction.

A brief diphasic wave was presumed indicative of the presynaptic terminal potential. In the case of 4 PSRs this wave form preceded a longer lasting negative potential which has been ascribed to the postsynaptic focal synaptic potential. These focal potentials were seen at +1 mm rostral to obex. In this plane focal potentials were seen dorsomedial (2 PSRs) and ventrolateral (2 PSRs) to the TS. The maximum amplitudes of terminal potentials at +1 mm rostral to obex were recorded dorsomedial (4 PSRs), dorsolateral (4 PSRs), and ventrolateral (2 PSRs) to TS.

This is the first electrophysiologic evidence that single PSRs project to several subnuclei of the TS and not merely to the dorsal respiratory group associated with the ventrolateral nucleus. In addition, the magnitude of the central projection of PSRs is greater at +1 mm rostral to the obex than at 0 or +2 mm rostral of obex. (Supported by USPHS grant NS 14847 and RCDA NS 00378).

300.7 PROJECTIONS TO AND FROM THE EXPIRATORY NEURONAL POPULATION OF THE CAUDAL MEDULLA IN THE CAT.
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Projections to and from the expiratory (E) neurons of the ventral respiratory group (VRG) in the caudal medulla were studied following the iontophoretic application of horseradish peroxidase (HRP) through a microelectrode (4 µ tip diameter) placed in a region where abundant E neuron activity had been recorded by the same electrode. Following a survival time of 48-52 hours, the brain stem, nodose ganglia and spinal cord were processed for HRP reaction product using the tetramethyl benzidine method in order to visualize both anterogradely and retrogradely transported HRP. Injection sites, 250µ to 500µ in diameter, were located in the caudal medulla in the region of the nucleus retroambigualis - nRA, (2.3 - 2.9 mm caudal to the obex, 2.5 -3.2 mm lateral to midline, 2.3 -3.2 mm below the dorsal surface). Retrogradely labeled neurons were found in: (a) the ipsi- and contralateral ventrolateral nucleus of the tractus solitarius extending 1.0-2.5 mm rostral to the obex, corresponding to the dorsal respiratory group (DRG); (b) the ipsi- and contralateral regions of the rostral medulla (3.0-4.5 mm rostral to the obex) located in the vicinity of the nucleus ambiguus, corresponding to the rostral portion of the VRG where abundant respiratory activity has been found. A few labeled neurons were found scattered in the reticular formation between the DRG and the rostral VRG. No labeled neurons were found in the contralateral nRA at the level of the injection site. HRP labeled fibers could be followed from the injection site to the contralateral ventral funiculus. These findings confirm the localization,by earlier antidromic mapping studies, of the projections to and from E neurons in the nRA. In addition our study demonstrates the bilateral efferent projection from the rostral VRG to the nRA. No labeling was found in the nodose ganglion indicating that vagal afferents do not project directly to the nRA.

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300.8 RESPIRATORY INHIBITION ASSOCIATED WITH TRANSIENT HYPERTENSION DURING SLEEP AND WAKING IN UNRESTRAINED CATS.
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Numerous studies, primarily in anesthetized, non-intact animal preparations, have demonstrated that transient hypertension produces respiratory inhibition and apnea via a sino-aortic baroreceptor reflex. In order to examine the influence of induced blood pressure elevation in an unanesthetized preparation, 5 adult cats were instrumented for chronic recording of sensorimotor and hippocampal EEG, EOG, EKG, crural diaphragm EMG, and lateral geniculate nucleus activity. Catheters were advanced into the aorta and vena cava from the femoral vessels. Electrode leads and catheters were terminated at the top of the head, allowing convenient connection to the recording apparatus. All cats were allowed at least one week of post-surgical recovery before recording sessions were initiated. At the time of recording, each cat was fitted with a piezo-electric strain gauge for measuring chest wall movements. Electrical and catheter connections were made via a cable which allowed free movement within a shielded recording chamber. Aortic pressure was recorded from the arterial catheter. Small injections of phenylephrine (typical dose: 30 ug phenylephrine in 0.3 ml isotonic saline over 3 sec) were administered via the caval catheter with a sage syringe pump. Transient hypertension was thus produced during quiet wakefulness (AW), quiet sleep (QS), and rapid eye-movement (REM) sleep. Physiological signals and a time code were recorded on polygraph paper and on magnetic tape. Behavioral observations were entered on the paper record. Intervals between the onset of inspirations were derived by measuring the time between the onset of chest wall expansions and by measuring the time between onsets of diaphragmatic EMG activity. Transient hypertension produced measurable slowing of respiratory rate during all states in all cats. The most pronounced slowing, including 5-10 second apneas, occurred following pressor injections which evoked arousals from QS. Diaphragmatic EMG amplitude and burst duration also transiently decreased during some periods of respiratory slowing. Such a decline in diaphragmatic activity together with a reduction in inspiratory duration suggests that tidal volume may be decreased. Furthermore, a decrease in respiratory rate and in inspiratory duration may indicate a delay in the onset of inspiration. This pattern of respiratory inhibition with transient blood pressure elevation may be an important factor in the pathophysiology of sleep apnea syndromes.

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300.9 EFFECTS OF A ROCKING BED ON BREATHING DURING SLEEP IN KITTENS. D.J. McGinty, M.S. London*, M. Stevenson*. Veterans Administration Medical Center, Sepulveda, CA, UCLA School of Medicine, Los Angeles, CA.

Disorders in breathing associated with sleep may result, in part, from deficiency in a brain stem arousal system. The medial reticular formation (RF) has been associated both with arousal mechanisms and with facilitation of breathing and is a possible substrate of the arousal effect on breathing. The vestibular system is known to have a widespread projection to medial RF neurons and was found, in acute studies, to modulate breathing. The present study investigated the effect of vestibular stimulation produced by a rocking bed on breathing and RF unit discharge during sleep in kittens.

Six 20 to 30 day old kittens were surgically prepared for chronic recording of standard sleep parameters and diaphragmatic EMG activity. Integrated diaphragmatic EMG activity was measured in both NREM and REM sleep during baseline and rocking at several frequencies. Three additional kittens were prepared with traecheal fistulas allowing insertion of a tracheal tube for pneumotachographic measurement of tidal volume. The "rocking bed" consisted of a suspended cage with a pendulum length of 40 to 60 cm and a lateral motion of 10 to 20 cm driven by a crank on a variable-speed motor.

Rocking produced a facilitation of diaphragmatic EMG activity in both NREM and REM in 5 of 6 kittens. Maximum facilitation was achieved at rocking frequencies of 0.5 and 0.55 times the baseline respiratory rate, with reduced or no facilitation at higher and lower frequencies. In kittens with fistulas minute volume was increased by 10-18% in NREM and REM during rocking compared to baseline.

Additional kittens were prepared for chronic microwire unit recording in the pontine RF. Cells with tonic activity showed modulation by rocking in NREM and tonic REM, but not phasic REM.

We conclude that vestibular stimulation produced by a rocking bed is a effective stimulus to breathing during sleep in kittens. We speculate that mechanism involves activation of medial RF neurons which have connections to brain stem respiratory neurons. Optimal facilitation occurred when the frequency of maximum tilt and acceleration (2 per rocking cycle) was equal to or slightly above the normal NREM breathing rate.

300.10 HISTOCHEMICAL PROPERTIES OF THE CAT DIAPHRAGM: DIFFERENCES BETWEEN ABDOMINAL AND THORACIC SURFACES. G. C. Sieck, R. R. Roy, R. M. Harper and V. R. Edgerton. Dept. of Anatomy, Neuromotor Control Lab. and Brain Research Institute, UCLA, Los Angeles, CA 90024.

The mammalian diaphragm is composed of anatomically distinct areas (sternal, costal and crural), reportedly varying in proportions of histochemical fiber types. Such regional variations in fiber type composition suggest possible differences in motor activation and function between diaphragmatic areas. The present study compared the fiber type proportions between the abdominal and thoracic surfaces in each part of the diaphragm. Diaphragms of 3 cats (2.5-4.0 kg) were excised and laid flat on moistened paper. 5 x 10 mm segments of muscle were cut from the sternal, right and left costal and right and left crural areas. After quick freezing in liquid nitrogen and isopentane, fiber types were categorized as fast twitch oxidative glycolytic (FOG), fast twitch glycolytic (FG) or slow twitch oxidative (SO) using myofibrillar ATPase, reduced nicotinamide adenine dinucleotide diophorase and α-glycerophosphate dehydrogenase staining procedures. Populations of 100 muscle fibers were sampled from the thoracic and abdominal surfaces of each region of the diaphragm.

Within each area of the diaphragm, the most apparent differences in fiber type composition occur between the abdominal vs. thoracic surface (abdominal-40.6%+7.3 fast (FG+FOG) vs. 59.4%+7.3 slow (SO); thoracic-70.5%+4.97 fast vs. 29.5%+3.8 slow). Thus, there was a gradation in the proportions of fiber types from predominantly fast fibers on the thoracic surface to predominantly slow fibers on the abdominal surface. In the center of the muscle, fiber type proportions were mixed. Unlike previous reports, there was little variation in fiber type composition between different diaphragmatic areas (proportions of fast fibers ranging from 34.7% to 53.0% on the abdominal surface and 63.0% to 75.3% on the thoracic surface). Variations in fiber type proportions between abdominal and thoracic sides suggest possible differences in the order of activation of motor units on each side. Previous studies have indicated that SO motor units in the diaphragm are recruited first during inspiration. The increased number of SO units on the abdominal side of the diaphragm suggests that initial activation of this side is important in inspiratory efforts. Motor units in the crura have been found to discharge continuously. Low threshold motor units (eg. SO units) firing continuously may be necessary to oppose the action of negative intrapleural pressure on the diaphragm. Delayed activation of fast-twitch motor units on the thoracic side of the diaphragm may facilitate this muscle's downward excursion during inspiration because of the higher force developed by these motor units. Supported by NIH grant HL 22418-04 and AHA Research Fellowship Award AHA-GLAA 659 to G.C.S.

300.11 ARE AMINO-ACID TRANSMITTERS IMPLICATED IN THE CENTRAL GENERATION OF THE RESPIRATORY RHYTHM ? AN IONTOPHORETIC STUDY OF MEDULLARY NEURONS. M. Denavit-Saubié, J. Champagnat and M.P. Surun (SPON : J.V. Passonneau). Laboratoire de Physiologie Nerveuse, CNRS, 91190 Gif-sur-Yvette, France.

Respiratory neurons located in the cat medulla present a rhythmic pattern of discharge in relation to the diaphragmatic activity. In these neurones bursts of spikes are spontaneously repeated and thus can be studied without artifical stimulation. The rhythmicity of these neurones arises from the periodic activity of reciprocal inhibitions and mutual excitatory connexions.

The pharmacology of these synapses was studied at the unitary level using microiontophoresis and pressure applications utilizing a mutibarrelled micropipette. Neurones recorded extracellularly in the anaesthetized bivagotomized cat were located histologically in the ventrolateral nucleus of the tractus solitarius and in the vicinity of the ambiguus nucleus. Inspiratory neurones were characterized by their discharge frequency which progressively increased during inspiration and disappered during expiration. All the neurons were sensitive to excitatory (L-Glu) and inhibitory (Gly, GABA) amino-acids.

Neuronal inhibitions which occur during expiration were specified using antagonists of inhibitory amino-acids. GABA and muscimol-induced inhibitions were blocked by bicuculline or picrotoxine, while, Gly, β-Ala or Taurine-induced inhibitions were selectively susceptible to strychnine. Two pharmacologically different inhibitions were found to be involved during expiration. 1) Strychnine reduced inhibitions that stop inspiratory discharge at the beginning of expiration. 2) Bicuculline or picrotoxine were active on inhibitions that maintain a low neuronal excitability throughout expiration. It can be concluded from these experiments that both glycine-like and GABA-like transmitters act complementarily to trigger and control the expiratory phase.

Neuronal excitations which occur during inspiration were reduced by local application of opiates and opioid peptides. These reductions of inspiratory activities were found to be antagonized by naloxone. Similarly L-glutamate induced neuronal excitations were stereospecifically reduced by opioids application. It can be presumed that transmitters involved in respiration related excitations, like L-Glu effects, are susceptible to modulation by endogenous peptides (Supported by CNRS grant n° 3586).

400 INTRACELLULAR PROTEIN KINASE INJECTION SIMULATES BIOPHYSICAL EFFECTS OF ASSOCIATIVE LEARNING ON HERMISSENDA PHOTORECEPTORS. J. Acosta-Urquidi, D. L. Alkon, J. Olds*, J. T. Neary, E. Zebley*, and G. Kuzma*. Lab. of Biophysics, NINCDS-NIH, Marine Biological Lab., Woods Hole, MA 02543.

Primary membrane changes (increased input resistance and enhanced depolarization following a light step) intrinsic to the Type B photoreceptors of the eye of the nudibranch mollusc Hermissenda crassicornis were observed to persist and to parallel behavioral changes on days following conditioning intact animals with paired light and rotation but not randomized stimuli (Crow and Alkon, Science 209: 412, 1980; West, Barnes and Alkon, Biophys. J., 33:93a, 1981). Changes in specific phosphoprotein bands (20,000 and 23,000 daltons) have also been detected in the eyes following acquisition of this long-term behavioral change (Neary, Crow and Alkon, Neurosci. 6:591, 1980). As a first test of the hypothesis that the observed changes in Type B cell membrane properties are due to changes of protein phosphorylation, protein kinase was injected into single axotomized Type B cells which were without impulses or synapses. Iontophoretic injection (-0.3 -0.9 nA, 30-60 sec) of the catalytic subunit of fresh protein kinase (CPK, bovine heart, Sigma, 1.38 units/µl in a carrier solution containing 0.95 M KAc and .05 M Tris buffer, pH 9.8) into Type B cell somata caused an increased input resistance (decreased slope conductance) compared to pre-injection values of 53% ± 2.9 SEM ($p<.005$, N=7), measured with negative pulses (0.1, 0.2, 0.3 nA, 400 msec) and of 29.2% ± 3.5 ($p<.01$, N=6) for positive pulses. Transient hyperpolarization (2-8 mV) followed CPK injection in 16/20 cells. CPK injection also enhanced the magnitude (240% ± 33, $p<.005$, N=6) of the long-lasting depolarization (LLD) (measured 30 sec after light off) which follows a 30 sec light step (10^4 ergs/cm^2-sec). Identical control iontophoretic injections of the carrier solution alone, inactive (>2-3 days old) CPK solutions, heat-inactivated CPK solutions or KAc did not affect input resistance and LLD (N=15). Bath application of 8-PCPT cAMP and 8 BT cAMP (5×10^{-4}M) also enhanced the LLD (105%, 9/9 cells, $p<.005$). The 8-PCPT cAMP (4/4 cells) also caused a clear increase of frequency and amplitude of the quantal voltage fluctuations known as "bumps". These results suggest that the observed phosphorylation changes provide a biochemical step for the production of the biophysical changes observed in the Type B cells during associative learning of Hermissenda. A possible causal sequence for this process might consist of (1) repeated stimulus pairing, (2) cumulative membrane depolarization of the Type B cell, (3) increased cyclic-AMP, (4) increased protein kinase activity, (5) increased protein phosphorylation, (6) decreased dark membrane G_K, (7) enhanced LLD response and (8) reduced positive phototaxis.

401 ^{3}H -ISOGUVACINE AS A MARKER FOR GABA NEURONS IN THE RETINA. E. Agardh* and B. Ehinger. Department of ophthalmology, University of Lund, Lund, Sweden.

Retinas from rabbit, goldfish and guinea-pig were exposed to ^{3}H -GABA, ^{3}H -muscimol, ^{3}H -nipecotic acid and ^{3}H -isoguvacine either by intravitreal injection in vivo or by incubations in a balanced salt solution and the distribution of radioactivity was then studied with autoradiography. All substances labelled a similar set of presumed amacrine cells. Incubating at 0°C, in 10^{-3}M ouabain, or in 10^{-3}M GABA inhibited the labelling by ^{3}H -muscimol whereas bicuculline 10^{-4}M, and glycine 10^{-3}M were less efficient as blockers. The result is interpreted as a neuronal uptake of ^{3}H -muscimol rather than as a GABA receptor binding. All the substances except ^{3}H -isoguvacine also labelled glia to such a degree that neuronal labelling was often disguised in rabbits and goldfish. Glial labelling by muscimol was less pronounced in guinea-pig. ^{3}H -isoguvacine gave a strong labelling of cells with the distribution of GABA neurons and only little glial labelling. ^{3}H -isoguvacine is actively accumulated by the retina and is releasable with 10 uM K^+.

402 DIVALENT IONS REGULATE ^{3}H-SPIROPERIDOL BINDING IN HUMAN PREFRONTAL CORTEX: DESENSITIZATION OF K_D 0.2 nM SITES DEPENDENT ON PRESENCE OF DIVALENT ION AND TEMPERATURE. A.C. Andorn*, J.J. Ondrejka*, L.H. Matt*, J.D. Reimnitz*, (SPON: J. Brodkey). Dept. of Psychiatry and Div. Hum. Genetics, Case Western Reserve Univ. Schl. of Med., Cleve. OH 44106.

^{3}H-Spiroperidol binds specifically to human postmortem prefrontal cortex. The association of the ^{3}H-ligand to the binding site at 37°C is not classical, in that an initial steady state is reached by 4 min. followed by a loss in specific binding (>30%) until a final steady state is reached at 50 +/- 10 min. The loss in binding appears related to a 3-fold decrease in the B_{max} of a single set of sites K_D 0.2 nM, between 4 and 60 min. The loss of binding over time is most rapid in the presence of divalent ion $Ca^{++} > Mg^{++} > Ni^{++}$. The loss of binding over time is also seen in the presence of monovalent ion, but both the rate and the % loss are reduced, as compared to divalent ions. The absoluted amount of specific binding at the initial steady state is greater in the presence of monovalent rather than divalent ion. Saturation analyses confirm these findings, showing that the increase in binding observed is related to an increase (2-fold) in the B_{max} of the sites K_D 0.2 nM at the initial steady state in the presence of monovalent as compared to divalent ion. Taken together, these data suggest that in the presence of divalent ion 50 % or more of the sites are already desensitized by the initial steady state (4 min), while in the presence of monovalent ion substantially less of these are desensitized. At 25°C, association studies are mroe classical, with a single steady state apparently reached by 20 min. and only a 10% loss in binding occurring between 20 and 60 min. The dependence of the rate of desensitization upon divalent ion and temperature, suggests that this event may be, or may be coupled to, an enzymatic event.

403 PATTERNS OF CONNECTIVITY AND SYNAPTIC STRENGTH IN CELLS OF THE SIXTH ABDOMINAL GANGLION POST-SYNAPTIC TO THE ABDOMINAL STRETCH RECEPTOR NEURONS OF THE CRAYFISH. Michael Bastiani and Brian Mulloney. Dept. of Zoology, Univ. of Calif. at Davis, Davis, CA 95616.

The SR neurons from all abdominal segments send axons into the ventral nerve cord and then posteriorly to the last (sixth) ganglion. The ipsilateral and contralateral SR neurons associated with abdominal ganglia one through four were isolated and selectively stimulated to determine the pattern of connectivity and synaptic strength between the SR neurons and cells in the sixth ganglion. A posterior to anterior gradient of connectivity and synaptic strength was discovered. For example, approximately 70% of the cells (N=100) post-synaptic to the SR neurons received EPSPs (mean=.35 mv) from the ipsilateral SR neurons of the fourth abdominal ganglion, while only 30% of the cells received EPSPs (mean=.11 mv) from the ipsilateral SR neurons of the first abdominal ganglion. In addition, connectivity and synaptic strength was greater on the ipsilateral side for any abdominal ganglion, e.g., the contralateral SR neurons of the fourth ganglion made connections with 43% of the post-synaptic cells (mean=.18 mv). Finally, a small minority of cells (4%) post-synaptic to the SR neurons exhibited the opposite pattern of connectivity and synaptic strength.

Cells exhibiting the normal pattern of connectivity and synaptic strength were identified as motor neurons and projection interneurons. A single cell with a reverse gradient in connectivity and synaptic strength was identified as a local interneuron.

It is proposed that the sequence of the SR neurons ingrowth into the sixth abdominal ganglion during development (the nearer SR neurons are assumed to arrive in the sixth ganglion earlier) and the time of maturation of the post-synaptic cells (motor neurons are assumed to mature earlier than local interneurons) can account for the patterns of connectivity and synaptic strength observed in the cells post-synaptic to the SR neurons.

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404 PROPERTIES OF MOTOR UNITS IN DYSTROPHIC MOUSE EDL. D.S. Bateson* and D.J. Parry* (Spon. T. Picton) Dept. of Physiology, University of Ottawa, Ottawa, Canada.

The extensor digitorum longus muscle (EDL) of the mouse consists almost entirely of type II fibres (fast-twitch). The motor unit composition of this muscle in both normal (C57BL) and dystrophic (dy^{2J}/dy^{2J}) mice has been examined. 46 motor units have been investigated in 11 normal mice of average age 13 wks and weight 31 gms. 22 motor units have been studied in 6 dystrophic mice of average age 12 wks and weight 22 gms. Anaesthesia was by Na pentobarbitone 75 mg/kg. I.P. initially, supplemented via an indwelling peritoneal cannula as required. Complete denervation of all muscles except EDL, was followed by laminectomy from L_1 to L_5. Motor units were isolated functionally by ventral root splitting of the L_3 & L_4 roots. Units were determined to be single by all or none contraction, EMG and antidromic ventral root action potential. Motor unit tetanic tension, expressed as a percentage of whole muscle tension for both normal and dystrophic mice showed little variation, 1.81% to 11.4% (mean value ± SD = 4.6 ± 2.2%) and 2.1% to 17.5% (mean ± SD = 6.9 ± 4.0%) respectively. This suggests that the C57 EDL contains about 22 motor units while dystrophic EDL is composed of about 15. Normal mice demonstrated a distribution of motor unit times to peak twitch tension that was unimodal. The mean value was 8.8 ± 1.2 msec (standard deviation). In EDL of dystrophic mice the mean time to peak tension was 9.7 ± 1.0 msec. These times to peak showed a tendency to fall into a bimodal distribution with mean values of 7.0 and 10.2 msec. The mean conduction velocity for normal mice was 70 m/sec (±12.8 SD) and 48 m/sec (±5.6 SD) for dystrophic mice. No simple relationship between tension and conduction velocity was observed.
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405 AN ENDOGENOUS SUBSTANCE IN BRAIN THAT INHIBITS SAXITOXIN BINDING TO VOLTAGE-SENSITIVE Na^+ CHANNELS. Daniel M. Becker* and Stanley M. Goldin, Department of Pharmacology, Harvard Medical School, Boston, MA 02115.

Cooperative inhibition by Na^+ of ^{3}H-saxitoxin (STX) binding to mammalian axolemma sodium channels was demonstrated; the cooperativity centered about a physiologically relevant range of Na^+ concentrations (Rhoden and Goldin, J.Biol.Chem. 254:11199,1979). This led to the hypothesis that the STX-specific site might act physiologically as receptor for an endogenous modulator of voltage-sensitive sodium flux. We report here the characteristics of an endogenous substance, partially purified from rat brain, which inhibits the binding of ^{3}H-STX to Na gate-associated binding sites in a bovine synaptic plasma membrane fraction (SPM), and which may prove to be such a modulator.

Aqueous extracts were prepared by evaporation of acetone from successive acetone-acid, acetone-water extracts of whole rat brain in a modification of the procedure of Carraway and Leeman (J.Biol.Chem. 248:6854,1973). Extracts were fractionated by gel filtration on Biogel-P2, and the fractions searched for their ability to inhibit high affinity saturable binding of [^{3}H]STX to SPM. Fractions demonstrating inhibitory activity were pooled and subjected to anion-exchange chromatography. The inhibitory substance was retained on DEAE-Sephadex and eluted in a sharp peak with choline sulfate.

The inhibitor was dialyzable, with an apparent molecular weight of less than 1000 as determined by elution position on Biogel P2. It is stable to boiling for at least 5 minutes but unlike inorganic ions, is completely destroyed at 600° after 24 h. The inhibitor is not destroyed by 45 minute incubation at 37° with 1 mg/ml pronase.

Binding of the factor to SPM reaches equilibrium within 5 min at 4°C. Dissociation, as measured by regeneration of STX-specific sites, was not seen after 12 hour dialysis at 4°C. The DEAE-purified anionic inhibitor does not contain significant amounts of ascorbic acid, known to irreversibly and nonspecifically denature other membrane proteins (e.g. Dunlap et.al., Mol.Pharmacol. 6: 105, 1979). Specificity of the factor's action was further defined by demonstration, under conditions similar to those used in the assay of STX binding, that the substance did not inhibit either the binding of ^{3}H-ouabain to the Na,K-ATPase of SPM or the specific binding of phorbol dibutyrate (Nagle et.al., Cancer Res. 41:89, 1981) to brain tissue. More detailed binding studies suggest competition to the Na^+ gate between the factor and ^{3}H-STX for binding.

406 BEHAVIORAL FREQUENCY DISCRIMINATION OF ELECTRICAL STIMULATION OF THE SUPERIOR OLIVARY COMPLEX. D. R. Berard* and K. R. Henry. Dept. of Psychology University of California, Davis, CA 95616.

We have found that electrical stimulation of the rat mid-superior olivary complex (SOC) results in behavioral frequency discriminations which are as accurate as those obtained with 80 dB acoustic stimulation over the frequency range of 200 to 3800 Hz. We chose the SOC as the target organ because it is one of the brain stem structures involved in the neural frequency following response (FFR). The FFR is a bioelectrical response which mimics the fundamental waveform of an auditory stimulus at frequencies from 0.2 to 3.9 kHz (rat) and 0.1 to 5.0 kHz (human). It has been proposed that phase locked neurons of the SOC may perform a cross-correlational analysis of volley input.

To behaviorally access the perceptual significance of the FFR, we have simulated the integrated FFR waveform by electrically stimulating the SOC with sine waves. The rats' ability to discriminate SOC stimulation was nearly identical (X^2=.42, df=15; P<.05 similarity) with their ability to distinguish the same frequencies of acoustic stimuli in order to receive reinforcing electrical stimulation of the medial forebrain bundle (MFB). Within the peak current limits of 15 to 25 μA, discrimination of SOC stimulation was improved by increased current intensity.

Discrimination of SOC electrical stimulation occured within the same frequency band as the FFR. We have estimated that over 15 contiguous critical bands for discrimination of SOC stimulation exist within the FFR frequency range.

Our data are consistant with the hypothesis that SOC stimulation provides a linear transformation of pitch information within the frequency window of 200 to 3800 hz, and that limited acoustic amplitude information can be encoded within a narrow range of stimulation current. It is possible that SOC stimulation may ultimately be used to restore partial auditory perception to the individual whose inner ear and auditory nerves have been damaged by age or other factors.

407 LONG-TERM PERSISTENCE OF UNILATERAL ANOMIA FOLLOWING COMPLETE CEREBRAL COMMISSUROTOMY. J.E. Bogen, A. Campbell* and A. Thompson*. New Hope Pain Center, Alhambra, CA 91801.

A prominent feature of the syndrome following complete cerebral commissurotomy in humans (Sperry, Gazzaniga and Bogen, 1969) is, in right-handers, an inability to name or to describe verbally objects palpated in the left hand, although the objects are handled appropriately and retrieved correctly (with the same hand) from an unseen collection. This unilateral anomia, which can be quite persistent (Bogen and Vogel, 1975), requires that the informed right hemisphere be unable to speak as well as being cognitively isolated from the speaking left hemisphere. Recent studies of callosotomized individuals (Sidtis et al, 1981) have shown in some a gradual emergence of right hemisphere capability for speech, raising the possibility that in individuals with complete commissurotomy, there might eventually emerge some right hemisphere speech. We have therefore retested, 13 to 18 years after operation, nine subjects who had complete cerebral commissurotomy as well as five subjects with partial commissurotomy (mainly the callosal trunk). The latter five subjects named objects in the left hand exactly as well as they did in the right. The former nine were correct with the left hand only 20% as often as with the right hand; in these nine patients, neither right hemisphere speech nor interhemispheric communication seems to have developed sufficiently to eliminate their left hand anomia.

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408 RAT MODEL FOR FETAL SURGERY. Robert A. Brodner. Department of Neurosurgery, Hahnemann Medical College and Hospital, Philadelphia, PA 19102.

A microsurgical model in the fetal rat has been developed in which various procedures including intracranial surgery and forelimb amputation are performed. Maternal blood pressure, pulse, and temperature are monitored throughout the experiment and sterile surgical technique is used. The uterus of pregnant rats in the third trimester is exteriorized and placed upon an operative stage immersed in a temperature controlled isotonic saline bath. Employing 25 to 40 diameters magnification and microinstrumentation, uterine incision is made and the amniotic membrane is opened separately. In one group of animals a craniectomy and cerebral incision are performed followed by separate closure of the fetal scalp and amniotic membrane with 10-0 nylon microsutures. In another group, a forelimb is amputated and, again, the amniotic membrane is closed separately. The uterine wall and abdomen are then sutured in layered fashion and pregnancy is allowed to continue to completion by natural delivery. The surgical pup is identified at birth and observed for five days. It is then terminated and formalinized for histological study.

Forty-two pregnant rats have undergone surgery upon one of their fetuses. In 30 maternal rats the fetal intracranial procedure was performed. Two of these mothers succumbed after surgery yielding a maternal survival rate of 93%. Postmortem examinations were performed. The group in which fetal forelimb amputation was done had a maternal survival of 100%.

The fetal survival rate in those undergoing direct intracranial surgery was 90%, and 92% in those fetuses subjected to forelimb amputation. Every surgical fetus was observed for five days after birth and all were noted to thrive.

The inaccessibility of the fetus in its natural state has often proved to be a significant obstacle in the study of the growth, development and physiology of the fetus. In these experiments, we have demonstrated the feasibility of performing sophisticated surgical procedures in the rat fetus while maintaining a relatively normal fetal-maternal relationship. A high percentage of animals operated in utero have been recovered in a viable condition at the end of the normal gestational period thus allowing postnatal evaluation and study. This model of fetal surgery has potential application in the expanding fields of prenatal research and treatment.

409 THE ALUMINUM FORMALDEHYDE (ALFA) HISTOFLUORESCENCE METHOD FOR DEMONSTRATING THE PERIPHERAL INNERVATION OF THE GILL OF A MARINE MOLLUSC. Edward J. Catapane. Dept. Natural Sciences, Medgar Evers College and the East Coast Neuroscience Foundation, Brooklyn, N.Y. 11225.

Neurophsiology studies show that the lateral ciliated cells of the gill epithelium of Mytilus edulis are innervated from the central nervous system via the branchial nerve. Serotonin and dopamine are postulated as the central and peripheral neurotransmitters mediating cilio-excitation and cilioinhibition, respectively. The Formaldehyde Induced Fluorescence (FIF) method for visualizing the cellular localization of biogenic amines demonstrated their presence in the central nervous system and large nerve trunks of M. edulis, but was unable to demonstrate the presence of nerve fibers within the gill filaments. To do this the recent modification of the FIF method, the Aluminum Formaldehyde (ALFA) Histofluorescence method was tried. The ALFA method produced more intensely fluorescing structures than the FIF method and revealed for the first time fine branches of the branchial nerve running beneath the gill epithelium, which contain biogenic amines. Microspectrofluorometric analysis showed two distinct emision spectra for these fluorescing structures which correspond to the spectra of serotonin and the catecholamines. This study is in agreement with the earlier neurophysiological studies illustrating the monoaminergic innervation of the gill. It also demonstrates the usefullness of the ALFA histofluorescence method for visualizing monoamine containing structures in tissues of marine invertebrates.

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410 BRAIN HISTAMINE H_1-AND H_2-RECEPTORS AND HISTAMINE-SENSITIVE ADENYLATE CYCLASE: EFFECTS OF ANTIPSYCHOTICS AND ANTIDEPRESSANTS. Joseph Coupet and Vera A. Szucs-Myers. Department of CNS Research, Medical Research Division of American Cyanamid, Lederle Laboratories, Pearl River, NY 10965

Several classes of psychoactive compounds have been investigated for their effects on histamine-sensitive adenylate cyclase in cell free preparations from the guinea-pig cerebral cortex. Their inhibitory actions on this enzyme system have been compared with their abilities to displace [^{3}H]-pyrilamine and [^{3}H]-cimetidine from histamine H_1-and H_2 receptor sites, respectively. The results of these studies show that compounds which inhibited the histamine-sensitive cyclase were also displacers of either [^{3}H]-pyrilamine or [^{3}H]-cimetidine or both [^{3}H]-ligands from their binding sites. In spite of the lack of a correlation between binding and cyclase antagonism, it was observed that compounds that displace both ligands showed greater inhibition of the cyclase than those that have affinities for sites labeled by one or the other ligand. It was concluded that the antihistamines, the antipsychotics and the antidepressants share a common property through their antagonism of H_1-receptors and that may be responsible for their sedative side effect.

411 EFFECT OF NERVE EXTRACT ON ATROPHY OF DENERVATED OR IMMOBILIZED MUSCLES. H.L. Davis. Department of Anatomy, University of Western Ontario, London, Ontario, Canada, N6A 5C1.

Changes in denervated muscles are due to disuse caused by paralysis of the muscle and the loss of neurotrophic substances. The relative roles of these two factors in the production of atrophy in denervated rats' extensor digitorum longus (EDL) muscles were studied. Muscles were denervated and/or immobilized (by fixation of the ankle) for 7 days. Some rats also received daily intramuscular injections of a saline extract of rats' sciatic nerves (2 mg protein/ml). Atrophy was assessed by measurement of wet weight, total protein and cross-sectional areas of types IIA and IIB fibers (in sections stained for ATPase). Both denervation and immobilization produced significant decreases in weight, protein, and areas of fiber. The group of rats with denervated EDL muscles had significantly greater atrophy than the group with immobilized muscles. In another group, denervated EDL muscles had significantly greater atrophy than contralateral muscles which were immobilized. However, when denervated muscles were injected with nerve extract, they did not differ significantly from contralateral, noninjected, immobilized muscles. Comparisons of the group of rats in which one EDL was denervated with groups in which one muscle was immobilized or was denervated and injected with nerve extract, indicated that loss of trophic influence was responsible for about 40% of the decreases in wet weight, total protein, and cross-sectional area of type IIB fibers, and the remaining 60% was due to disuse. Loss of trophic influence was responsible for only about 5% of the atrophy of denervated type IIA fibers. Therefore, inactivity and loss of neurotrophic influence were responsible for the atrophy which occurred in denervated skeletal muscles, and these two factors influenced the two types of fiber differently. The component of denervation atrophy due to loss of trophic influence could be completely prevented by injection of substances extracted from peripheral nerves.

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412 GLUCOCORTICOID BINDING IS DECREASED IN HIPPOCAMPUS BUT NOT CORTEX OF AGED RATS. C. H. DeFiore and B. B. Turner. Depts. of Biology & Psychology, Virginia Polytechnic Institute & State University Blacksburg, VA 24061.

The hippocampus (HC) has been implicated in spatial orientation, memory, discriminative learning, and extinction tasks. A significant percentage of the aged human population show moderate to severe impairments in these areas of performance. The HC has the highest number of glucocorticoid receptors in the brain, and glucocorticoids have been hypothesized to play a modulatory role in several HC dependent behaviors. A decrease in HC glucocorticoid receptors could mediate some of the reported behavioral deficits in the aged. A previous report of cytoplasmic binding in in aged mice found no change in glucocorticoid receptors in the hippocampus or other brain regions. This study may have been compromised by procedural considerations as evidenced by the low levels of binding obtained. We wished to determine in the rat whether the number of glucocorticoid receptors or their affinity change with age in the HC or the cerebral cortex (CX)

Sprague-Dawley male rats, 6 and 24 months of age, were adrenalectomized 12 hr before sacrifice. Rats were anesthetized with Metofane and cardiac perfusion performed with 60 ml of cold dextran-saline. Tissues from 2 young rats and 2 aged rats were used in each experiment. Brain regions were dissected on a chilled glass plate and homogenized in Tris-EDTA buffer pH 7.4 containing 10% glycerol and centrifuged at 105,000xg for 60 min. Aliquots of supernatant were then incubated in 7 concentrations of ^{3}H-corticosterone (2-30 x 10^{-9}M) and additional aliquots of 1 and 2 x 10^{-8}M unlabeled steroid to determine non-specific binding. Free steroid was removed by passage through LH-20 columns.

Reciprocal plot analysis indicated that not only does the maximal binding (B_{max}) change with age, but the apparent dissociation constant (K_d) also changes. The aged animals showed a decrease in the number of receptors compared to the young: 146±4 fmoles/mg protein (old) to 211±2 fmoles/mg protein (young), $p<.01$. The apprent K_d increased with age from 2.12±.2 to 4.62±.7 x10^{-9}M ($p<.03$). There were no differences in B_{max} or K_d between the two age groups with regard to binding in the CX. The relative deficit in HC binding thus appears to be a regional, specific effect.

These data 1)provide evidence that cytoplasmic glucocorticoid binding is substantially reduced in the aged HC, both with respect to receptor number and affinity and 2) provide a possible physiological mechanism for the functional deficits occurring in the HC with age. (Supported by a grant from the Gerontology Center, VPI & SU).

413 DISTRIBUTION OF NEUROTOXIN BINDING ACTIVITY IN RAT CNS MYELINATED AXONS. G.H. DeVries and M. Lazdunski*, Department of Biochemistry, Medical College of Virginia, Richmond, Virginia, USA and Centre de Biochimie, Universite De Nice, Nice, France.

Myelinated axons were isolated from young adult rat brainstem by the flotation procedure (DeVries, Neurochem. Res. 5:3, 1981), subjected to hypotonic shock and fractionated on a 20%-40% continuous sucrose gradient in a zonal rotor. Ten separate fractions were harvested from the gradient based on the isopycnic density of each membrane constituent of the myelinated axons. The maximal binding of a ^{3}H ethylene diamine tetrodotoxin derivative (^{3}H TTX-EN) (Eur. J. Biochem. 104:617, 1980) was determined for each fraction. In addition the specific binding of a ^{125}I labeled sea anemona toxin of Anemonia sulcata (^{125}I ATX_{II}) at a concentration of 10^{-7} M as well as the specific binding of ^{125}I labeled scorpion toxin of Androctonus australis (^{125}I AaH) at a concentration of 10^{-9} M were determined for each fraction. Under these conditions the specific binding of ^{125}I ATX_{II} was highest in all membrane fractions ranging from 1 to 10 pmoles/mg protein. The maximal binding of ^{3}H TTX-EN was about 1 pmole/mg and was found in membrane fractions whose densities ranged from 30% to 35% sucrose. The binding of ^{125}I AaH ranged from 10 fmoles/mg to 30 fmoles/mg and had a bimodal distribution in the density gradient with a peak of binding activity at 37% sucrose and another at 30% sucrose. The bulk of the ^{125}I ATX_{II} binding activity was found in fractions with a density which ranged from 28% to 32% sucrose. Clearly the binding activity of the three neurotoxins was differentially distributed in the gradient so that membrane fractions which were more enriched in the binding of one particular toxin could be identified. However, the maximal binding of all toxins was found in the 28% to 32% range of sucrose densities; we have previously reported that this fraction is enriched in axolemma (DeVries et al., Transact. Amer. Soc. Neurochem. 11:130, 1980). These results suggest that each of the neurotoxin binding sites can be dissociated from each other by density gradient centrifugation although the mechanism by which this occurs is not certain. (Supported by NIH grant 10821 and a Fogarty Foundation Fellowship)

414 MULTIPLE ^{3}H-SPIROPERIDOL BINDING SITES IN HUMAN TEMPORAL CORTEX: COMPARISON WITH OTHER BRAIN REGIONS. L.L. Dickey*, A.C. Andorn*, L.H. Matt*, J.J. Ondrejka*,(SPON: V. Rowland).Dept. of Psychiat. Case Western Reserve Univ. Schl. of Med., Cleve. OH 44106.

^{3}H-spiroperidol binds specifically to homogenates of normal postmortem human temporal cortex, Brodmann areas 41-42, a region associated with the production of auditory hallucinations (Penfield and Jasper, Epilepsy and the Functional Anatomy of the Human Brain, Little, Brown,Co., 1954). This binding is saturable, stereoselective with regard to (+) and (-) butaclamol, reversible, and is linear over defined protein concentrations. Under standard assay conditions (Andorn and Maguire, J. Neurochem.,35, 1105-1113,1980), binding reaches steady state by 2.5 +/- 0.5 min. but a 20% loss in specific binding occurs over time until another steady state is reached by 40 +/- 2 min. This is similar to our observations in the prefrontal cortex, and suggests that desensitization of binding sites might be occurring. Both steady states are reached earlier in temporal cortex. These findings suggest site densities, rates of desensitization, or site K_D could differ between the two cortical areas. Saturation analyses at both steady states indicate a multiplicity of high affinity sites, K_{Dapp} range from 40 +/- 20 pM to 0.7 +/- 0.2 nM, with respective B_{max} of 50 +/- 20 and 400 +/- 100 fmol/mg protein. These K_D are similar to those observed in the same conditions in the prefrontal cortex. The overall B_{max} is slightly greater in this region of the temporal cortex than in the prefrontal cortex. Preliminary evidence indicates that this may be due to a larger B_{max} for sites K_D 0.7 nM. Their relatively increased density in this area of the temporal cortex may indicate that they have significance for the etiology of psychotic symptoms. Both the equilibrium and non-equilibrium binding studies performed in this region indicate that temporal cortical sites are more similar to prefrontal cortical sites than to caudate sites. These findings demonstrate ^{3}H-spiroperidol binding sites in a brain region associated with a discrete psychotic symptom. Further, they suggest a similarity in binding site properties between regions involved in the modulation of perceptual experience.

415 EFFECTS OF ORAL PHOSPHATIDYLCHOLINE ON MOUSE BRAIN CHOLINE AND ACETYLCHOLINE. E.F. Domino, B.N. Mathews, A. Ortiz, S.K. Tait, and F. Fucek. Dept. of Pharmacology, Univ. of Michigan, Ann Arbor, MI 48109 and Lafayette Clinic, Detroit, MI 48207.

The report of Hirsh and Wurtman (Science 202: 223-225, 1978) that phosphatidylcholine (PC) elevates rat brain acetylcholine (ACh) stimulated a great deal of research on the possible value of PC in various human brain disorders. The purpose of the present study was to reevaluate the effects of oral PC on brain choline (Ch) and ACh in another species. Male Swiss Webster mice, 30-50 g body weight, were habituated for at least 1 wk to a 0700-1900 light, 1900-0700 dark cycle. Food and water were provided ad lib until 16 hr before the experiment, when food was removed. Mice were housed 15 to a cage. They were killed between 0800 and 1200 by focused microwave irradiation to the head. Oral PC in a dose of 250 mg/kg of Ch was given 1, 2, 4, 8, 12 and 24 hr in a 1:2 suspension with 0.9% NaCl to groups of 12-14 mice. Control groups received equal volumes of 0.9% NaCl. In some experiments, the effects of PC administration to alter the known brain ACh depleting effects of scopolamine hydrobromide (1 mg/kg, i.p., base) were also determined. In this case, the PC was given orally 4 hr before and scopolamine, i.p., 1 hr before microwave irradiation. Brain temperatures after microwaving were measured to assure adequate protein denaturation. Brain Ch and ACh were isolated by ion pair extraction in dipicrylamine in dichloromethane, demethylated by sodium benzenethiolate and measured by GC-NP detection as described by Kosh et al. (J. Chromatogr. 163: 206-211, 1979). Brain Ch levels significantly increased 4 ($P<.001$) and 8 ($P<.01$) hr after PC. However, there was no change in brain ACh. In a second series killed 1, 3, 6, and 12 hr after pretreatment with the same large dose of PC only the 6 hr pretreated mice showed a significant increase in brain Ch ($P<.01$), a finding consistent with the first series of experiments. The known depleting effects of scopolamine pretreatment on brain ACh were confirmed ($P<.001$) in a third series of experiments in which 1 mg/kg were given i.p. 1 hr previously. Scopolamine also caused a decrease in brain Ch ($P<.001$). Pretreatment with PC reversed the depletion of brain Ch induced by scopolamine, but had no effect on brain ACh. The results obtained indicate that although mouse brain Ch can be increased by oral PC there is no effect on brain ACh. If the purported beneficial effects of oral PC in various human disorders are true, they may not be due to a simple elevation in brain ACh, unless human brain is more like that of the rat than the mouse. Obviously, further studies related to possible animal species differences need to be undertaken.

416 TISSUE DISTRIBUTION OF THE 4-HYDROXYCYCLOHEXYL METABOLITE OF PHENCYCLIDINE. Mark S. Franczak, R. Stanley Burns* and Michael H. Ebert*. Lab. of Clin. Science, NIMH, Bethesda, MD 20205.

The tissue distribution of 4-phenyl-4-piperidinocyclohexanol (PPC), the major metabolite of phencyclidine (PCP) in man, was examined in the rat. In the Rhesus monkey and chronic spinal dog, PPC has properties similar to central nervous system stimulants, and a different spectrum of activity from the parent compound, PCP.

A 5 mg/kg dose of PPC was administered I.V. by tail vein injection, and animals were sacrificed by decapitation after 5, 15, 30, 60, 90, 120, 150, 180, 210, and 240 minutes. Plasma was obtained by the collection of thoracic stump blood into heparinized tubes. Following the addition of the internal standard, 0.3 to 1.5 grams of brain, heart, lung, liver, kidney, adipose, and muscle tissue were homogenized in 10 ml of 0.2 N sulfuric acid. PPC values were determined by gas chromatography-mass spectrometry using a deuterated (d_5) PPC internal standard.

The kinetic analysis of mean concentration versus time yields a plasma elimination half-life of 88 minutes, steady state volume of distribution of 11.6 liters/kg and a plasma clearance of 78.4 ml/kg/min. Disappearance half-lives of PPC in brain and adipose tissue were similar to the plasma elimination half-life with calculated values of 80 and 76 minutes, respectively. During the post-distributive phase (90 to 240 minutes), the brain to plasma concentration ratio was 1.5 to 3.0. The concentration ratio of adipose tissue to plasma was 1.5 to 2.0. The highest levels of PPC were found in the lung, kidney and liver, respectively.

PPC rapidly distributes into brain tissue, achieving peak levels within 5 minutes. The compound selectively partitions into brain resulting in levels up to 3 times greater than those in plasma during the post-distributive phase. Other studies in our laboratory indicate that in the Rhesus monkey, PPC produces an excited state with motor restlessness, increased startle response, and purposeless movements resembling athetosis. The presence of PPC in high concentrations in brain together with central nervous system stimulant properties may explain the mixed stimulant and depressant effects of the parent drug, phencyclidine. In man, PPC may be responsible for the behavioral toxicity of phencyclidine in the form of an excited state.

417 EFFECTS OF AMBIENT CATIONS ON HYPERSEROTONERGIC ANXIETY IN A HUMAN POPULATION. A. James Giannini, R. H. Loiselle* Psychiatry Dept., Northeastern Ohio Universities College of Medicine, Rootstown, OH 44222.

Previous investigations in this laboratory (Giannini, 1979; Giannini and Dvoredsky, 1981) have demonstrated a correlation between high-concentration cation atmospheres and hyperserotonergic anxiety in sensitive human populations. This study attempted to study the relationship between serotonergic metabolism and anxiety produced in an ionized environment in a general population. Twenty young male volunteers signed standard consent forms to participate.

The group was screened for serum serotonin (5HT) and 24 hour urine 5-hydroxyindoleacetic acid, (5-HIAA). Levels for all subjects were found to be within established norms. They were then collectively exposed to a cationized atmosphere for a fifteen-hour period. Serum 5HT and 24-hour urine 5-HIAA levels were then measured. Ten of the patients complained of anxiety and of these ten, two reported vertigo. Physical examination revealed a resting tremor in all ten patients reporting anxiety and none in the nonanxious group. No other signs associated with a hyperserotonergic state were noted. No significant changes in 5HT and 5-HIAA were noted in this group after exposure. Also there were no significant differences between the anxious and nonanxious subgroup.

This finding contrasts with earlier studies which correlated highly cationized atmospheres, anxiety, and increased levels of serotonin. They also differ with the findings of Dirnfield (1954) who reported increased 5-HIAA and Giannini and Dvoredsky (1981) who reported decreased levels. A possible explanation for these divergent findings rests on the fact that previous studies relied on self-selected subjects who were included in study groups solely on the basis of presenting hyperserotonergic symptomatology. This randomly selected group had no such predetermined sensitivity. Only one-half the group experienced anxiety and even in this subgroup there was no noted change in serotonin metabolism. Since cerebrospinal fluid levels for 5HT and 5-HIAA were not drawn, it cannot be determined if changes in central 5HT metabolism were greater than those noted in the periphery. It is hypothesized that sensitivity of serotonin systems to the effects of elevated atmospheric cation levels is quite variable in different individuals and that central sensitivity is greater than peripheral sensitivity.

418 APERIODIC IMPULSE PATTERNS IN A CUTANEOUS AFFERENT UNIT MODEL M.D. Goldfinger. Dept. of Biology & School of Medicine, University of Missouri, Kansas City, Missouri, 64110

The branching of many skin afferent axons is a substrate for the superposition of electrical activity elicited from each mechanosensitive branch terminal. The present work uses a mathematical afferent unit model(1)to consider the generation of aperiodic discharges of the parent axon elicited by repetitive mechanical stimulation of 2 or more (up to 20) terminals.

During multiterminal stimulation, the interspike interval distribution(IID)differs from the merged interstimulus interval distribution, due to absolute refractoriness(ARP=2 ms), recovery cycles, and mechanoreceptor fatigue (see II below). For simplicity, the amplitude of all stimuli used supports 1:1 firing for any period exceeding ARP. The general result of superposing several separate spike trains is a Poisson process (2). Two ways of approaching this ideal are described.

I. Strictly periodic inputs: During multiterminal stimulation using unequal respective periods, the mean interspike interval ($\overline{T}$) decreases with larger numbers(N) of terminals and/or by smaller differences between individual periods. All interspike interval values are between ARP and the shortest input period. With increasing N, an exponential IID falling limb is approached. The Expectation Density (ED) includes deadtime, plateau level, greater-than-plateau peaks at multiples of individual input periods, and minima due to ARP. With greater N, the ED peaks blend into the plateau noise.

II. Random variation of input periods: The waiting time for the next successive stimulation of each terminal is varied equiprobably between periods P and P' ($0<P'<P$). All interspike interval values are between ARP and P. With larger N and/or smaller P'/P, $\overline{T}$ decreases and the spike train becomes increasingly Poisson-like. Mechanoreceptor fatigue can cause a finite IID rising limb and a slowed risetime to ED plateau. Larger P'/P (eg,31 ms/33 ms;N=20) yields spike trains with ED periodicities.

These results can be speculatively applied to the Type I SA response elicited by maintained dome pressure. Assuming each final-order axon branch is, in effect, activated sequentially and can fire independently, a Poisson-like discharge pattern(eg,(3)) could occur if: (i)several branches fire with different periods; or (ii) a smaller number of branches fire randomly within a range of periods determined by the maintained stimulus intensity.

Ref.'s: (1)Goldfinger & Fukami, J. Neurophysiol.45:1096-1108,1981
(2)Cox & Smith, Biometrika 40:1-11,1953
(3)Iggo & Muir,J. Physiol.(London)200:763-796.

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419 ALTERATION OF SPINAL CORD METABOLISM AND BLOOD FLOW IN SPINAL CORD INJURY. N. Hayashi, B. A. Green, J. Mora*, M. Gonzalez-Carvajal*, K. Abe* and K. Kogure Research Lab, Depts. of Neurosurgery and Neurology, Univ. of Miami Sch. of Med., Miami, FL 33101.

Spinal cord tissue metabolism and blood flow were studied in the gray and white matter of the rat following irreversible spinal cord injury. Local spinal cord blood flow (lSCBF), local tissue oxygen tension (lTO_2) and relative local tissue oxygen consumption ($r\text{-}lTO_2C$) were simultaneously measured in 140 rats at different time intervals using a microelectrode technique.

Pathophysiological parameters used to determine factors associated with secondary spinal cord damage included alteration of spinal cord tissue ATP, tissue pH and tissue NADH. Analyses were performed within the lesion site as well as proximally and distally using topographic techniques. Quantitative alterations of coenzyme Q and alpha-tocopherol (as an indicator of free radical reaction) were measured at different time intervals following injury.

Normal mean values of lSCBF in gray and white matter were 63 and 20 ml/100g/min., respectively. Values for $r\text{-}lTO_2C$ in gray and white matter were 3.6 and 1.1 ml/100g/min., respectively. The normal mean values for coenzyme Q_{10} and alpha-tocopherol in the rat were 12 μg/g and 12.5 μg/g, respectively.

A 100 gram-centimeter force irreversible injury at T_7 in the rat was utilized. Gray matter was damaged more than white matter within the lesion. $R\text{-}lTO_2C$ was altered more than lSCBF as evidenced by decreased tissue ATP and decreased coenzyme Q levels after 30 minutes. At two hours post injury, an NADH hyperoxidation (as a black hole) and a decrease in alpha-tocopherol also were observed. Proximal and distal to the lesion, less homogeneous patterns of lSCBF and tissue metabolism were noted. The significance of free radical reaction as a component of the sequelae has also been documented. Our data suggests that the reversibility of the lesion is determined more by the severity (reversibility) of metabolic disturbances (i.e. the maintenance of oxygen metabolism) than blood flow alterations.

420 CONTROL OF THE BLADDER AND EXTERNAL URETHRAL SPHINCTER BY THE SPINAL CORD. F. Jolesz*, P. Ruenzel* and E. Henneman. Dept. of Physiology, Harvard Medical School, Boston, MA 02115.

Severe injury to the spinal cord results in paralysis of skeletal muscles whose motoneurons lie below the level of the lesion. The specialized muscles of the external urethral sphincter and nearby perineal regions, however, become spastic after spinal transection and prevent emptying of the bladder by the detrusor muscle. The reflex discharges elicited by electrical stimulation of the pudendal nerve branches supplying the external sphincter have been recorded from the distally severed ventral roots of the first, second and third sacral segments. Electromyographic recordings from the external sphincter have also been made. Considerable electrical activity remains in these muscles after unilateral section of the sacral ventral roots. This activity is maintained after spinal transection as previously reported. In the second sacral ventral root and to a lesser extent in the third prolonged polysynaptic electrical responses are elicited by single shock stimulation of the pudendal nerve. Mechanical stimulation of the surface of the urethra, anus and adjacent perineum or an increase in bladder volume usually facilitates these reflexes. To date no monosynaptic reflexes have been elicited by pudendal stimulation. Potentiation of latent monosynaptic reflexes has been attempted by tetanizing the pudendal nerve at 500/s prior to testing with single shocks. No concealed monosynaptic reflexes were revealed by this procedure. It is tentatively concluded that there are no monosynaptic connections from the muscles of the external urethral sphincter to its motoneurons. This distinguishes the sphincter from most other skeletal muscles. Control recordings of the segmental reflexes of the sacral roots elicited by stimulation of the dorsal roots indicate the existence of well defined monosynaptic reflexes. Other skeletal muscles with motor neurons at this level of the spinal cord, therefore, have quite normal patterns of reflex activity. The absence of muscle spindles and monosynaptic reflexes and the presence of large polysynaptic reflexes indicate that reflex control of sphincter muscles may be quite different from that of skeletal muscles in general. The "spontaneous" activity of the sphincter muscles and the increase in their tone after spinal transection may be due to the polysynaptic reflexes we have noted. We are investigating procedures designed to reduce this polysynaptic activity and relax the external sphincter so that the detrusor muscle can expel urine in a normal manner.

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421 SEROTONERGIC AND CHOLINERGIC INTERACTION IN THE REGULATION OF CORTICOTROPIN RELEASING HORMONE IN VIVO. J. P. Kile and B. B. Turner. Depts. of Biology & Psychology, Virginia Polytechnic Insitute & State University, Blacksburg, VA 24061.

Both serotonin and acetylcholine are known to stimulate the secretion of corticotropin releasing hormone (CRH). However, in vitro studies suggest that serotonergic stimulation of CRH secretion may be mediated instead via acetylcholine neurons that synapse directly onto CRH neurosecretory cells in the hypothalamus. This study investigated the possibility of a cholinergic system in the hypothalamus controlling the stimulation of CRH release by serotonin in the intact animal.

Male Sprague-Dawley rats were housed in single cages in a room with controlled access (lights on 0600-2000 hrs) 1 week prior to experimentation. Eleven groups of rats (n=10) were injected at 0630 hr with one or more of the following agents: saline (SAL), physostigmine (PHY), mecamylamine (MEC), scopolamine (SCO), 5-hydroxy-tryptophan (HTP), or methysergide (MET). One hr post-injection, rats were decapitated, and trunk blood collected for plasma corticosterone determination by radioimmunoassay.

In unstressed rats, PHY ($p<.001$), SCO ($p<.01$), HTP ($p<.001$), and MET ($p<.05$) groups had significantly higher corticosterone levels than the control group (SAL). When drugs were injected in combination, PHY-HTP and MEC-HTP produced increased levels of plasma corticosterone which were lower ($p<.05$) than those of the PHY group, but not different from the HTP group. MEC only slightly inhibited the HTP effect. When SCO was given with MEC and HTP this inhibition was negated. The PHY-MET group has significantly lower corticosterone levels ($p<.01$) than the PHY rats, while MEC-MET group levels were reduced to control levels.

In stressed rats (horizontal shaker 40 min, plus Metofane anesthesia 20 min), the SAL group had corticosterone levels six fold higher than in unstressed SAL rats. Similar effects to those in unstressed rats were observed for PHY, HTP, and MET groups. However, HTP group corticosterone levels were 25% higher than the PHY group levels. Corticosterone concentration in SCO and MEC groups was not significantly different from the SAL rats. MEC was able to abolish the HTP-induced rise in corticosterone. SCO when given with HTP had no significant effect.

These data suggest that: 1) in contrast to in vitro studies, serotonin actually mediates acetylcholine stimulation of CRH; 2) there is also direct control of CRH release by both neurotransmitters; 3) nicotinic receptors stimulate, whereas muscarinic receptors inhibit CRH secretion; 4) serotonin is more important in regulating CRH release during stress than in basal conditions. (Supported by an NIH BRSG grant from VPI & SU).

422 MEASUREMENT OF BLOOD FLOW BY WASHOUT OF MICROWAVE INDUCED HEATING. P. M. Lawner, L. D. Braun* and W. H. Oldendorf. Departments of Neurosurgery and Neurology, UCLA School of Medicine and Research Service, Brentwood VA Medical Center, Los Angeles, CA 90073.

A technique was developed to measure cerebral blood flow (CBF) using the washout of heat delivered by a microwave source.

Ten adult Wistar rats (275-325 g) were anesthetized with pentobarbital 50 mg/kg i.p.. A 0.25 mm diameter thermistor (Thermometrics, Edison, NJ) was placed into the right cerebral hemisphere using preset external coordinates, and a second thermistor placed through the femoral vein into the venae cava. Using a wheatstone bridge and a potentiometric recorder, the temperature difference between the two thermistors was plotted. The animal's head was exposed to a microwave source (Gerling Moore-Metabostat, Palo Alto, CA) attenuated to achieve a brain temperature elevation of $0.5^{o}C$ in 2 s, and the temperature washout curve was utilized to calculate CBF using the T 1/2 method. Three to five determinations were performed in each animal.

In a second group of 12 rats, under identical anesthetic conditions, CBF's were measured in a 125 mm^3 volume around the thermistor tip using a ^{14}C-butanol uptake technique (Van Uitert, R.L. and Levy, D.E., Stroke 9:67, 1978).

Mean CBF using thermal washout was 55.59 $\pm$ 7.47 S.D. (ml/100 g/min) while ^{14}C-butanol flow in the same area was 52.12 $\pm$ 5.87 (difference $p > 0.2$).

This technique appears to be a simple, inexpensive and reliable method to measure CBF with an ideal "indicator" delivered directly to brain parenchyma.

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423 BRAIN UPTAKE OF ERYTHROSIN B, A FOOD DYE, IN ADULT RATS. H. Levitan, Z. Zilyan*, Q. R. Smith, and S. I. Rapoport (SPON: E.J. Elliott) Lab. of Neurosciences, National Institute on Aging, Baltimore, MD. 21224.

Food colors have been held partly responsible for behavioral disorders such as hyperactivity and minimal brain dysfunction in children, and at least one widely used food color, erythrosin B (FD&C Red No. 3) can change neuronal membrane physiology, neurotransmitter release and uptake, and the activity of brain Na-K-ATPase when applied directly to cells or subcellular organelles. There is no information however on whether significant levels of this dye appear in the brain after consumption or administration. In order to determine whether erythrosin B crosses the blood-brain barrier and enters the brain in detectable amounts, we have injected 14C-erythrosin B directly into the circulation of unanesthetized, male, mature (3 month old) Osborn-Mendel rats. A tracer amount of 14C-erythrosin B was injected into the femoral vein, either alone or 10 minutes following injection of a saturating concentration of non-radioactive dye. Blood radioactivity was monitored and the brain removed after 10 min, 30 min, or 2 hours. Brain radioactivity, which was measured after correcting for intravascular tracer, was negligible. In other experiments 14C-erythrosin B and 3H-sucrose were infused directly into the carotid artery of rats about 1 min after the ipsilateral cerebral hemisphere had been perfused with a saline solution to remove blood. These animals were decapitated 1 minute later and their brain was removed. In none of the 14 brain regions examined did the concentration or erythrosin B significantly exceed that of sucrose, a substance used as an indicator of brain blood volume because it penetrates the blood-brain barrier poorly. We conclude that erythrosin B does not penetrate the blood-brain barrier or enter the brain of mature rats in measurable amounts.

424 A CENTRAL INTEGRATIVE DISORDER OF SYNTHETIC FUNCTIONS: UNDERLYING PSYCHOPATHOLOGY. R. S. Lourie, C. Schwarzbeck, III*. Mental Health Studies Center, NIMH, Adelphi, Maryland 20873.

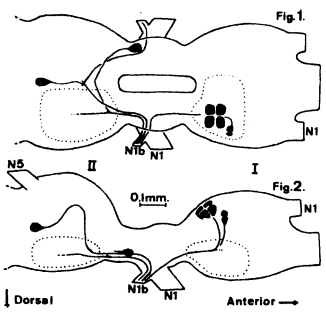
This is a report of a clinical and neuro-psychological five-year study of youngsters with a usually correctibel defect in a brain's synthetic functioning. It describes a fundamental central integrative disorder (CID), in which cognitive functions are interfered with in the presence of stress and anxiety. It results in thinking and acting becoming disorganized or even paralyzed, which then becomes the basis for a wide range of psycho-pathological patterns. In a Clinical population, 25% of patients up to 18 years of age were found to have this constitutionally based difficulty, but never as one of the wide range of presenting symptoms. It occurs in degrees from mild to severe with individual differences in the degree of stress and anxiety necessary to trigger the disorganization. It has been found in adult patients as well, but has not been studied as systematically.

Once this process starts, it usually results in a feeling of helplessness in which the mind goes blank and thinking stops or cannot be collected. It is then experienced (in a paraphrase of Kurt Goldstein's concept) as a "more or less catastrophic reaction." It inevitably is responded to with individually determined patterns of defense. The types of defenses used, in interaction with other inner and outer forces and life experiences lead to a wide variety of psychopathological symptoms and syndromes. The ones encountered in this study range from neuroses, psychosomatic reactions, character disorders, and psychoses to behavior difficulties and learning disabilities. Extreme acting out even leading to adolescents committing murders has been found in individuals whose disorganization has led to dissociated and/or hypnogogic states. Even when the disorganization is moderate in degree but appropriate environmental supports are not available, the child has difficulty in dealing with developmental stages in which anxiety is expectable. Therefore, unresolved sources of anxiety persist. Thus, attachment and impulse disorders, separation concerns, fears of body damage and anxiety stemming from oedipal conflicts can color the resulting symptom picture. There often is resulting poor self-image and other narcissistic problems. These patterns then in turn can provide additional sources of anxiety.

Individuals who have CID not infrequently are resistant in traditional psychotherapy to giving up defenses which would leave them feeling helpless. Corrective approaches to the disorganization can then expedite the use of other therapeutic modalities. Some remedial techniques have been developed.

425 NEURONS INNERVATING SOME FLIGHT MUSCLES IN A NOCTUID MOTH Bent M. Madsen* and Lee A. Miller. Biologisk Inst., Odense Universitet, DK-5230 Odense M, Denmark.

Tympanate moths can hear and avoid bats (Roeder, Science, 154:1515-1520, 1966). We are now investigating the neuroethology of evasive behavior shown by moths when exposed to ultrasound. Here we describe the structure of 8 neurons innervating the dorsal longitudinal muscles of the mesothorax in the moth Mamestra brassicae. We used standard cobalt staining techniques with intensification. Cobalt entered through the cut axons of the appropriate nerve (IIN1b). We made camera lucida drawings of 25 preparations. The results are presented schematically in Fig. 1 (ventral view) and Fig. 2 (lateral view, left side). We found 6 motor neurons (MN) in the posterior part of the prothoracic ganglion (I). Their cell bodies lie near the ventro-lateral surface of the ganglion. Each soma has a neurite that runs almost vertically to the dorsal area of the ganglion where it branches profusely (area within the dotted lines in I). A seventh MN was stained in the mesothoracic ganglion (II). The soma is located in the posterior end of the contralateral connective between I and II. The soma occupies the same position in stained, but unfixed preparations. The neurite branches profusely in the ipsilateral, dorsal neuropile (area within the dotted lines in II). An eighth neuron was stained near the midline of ganglion II. Its soma lies in the central posterior region of II. The neurite runs anteriorly and bifurcates into two axons; one exiting in each IIN1b root. Our results resemble those from the moth Manduca sexta. However, the soma of the contralateral MN lies within II in M. sexta (Casaday and Camhi, J. comp. Physiol. 112: 143-158, 1976). The medial cell in II is probably a dorsal unpaired medial (DUM) neuron (Davis and Alanis, Comp. Biochem. Physiol. 62: 777-788, 1979).



426 BLOOD CHOLINE LEVELS AND DELAYED RESPONSE PERFORMANCE IN YOUNG AND OLD RHESUS MONKEYS. J.G. Marriott, E.F. Domino, S.K. Tait*, B.N. Mathews*, F. Fucek* and J.S. Abelson. Warner-Lambert/Parke-Davis & Univ. of Michigan, Ann Arbor, MI, and Lafayette Clinic, Detroit, MI.

Numerous studies in both animals and humans have implicated cholinergic neurotransmission in the functioning of such higher cognitive process as learning and memory. In addition, evidence indicates that declines in cholinergic function may be involved in the impaired cognitive performance of normal geriatric subjects and of patients with senile dementia of the Alzheimer's type.

The present studies investigated the relationship between blood choline levels and cognitive performance in a colony of young and aged rhesus monkeys. The young adult monkeys had a mean age of 8 years, the geriatric animals 19 years. This colony of non-human primates has been used for several years to study age-related cognitive impairments. Delayed response performance of these animals was measured using automated test procedures. This behavioral task has repeatedly shown significant and reliable memory impairments in aged monkeys relative to the performance of young animals having a similar amount of experience. Chemical demethylation, gas chromatography-nitrogen detection was used to measure red blood cell (RBC) and plasma choline concentrations in these same subjects, as a peripheral marker of low affinity choline uptake.

Mean plasma choline concentrations appeared slightly higher in the geriatric animals compared to young controls ($P<.03$). RBC choline was slightly lower in old versus young animals; however, statistical analyses of this measure and RBC/plasma choline ratios showed no significant age differences. Further analyses were made comparing blood choline concentrations to delayed response performance in young and old monkeys. Although there was a trend for poor performing animals in both age groups to have higher blood choline concentrations, no significant correlations between these measures were foung using traditional statistical analyses. Administration of up to 10 g/kg of lecithin, the primary dietary source of choline, had only slight effects in raising blood choline levels of young monkeys. No improvement in performance on the memory task was found following lecithin administration in aged monkeys. In summary, blood choline levels in adult rhesus monkeys do not change appreciably with age, and cognitive declines commonly seen in aged subjects are not closely related to blood choline levels.

427 ZINC LEVELS IN TISSUES FROM ALLOXAN DIABETIC RATS. M. E. McNeill[1], E. C. Simpson[3]*, J. T. Bray[2]*, C. Smith[1]*, L. A. Webb[2]* and C. R. Morgan[1]*.

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Secondary complications associated with diabetes mellitus such as retinopathy, atherosclerosis and infertility are similar to complications that arise in zinc (Zn) deficient animals. Many patients with diabetic skin lesions show improved wound healing when treated with Zn supplement. Therefore the present study was done to probe the relationship between Zn and diabetes by determining the concentration of this trace element in selected tissues. The tissues sampled were determined by three criteria: (1) pathology associated with diabetes mellitus--the pancreas retina, testes, serum, aorta (2) centers possibly involved in modulating insulin secretion--brain, hypothalamus, hypophysis and (3) tissue known to have a high Zn concentration--pineal.

Eight male Sprague-Dawley rats made insulin deficient with an i.v. injection of alloxan (45 mg/kg) were paired with age-matched controls, housed with lights on 0700 - 1900 hrs. and fed rat chow and water ad libitum. Terminal body weight and blood glucose levels were determined at sacrifice 10 months later. Tissue samples were obtained and all samples except serum were freeze-dried at -38°C for 3 days. The dry samples were weighed and the Zn concentration was determined by flame atomic absorption spectrophotometry.

Comparisons were made using the Mann-Whitney U Test. The following table lists those tissues which showed significant differences.

		Body Weight g	Blood Sugar mg/dl	ZINC ug/g (dry weight) AH	HYPO	BRAIN	AORTA
Alloxan Diabetic	n=8	261.1	471.0	89.3	53.5	20.6	90.0
	SEM	±21.2	±17.9	±6.55	±0.53	±0.20	±4.44
Control	n=8	495.5	131.4	77.4	48.5	23.4	76.9
	SEM	± 6.01	±19.4	±1.08	±1.74	±0.36	±4.02
				p=.02	p=.01	p=.0002	p=.038

AH = adenohypophysis; HYPO = hypothalamus

Zn levels were decreased in the pancreas, testes and pineal and were increased in serum and retina (these changes were not highly significant). An inhibitory effect of Zn on in vitro insulin secretion has been reported and attributed to interference of the intracellular function of calcium in the beta cell (Ghafghazi, T., M. L. McDonald and P.E. Lacy, Diabetes, 30:341, 1981). Significant changes in Zn levels in selective tissues in insulin deficient rats suggests that Zn may play a role in endocrine function.

428 A PHARMACOLOGICAL,ADRENERGIC, AND PEPTIDERGIC EVALUATION OF DRINKING RELATING TO THE SUBFORNICAL ORGAN.

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(Department of Physiology and Pathology, School of Dentistry - UNESP - Araraquara, 14.800, S.P. Brasil).

It has been shown that the subfornical organ (SFO) is sensitive to angiotensin II (A II) in inducing thirst (Simpson and Routtemberg, Brain Research, 79, 1974). Adrenergic stimulation also plays a part although at a lesser intensity (Menani, Saad and Camargo, Abstracts - Brazilian Society of Physiology,58-59, 1979). The main point in question is to verify the existance of an interaction between the adrenergic receptors and the angiotensin of the SFO in the ingestion of water. In the experiment performed, in the present investigation A II was injected into the SFO of rats with the object of inducing thirst. Next separate experiments were done using regitine and propranolol. A dose of A II had previously been injected into the SFO. The dose of 4 ng of A II provoked water ingestion which was reduced by the application of 20 nmol of propranolol and totally blocked by doses of 40 and 80 nmol. The ingestion of water in relation to the three doses of propranolol showed significantly different results in relation of one to the other: $F(2.21)=0.94$; $P<0.01$. The same results were obtained when we compared the data of the A II and the isotonic solution with the data of the doses of propranolol $F(3.30)= 33.60$; $P<0.01$. The application of 4 ng of A II into the SFO provoked a water ingestion of 3.9 ± 0.5 ml. When a dose of 80 nmol of regitine was applied previous to A II, there was an ingestion of 2.8 ± 1.4 ml, show no significant difference ($P>0.05$). Both the results of this research and also the results of research done by others show that the response initiated in the cholinergic and peptidergic receptors to induce water ingestion (SFO) could be divided at some point by catecolaminergic vias of the beta type.

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429 POSTNATAL DEVELOPMENT OF THE GIGANTOCELLULAR TEGMENTAL FIELD (FTG) OF THE RAT. A.J. Miller and G. Fry*. Ctr. Craniofacial Anomalies, Univ. of Calif., San Francisco, CA 94143.

Presynaptic boutons of the FTG nucleus of the brain stem reticular formation were analyzed for changes in numerical and volumetric density during early postnatal development. A bouton was defined as that cellular structure occupied by three or more synaptic vesicles in which its membrane and that of an adjacent neuron were separated by a cleft and their cytoplasm demonstrated an increased density. Sprague-Dawley rats at postnatal ages of 1,5,10,15 and 30 days and young adult animals were perfused with a mixed aldehyde solution of 2% formaldehyde-2% gluteraldehyde in a 0.1M phosphate buffer. The tissue was prepared for electron microscopy by standard techniques. Both the myelin and boutons were analyzed using a stereological approach. The FTG was sampled at one rostro-caudal level: the transition between the medulla and pons (caudal level of VII nucleus).

Myelinated tissue did not appear until day 5, and then rapidly increased in volumetric fraction (V_{V_i}) to reach the adult level (40-45%) by day 30. The number of boutons per area (N_{A_i}) and volume (N_{V_i}) increased 3 fold within the first 15 postnatal days and reached the adult level by the 15th day ($N_{A_i} \doteq 1$ bouton/10 μm^2; $N_{V_i} = 9.3 \times 10^8$ boutons/mm^3). The percentage of FTG volume occupied by the boutons (V_{V_i}) did not increase until after day 15 but never exceeded 10% of the total volume.

The significant correlation of the Sudden Infant Death Syndrome with sleep and a critical postnatal age suggests that development of pathways within the central nervous system related to sleep,respiration and upper respiratory tract control is a key factor. Comparison of relative immature brain weights to adult size have suggested that the brain of a 13-day-old rat is comparable to that of a 15.5-month-old human infant. Our study indicates that the most rapid change in synaptic density of the FTG region is prior to this age. This suggests that during the most critical ages of this Syndrome, central neurons, particularly in the brain stem, are undergoing a rapid change in synaptic growth and contact.

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430 ANALYSIS OF SUPRAPONTINE MODULATION OF HYPOGLOSSAL AND PHRENIC MOTONEURON POOLS IN HEMIDECEREBRATE CAT

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It is well known that the suprapontine(SP) brain structures have a modulating influence on medullo-spinal afferent and efferent nerves. In the present one we examined the effect of SP influence on respiration. We studied SP influence on respiratory efferent output in lightly chloralose anesthetized hemidecerebrate(HD) cats and examined the hypoglossal and phrenic nerve activity at different levels of CO_2 breathing. Both these nerves show respiratory related activity which increases in amplitude with hypercapnia. We monitored integrated electrical activity of whole hypoglossal and phrenic nerves of both sides during 100% O_2 breathing and also at different CO_2 levels(5-15% CO_2 in O_2). Prior to HD, the hypoglossal and phrenic activity on both sides were similar. The animal was then hemidecerebrated at midcollicular level and retested 2 to 4 hours later. Measurement of hypoglossal and phrenic nerve activity on both the HD and intact side were thus repeated using 100% O_2 and different levels of CO_2 in order to increase neural drive. HD decreased ipsilateral hypoglossal nerve activity($p<0.02$) and increased ipsilateral phrenic nerve activity($p<0.05$) during 100% O_2 breathing and with low level of CO_2. When the animal breathed CO_2, the hypoglossal nerve activity increased alinearly on both sides, less at lower level of CO_2 than at higher levels. Between 5-10% CO_2 level the hypoglossal activity on the intact side was steeper($p<0.001$) than the HD side. However, at higher CO_2 levels($\sim$10%) the difference between the two hypoglossal nerves diminished and at 15% CO_2 not significantly different($p<0.1$). On the other hand, significant difference between the two phrenic nerves were not evident above 11% CO_2. Differences in activity between the intact and the HD side could also be eliminated by increasing the depth of anesthesia.

It is concluded that the hypoglossal motoneuron pool receives a facilitory and the phrenic motoneuron pool an inhibitory influence from SP structures. The influence of SP structures on these motoneuron pools, however, depends on the prevailing level of CO_2. At CO_2 levels of 5-10% the difference between the two sides increases, indicating that the SP influence increases. Above 10% CO_2 the difference between the two sides diminishes, suggesting that either the peripheral motoneuron pools overcome the SP influence or else the SP influence wanes. Abolition of the SP influence by increasing depth of anesthesia suggests that these modulatory influences are transmitted via polysynaptic pathways.

431 DIFFUSION IN THE BRAIN CELL MICROENVIRONMENT. C. Nicholson and J.M. Phillips*. Dept. of Physiol. and Biophys., New York Univ. Med. Ctr., 550 First Avenue, New York, N.Y. 10016

The widespread use of ion-selective microelectrodes (ISMs) in the brain cell microenvironment necessitates a re-evaluation of the concept of diffusion in the extracellular space of the brain. We have done this both at the theoretical and experimental level.

We used the concept of volume averaged variables, as applied to porous media (Lehner, 1979, Chem. Eng. Sci. 34:821) to derive an extended form of Fick's Law for the complex environment of the brain. This approach yields two new parameters that reflect the geometry of the extracellular space. The first is volume fraction which indicates the amount of intercellular space available to the diffusing ion. The second parameter is tortuosity which relates to the increase in average path length of a diffusing particle as it circumnavigates cellular obstructions.

Using point iontophoresis (Lux & Neher, 1973, Exp. Brain Res. 17: 190) and ISMs selective for ions not normally present in the brain, we experimentally confirmed the theoretical concepts in the rat cerebellum. We used the cations tetramethylammonium and tetraethylammonium and the anions hexafluroarsenate and alpha-naphthalene sulfonate. The volume fraction was 0.21 ± 0.02 (mean$\pm$SEM), the tortuosity was 1.55 ± 0.05 and we found no significant difference using cations and anions.

Our results show that the brain cell microenvironment allows diffusion of small ions, and probably of other neuroactive molecules, in conformity with an extended form of Fick's Law. Under many conditions, the volume fraction and tortuosity combine so as to substantially increase the effective strength of an ionic source. These studies are also consistent with the view that the migration of K^+ in the brain involves processes in addition to simple diffusion (Nicholson, Phillips & Gardner-Medwin, 1979, Brain Res. 169:580). Supported by USPHS Grant #NS13742.

432 SYNAPTOGENESIS IN HUMAN NORMAL AND DOWN'S SYNDROME NEOCORTEX. T.L. Petit, J.C. LeBoutillier*, D.P. Alfano and L.E. Becker*. Department of Psychology, University of Toronto and Department of Pathology, Hospital for Sick Children, Toronto, Ontario, Canada.

Delineation of the normal patterns of central nervous system development is a necessary prerequisite to elucidating the possible neural mechanisms underlying various neuropathological conditions. Previous observations on normal and aberrant features of brain development have indicated alterations in dendritic growth and branching, reduced numbers of dendritic spines and altered dendritic spine morphology in some cases of human mental deficiency. This investigation was undertaken to examine synaptic development in human normal and Down's syndrome neocortex.

Post-mortem tissue was obtained from six normal and four Down's syndrome brains ranging in age from twelve weeks post-conception to four weeks postnatal. Tissue was processed using routine osmium and EPTA staining procedures and photomicrographs were systematically taken throughout the molecular layer of the sensorimotor neocortex. Synaptic density was determined by counting the number of synapses per 15,000X field. Pre- and post-synaptic length, width and area, and synaptic cleft width were measured using 300,000X photomicrographs from EPTA stained tissue.

An increase in synaptic density over the range of ages examined was observed for both normal and Down's syndrome tissue. No obvious differences were apparent in the development of synaptic density in Down's syndrome tissue. Synaptic length, width and area were observed to increase, while synaptic cleft width remained fairly constant throughout the range of ages examined in both normal and Down's syndrome tissue. All parameters appeared to be reduced in Down's syndrome tissue during the later stages of development examined. However, due to the small number of brains, extending over a wide range of developmental ages, it is as yet difficult to reach any firm conclusions regarding altered synaptic development in Down's syndrome neocortex.

This research was supported by grants from the Ontario Mental Health Foundation, the Physicians' Services Incorporated Foundation and the Natural Sciences and Engineering Research Council of Canada.

433 MIMICRY OF ADRENERGIC STIMULATION BY C5a ANAPHYLATOXIN IN THE RAT BRAIN. Nicole Schupf and Curtis Williams, Dept. Psychology, Manhattanville College and Dept. Biology, SUNY Purchase, Purchase, NY 10577.

A soluble exogenous antigen reaction (EAR) system has been shown to alter eating patterns when administered directly to the perifornical hypothalamus. Treatment with rabbit specific antibody and antigen, ovalbumin (OA), induced eating in saline treated rats and increased the eating response to norepinephrine (NE). These effects mimic those of focally applied NE. The aggregation of PMN cells at the site following EAR treatment suggests the activity of leukotaxins and the possibility that the complement cascade had been activated by the immune complexes with the production of the anaphylatoxins C5a and C3a. Both peptides are cytoactive in the periphery, causing smooth muscle contractions, vasoconstriction and degranulation of mast cells.

Should the psychopharmacological effects of the EAR be mediated by the anaphylatoxins, fresh rat serum activated by immune complexes would induce a similar response at the site. We treated fresh rat serum with aOA·OA complexes to produce classical anaphylatoxin (rat CAT). As with the EAR system, there was a significant increase in food intake following injection of rat CAT (1µl, 1/8 dilution activated serum) into the perifornical hypothalamus. Food intake following injection of heat-inactivated (56°, 30 min) rat serum treated with immune complexes, in which CAT cannot be formed, was comparable to that following a saline injection.

Serum carboxypeptidase rapidly cleaves the carboxy-terminal arginine from the anaphylatoxins. C3a(des arg) is inactive while, C5a(des arg) in the rat retains residual leukotactic and anaphylatoxic properties. The anaphylatoxic activity of the rat CAT, therefore, is considered to be primarily C5a(des arg). We injected purified human C5a (kindly supplied by Dr. T.E. Hugli) into the perifornical hypothalamus 20 minutes prior to a saline injection. C5a (0.6 - 5.0 pmol in 1 µl) caused a significant and dose dependent induction of eating behavior while a slight non-significant suppression of water intake was seen. These results, again, mimic the activity of NE at this site. Preliminary results with receptor blockers support the alpha-adrenergic association of the C5a effect: C5a induced eating is blocked by phentolamine but not by propranolol nor by haloperidol.

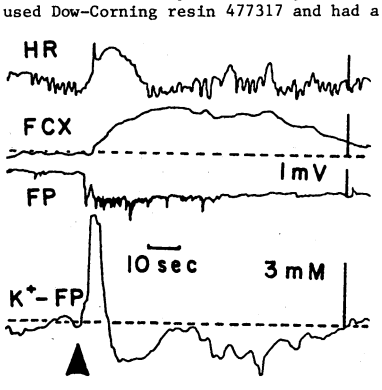
The results of these experiments suggest that immune complexes formed or deposited in the brain might entrain a variety of behavioral disturbances depending on the specific sites affected. The results further suggest that the effect may depend upon focal complement activation and that associated production of anaphylatoxin interferes with catecholaminergic function.

434 THE EFFECT OF AMYGDALOID LESIONS ON CHOOSING BETWEEN SIGNALLED AND UNSIGNALLED INESCAPABLE SHOCK BY RATS. R.N. Shull and M.G. Gaston*. Dept. of Psych., Calif. State Univ., L.A., CA 90034.

It has been clearly demonstrated that many types of animals prefer signalled over unsignalled electric shock, whether that shock is avoidable, escapable, or inescapable, and, for rats, a preference for signalled shock has been found, using a variety of different apparatus and procedures (Badia et al., Psych. Bull. 86:1107, 1979). The present experiment was designed to determine whether amygdaloid lesioned rats would acquire a preference for that side of a two-compartment box in which unavoidable and inescapable electric shock was signalled by a tone over the opposite side of the same box in which shock was unsignalled. Subjects tested were 19 male Long-Evans hooded rats (approx. 350-450 g.). Nine of these had been randomly chosen to receive bilateral electrolytic (2 mA for 30 s) amygdaloid lesions (two week recovery period), while five underwent sham operations and five were left intact. Histology later showed that all lesioned animals sustained substantial bilateral amygdaloid damage with the greatest destruction occurring in the basolateral nucleus. An experimental session (six sessions per subject) consisted of 30 scrambled shocks (0.8 mA, 3 s. duration) delivered to a grid floor according to a variable interval 75 s. schedule. When a subject was on the side of the box with vertical stripes, shocks were unsignalled; when on the side with horizontal stripes, shocks were immediately preceded by a 5 s., 80 db, 1350 Hz tone. Sham-operated and intact controls did not differ significantly on any measure and their data were pooled for analyses. Two-tailed t-tests demonstrated that while controls showed a significant preference for the signalled side ($t[9]=11.13$, $p<.001$), the lesioned animals failed to show a significant preference for either side ($t[8]=-1.02$). Visual observation of the subjects during the testing sessions showed that the external behaviors of the lesioned rats did not differ from those of the controls except that the lesioned rats made significantly fewer crossovers per session ($F[1,18]=12.39$, $p<.005$), although all subjects ran from one end of the box to the other in a repetitive fashion during shock delivery and displayed some degree of conditioned vocalization to the tone after several tone-shock pairings. It seems, then, that the lesioned animals in the present experiment were capable of displaying "normal" emotional behavior but "abnormal" instrumental behavior as compared to controls. The lesioned animals' failure to show a preference could have been due to a deficit in their ability to utilize environmental cues, suggesting that the amygdala is involved in the coupling of internal motivational-emotional states to appropriate overt motor behaviors.

435 MEANINGFUL STIMULI EVOKE EXTRACELLULAR POTASSIUM SHIFTS IN CONSCIOUS CAT FRONTAL CORTEX. James E. Skinner and Mark Molnar*. Neurophysiol. Sect., Neurol. Dept. and Neurosci. Prog., Baylor College of Med., Houston, TX 77030 and Inst. Psychol., Hungarian Acad. Sci., Budapest.

Previous observations in our laboratory have shown: 1) meaningful stimuli (e.g., novel, conditioned or reinforcing stimuli) will evoke an extracellular negative slow potential in the frontal cortex that lasts for 20-30 secs; 2) event-related membrane potential and input resistance shifts in frontocortical neurons accompany the slow extracellular potentials; and 3) event-related cyclic AMP concentrations rapidly rise in the underlying cortex and then slowly decline as a negative linear correlate of the slow potential amplitude. Our present results show that extracellular potassium shifts are also temporally correlated with the extracellular event-related slow potential. In a few locations, rapid decreases occur that are followed by slow recovery. When the ion-sensitive electrode is in a different location, the same stimulus can evoke a different pattern of potassium shifts as shown below. In this figure, there is an initial brief increase (K^+ - FP) followed by a decrease that persists for the duration of the negative polarity of the extracellular field potential recorded at the same location (FP). This latter pattern seems to be the most common type encountered in the anterior sigmoid cortex at a depth of 0.5 to 1.0 mm. FCX is the record from a nearby surface to depth transcortical pair of electrodes. HR shows the heart rate response. The potassium ion-selective electrode used Dow-Corning resin 477317 and had a tip diameter of 5 microns. The stimulus (arrow) was the presentation of sardines. Identical responses were obtained in three separate trials. The best temporal correlate of the long duration negative FP is the decrease in extracellular K^+. This result is compatible with a membrane mechanism of inactivation of a potassium current, as is the case for the slow-EPSP. Fig. 1. Event-related extracellular potassium shifts. K^+ - FP = actual potassium concentration change. (HL17907 & NS11535)



436 THE EFFECT OF PERINATALLY ADMINISTERED ETHANOL ON THE POSTNATAL DEVELOPMENT OF THE PURKINJE CELL IN THE MOUSE CEREBELLUM. D. E. Smith and D. L. Davies. Department of Anatomy, Louisiana State University Medical Center, New Orleans, LA 70112.

While the gross teratologic effects of alcohol on development have been known for decades, it has been only recently that investigators have begun to study the subtle microscopic changes which occur when the developing nervous system is subjected to an alcoholic insult. In the cerebellum, there is a reduction in the Purkinje and granule cell populations following the administration of alcohol during the perinatal period (Bauer-Moffett and Altman, Exp. Neurol. 48:378, 1975; IBID, Brain Res. 119:249, 1977; Barnes et al., Soc. Neurosci. Abst. 5:152, 1979). In order to examine possible changes in cerebellar morphology, a series of experiments were initiated using a strain of mice known to have a high preference for and behavioral tolerance to ethanol (C57BL/6J). Time-pregnant mice were divided into three groups: controls fed mouse chow and water ad libitum; experimentals fed a chocolate-flavored liquid (Nutrament) formulated to contain 25% of its caloric value as ethanol (5.4% v/v); and nutritional controls pair-fed isocaloric amounts of the same diet with sucrose replacing the ethanol. Administration of ethanol and sucrose diets began on gestation day 12 and continued until postnatal day 7; at which time, the ethanol and sucrose diets were discontinued and the groups returned to a chow and water diet. Litters were sacrificed on postnatal days 7, 14 and 21 for evaluation of cerebellar development. Some pups were processed for Golgi analysis, while others were perfused with a buffered paraformaldehyde-glutaraldehyde solution for ultrastructural examination. The material was routinely sectioned in the parasagittal plane. The most striking observation in the Golgi material was the altered morphology of the Purkinje cell. Normally, the Purkinje cell exhibits perisomatic spines at 7 days, but a rudimentary apical dendrite is also present (Meller and Glees, Neurobiology of Cerebellar Evolution and Development, p. 783, 1969). Perisomatic spines are present in the ethanol-treated group, but there is no evidence of an apical dendrite. By 14 days, the control Purkinje cells have well-developed arbors, but the ethanol-treated group displays an arbor that is more truncated and disoriented. In addition, some of the Purkinje cell bodies display grotesque silhouettes. At 21 days, there is less discernible difference between the three groups suggesting that the plasticity of this system allows for recovery from this form of alcoholic insult.

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437 STRUCTURE, INPUT, COUPLING AND PHYSIOLOGY OF THE GIANT DESCENDING NEURONS (GDNs) OF THE FLY (Musca). N.J. Strausfeld, J. Bacon, U. Bassemir* and D. Nässel*. EMBL, 69 Heidelberg and MPIV, 8131 Seewiesen, W. Germany.

Amongst descending neurons connecting cerebral to thoracic neuropil there is a prominent pair of cells whose axons lie most dorsal in the connective and have the largest diameters (20-35 µm). We are studying the structure and function of these cells (GDNs) using Co^{2+}, HRP and Golgi staining, and by intracellular recording with Lucifer-filled electrodes. Dendrites and terminals have been mapped in relation to several classes of optic lobe neurons, antennal receptors and motoneuron axons to dorsal longitudinal flight muscle. Cobalt transneuronally stains GDNs from some antennal receptor axons whereas Co^{2+}, HRP and Lucifer transneuronally stain one class of small-field visual cells of the lobula (Col A's) from the GDNs. After identifying GDN-Col A assemblies by light microscopy, E.M. studies on the same preparation reveal that although many profiles are presynaptic to GDN dendrites, only certain profiles had Co^{2+}-Ag deposits in mitochondria and in presynaptic sites. Postsynaptic sites in GDN, opposite Co^{2+}-Ag-containing Col A terminals, show extensive accumulations of Co^{2+}-Ag. Thus Co^{2+} is resolved at certain synapses only. There is no evidence of extracellular leakage. Lucifer also passes between the same cell types. Although Col A cells contain synaptic ribbons and vesicles the above observations indicate a dual nature of GDN-Col A and antennal-axon GDN-junctions, and their possible electrotonic and chemical nature is being investigated.

We have made intracellular recordings from GDNs in the ventral cord. Large (<15 mV) psp's were observed in response to mechanical stimulation of the antennae and to visual stimulation of the compound eye. Spike activity in GDNs could not be evoked by these stimuli even though other identified DNs did spike. However, the GDN would spike on release from hyperpolarization when recording near its terminal. Possibly the GDN is integrating and non-spiking in the ventral cord, and spike initiation is in the mesothoracic ganglion. Alternatively, the state of arousal in the C.N.S. of constrained preparations may be insufficient to reveal true spiking nature of the GDN. These aspects of physiology, and the post-embryonic development of GDNs, are currently being investigated.

Comparative anatomy of descending pathways in Musca, Calliphora and Drosophila reveal certain species specific features of GDN morphology of terminal processes in the pro- and mesothorax. However, in Musca and Drosophila, there is apparent coupling of GDN pairs via a point of contact in the mesothoracic ganglion as revealed by Lucifer and Cobalt, respectively. Certain other descending neurons are dye-coupled ipsilaterally to the GDN dendrites of Musca.

438 THE EFFECT OF HIPPOCAMPAL ABLATIONS IN RATS ON REWARD MAGNITUDE SHIFTS. P. N. Strong, E. Swain*, P. Taylor*, and M. Raboy*. Psychology Department, University of South Florida, Tampa, FL 33620.

Contrary to Franchina & Brown we found that hippocampectomized rats do respond to magnitude of reward shifts but, as we predicted, they do not show a negative contrast effect. Furthermore, unlike naive rats, hippocampectomized rats appear to show a positive contrast effect which may be due to their insensitivity to the stimulus generalization decrement due to reward magnitude changes. Significant differences between our motivating and feeding procedures compared to Franchina & Brown appear to explain the differences between the two studies.

439 ROLE OF MONOAMINES IN POSITIVE FEEDBACK OF ESTROGEN ON LH SECRETION. R.F. Walker* (SPON: B. Peretz) Sanders-Brown Research Center on Aging and Dept. of Anatomy, U. Kentucky Med. Ctr., Lexington, KY 40536

These experiments were designed to investigate how monoamines control daily phasic LH secretion in estrogen-treated, ovariectomized rats. Drugs which block catecholamine (α-methyl-p-tyrosine, AMT, 200mg/kg) or serotonin (p-chlorophenylalanine, pCPA, 250mg/kg) synthesis were given 3 days after ovariectomized rats received sc implants of estradiol pellets. Either drug, given alone, blocked the LH surge on the subsequent day, however AMT, caused a greater depression of basal LH levels (120ng/ml vs 170ng/ml at 1300h). Phasic LH secretion was restored by administration of monoamine precursors 5-hydroxytryptophan (5-HTP) for serotonin or l-dihydroxyphenylalanine (l-DOPA) for catecholamines. In another study, the restorative effect of 5-HTP or l-DOPA on phasic secretion of LH was tested in estrogen implanted rats in which the LH surge was blocked by AMT and pCPA treatment. LH surges were blocked and basal serum LH levels were depressed when total monoamine synthesis was blocked by both drugs. Neither 5-HTP nor l-DOPA alone, restored phasic LH secretion, though basal serum LH levels were elevated by l-DOPA treatment (301ng/ml vs 150ng/ml at 1300h). In the final study, 5-HTP or l-DOPA was injected into ovx rats at 1200h, 12 days after receiving sc implants of estradiol; a time when the daily LH surge is greatly diminished in amplitude (2800ng/ml on day 3 vs 350ng/ml on day 12). 5-HTP was more effective than l-DOPA in restoring phasic LH secretion (29% vs 20%). However, LH surges were reinstated in 89% of the 5HTP treated rats if they were pretreated with pCPA. Pretreatment with αMT of rats receiving l-DOPA, was not effective in restoring LH surges. Estimation of hypothalamic serotonin content at several time points over 24 hours in rats implanted with estrogen for 12 days showed that the serotonin circadian rhythm, which is required for phasic LH secretion, is damped by prolonged and continuous estrogen exposure. These data suggest that estrogen induced phasic LH secretion occurs in response to a daily serotoninergic signal superimposed upon a background of catecholamine-mediated, basal LH secretion. Furthermore, extinction of the daily LH surge in ovx rats bearing estrogen implants, may follow attenuation of the serotonin circadian rhythm resulting from chronic steroid exposure.

440 AUTORADIOGRAPHIC ANALYSIS OF I^{125}-PROLACTIN BINDING AND UPTAKE BY EPENDYMA OF THE RAT CHOROID PLEXUS. R.J. Walsh and B.I. Posner*. Dept. of Anatomy, George Washington Univ., Washington, D.C. 20037, Dept. of Medicine, Royal Victoria Hospital, Montreal, Canada.

Light and electron microscopic autoradiography were used to localize I^{125}-prolactin that was specifically bound to ependyma of the rat choroid plexus. Experimental adult male rats received a jugular vein injection of ovine I^{125}-prolactin (3.22 x 10^8dpm) while control animals received an identical dose of I^{125}-prolactin plus a 500-fold excess of unlabelled prolactin. Two experimental and two control animals were sacrificed by intracardiac perfusion of Ringer's lactate followed by glutaraldehyde fixative 3, 20, 40, and 60 minutes after hormone injection. Choroid plexus was removed and processed to Epon. One micron, iron hematoxylin prestained sections were coated with NTB-2 nuclear emulsion, exposed for 21 days, and analyzed in the light microscope by counting silver grains per 6960 μm^2 unit area. Thin sections were coated with a monolayer of Ilford L4 emulsion, exposed for 3 months, developed for fine silver grains with gold latensification (Kopriwa, Histochemie, 37:1 (73)), and analyzed by the direct scoring method.

Quantitation of silver grains at the light microscopic level indicated that excess unlabelled prolactin caused an 85% inhibition in the intense autoradiographic reaction observed over ependyma of all experimental animals, reaffirming the presence of specific prolactin binding sites on choroid ependyma (Walsh et al., Science 201:1041 (78)). Electron microscopic autoradiographs from experimental animals revealed the majority of silver grains (67%) were localized to the basal and lateral plasmalemma of the ependyma at 3 minutes post-injection. With increasing time, there was a dramatic decrease in the incidence of silver grains over the plasmalemma such that only 16% were localized to this portion of the cell 60 minutes after hormone injection. Concommittant with a decrease in silver grains over plasmalemma, there was an increase in grains localized to cytoplasmic vesicles and tubules (21% at 60 min.) and lysosomal bodies (37% at 60 min.). All other cytoplasmic organelles and the apical plasmalemma exhibited insignificant radiolabel at all times. The results are consistent with other studies regarding the localization and fate of polypeptide hormones at target cells, i.e., maximum hormone binding occurs at the plasmalemma shortly after initial exposure to the hormone with subsequent internalization and translocation to intracellular vesicles, vacuoles, and lysosomes.

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441 GROWTH HORMONE RESPONSE TO APOMORPHINE ADMINISTRATION AND IN VITRO LITHIUM RATIOS PREDICT SUCCESS OF LITHIUM THERAPY IN THE SCHIZOPHRENIAS. Frank P. Zemlan, Jack Hirschowitz*, Robert Hitzemann and David L. Garver. Department of Psychiatry, University of Cincinnati College of Medicine, Cincinnati, OH 45267.

Blood samples from 31 patients with RDC schizophrenic symptoms were assayed for peak plasma growth hormone (GH) response to apomorphine administration (0.75mg s.c.) and the in vitro lithium ratio. Patients then entered a 2 wk lithium carbonate (Li) trial with symptomatology assessed with the serial New Haven Schizophrenic Index (NHSI). Designated Li responders were patients showing a >35% improvement in NHSI scores. Of the 31 patients, 6 responded to Li therapy (n=5 DSM-III Schizophreniform Disorder, n=1 Schizophrenic Disorder) and 25 did not respond (n=1 Schizophreniform Disorder, n=24 Schizophrenic Disorder).

The mean GH peak for Li responders was 38.7 + 5.7ng/ml, while the peak level for non-responders was 18.4 + 2.5ng/ml ($p<0.002$). If 20ng/ml is taken as the cut-off peak GH response, then all Li responders fell above this point. For patients with a peak GH response $\geq$ 20ng/ml a significant decrease in serial NHSI scores was observed ($p<0.01$) with no effect of the Li trial seen in patients with a peak GH $\leq$ 20ng/ml.

Mean in vitro lithium ratios for the Li responders was 0.452 + 0.057 while the ratio for nonresponders was 0.356 + 0.026. If 0.390 is taken as the cut-off lithium ratio, then 80% of the Li responders fell above this point. The percent improvement in NHSI scores among patients meeting only the GH criterion was 32%, while among patients meeting both the Li ratio and GH criteria, a 67% improvement was observed during the Li trial. The doubling in the percent improvement among patients meeting both criteria was due to the fact that all Li non-responsive patients with peak GH response $\geq$ 20ng/ml were excluded employing the additional Li ratio criterion. That is, using peak GH response alone to predict success of the Li trial, 50% of the predicted Li responders did not turn out to be actual Li responders. Using both biological markers to predict response on the Li trial, a purer population of Li responsive patients was isolated.

The present data suggest that these two biological markers can isolate a subgroup of schizophrenic patients responsive to Li treatment, as well as, the existence of biological abnormalities characteristic of Li responsive schizophrenic patients.

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